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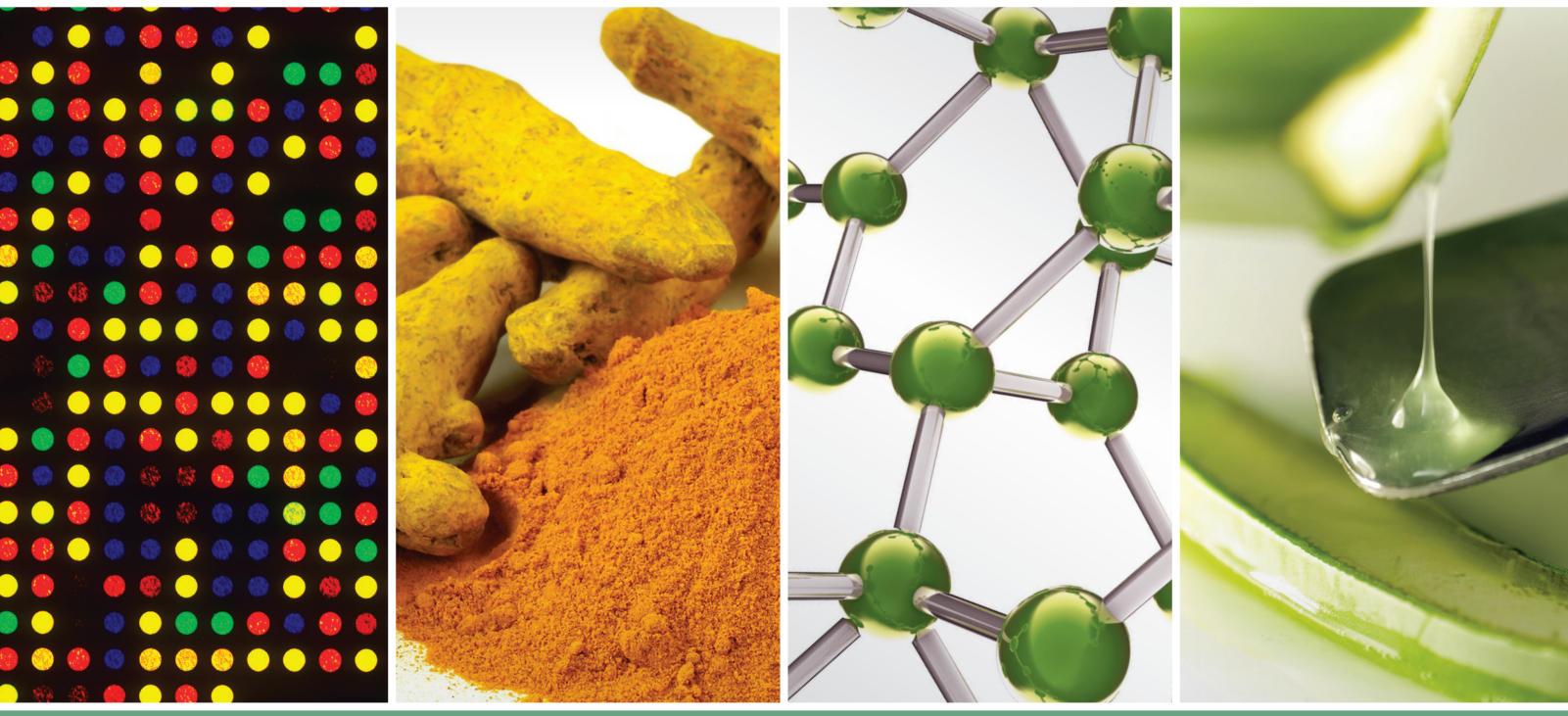
Traditional Medicines in the World: Where to Go Next?

Guest Editors: Si-Yuan Pan, Gerhard Litscher, Kelvin Chan, Zhi-Ling Yu, Hou-Qi Chen, and Kam-Ming Ko



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Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2014, Article ID 739895, 4 pages
http://dx.doi.org/10.1155/2014/739895

Editorial

Traditional Medicines in the World: Where to Go Next?

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Received 27 August 2014; Accepted 27 August 2014; Published 29 December 2014

According to the WHO, 65–80% of the world's healthcare practice involves the use of traditional medicine (TM), commonly referred to as complementary and alternative medicine (CAM), in some way. Today, TM has become an indispensable part of our health management. It has been well known that TM covers a wide array of therapies and practices which vary from culture to culture and country to country. It is more important that TM differs from conventional medicine (CM) in both theory and practice. However, the research and development of almost all TM systems mostly follow the track that had been laid down by CM nowadays. It is clearly not appropriate for the future development of TM. Therefore, a well-structured strategy for research, practice, and development is instrumental to optimize the utilization of TM which reflects its superiority over CM in some ways. Now it is time for us to start thinking about "where is TM heading?" and "how should TM reach its destination?"

This special issue focuses on TM development and practice strategy in the theory and experiment.

N. T. Y. Leung et al. contributed a paper entitled "*Potential therapeutic effects of meditation for treating affective dysregulation*." Affective dysregulation is at the root of many psychopathologies. The root of these disorders appears to be an attenuated, top-down cognitive control from the prefrontal cortices over the maladaptive subcortical emotional processing. The mainstream treatment approaches are antidepressant medication and cognitive behavioral therapy. Because of its emphasis on present-moment awareness and acceptance, a growing body of research has explored the potential clinical applications of meditation for disorders stemming from affective dysregulation. Current evidence reveals that meditation may prevent and intervene in mood and other affective disorders. Increasing research has suggested that the cultivation of awareness and acceptance along with a nonjudgmental attitude via meditation promotes adaptive affective regulation. This review examined the concepts of affective regulation and meditation and discussed behavioral and neural evidence of the potential clinical application of meditation. Lately, there has been a growing trend toward incorporating the *mindfulness* component into existing psychotherapeutic treatment. Promising results have been observed thus far. Future studies may consider exploring the possibility of integrating the element of *compassion* into current psychotherapeutic approaches.

In their paper entitled "*Acupuncture and depth: future direction for acupuncture research*," Y. L. Goh et al. concluded that the plentiful variables that exist in acupuncture research are the reason for the lack of scrutiny on the depth of acupuncture. The authors stated that, as research progresses, attention needs to be given to the amount of tissue invaded and types of tissues excited. Along with the advancements in imaging, laser technologies, and so forth, research on

acupuncture depth could also progress. They suggest that future acupuncture research, especially RCTs, should take into consideration the depth of insertion. In addition, the use of bibliometric method is crucial for future development of traditional medicine research trends too.

The paper entitled "*Antidiabetic and antioxidative effect of Jiang Tang Xiao Ke granule in high-fat diet and low-dose streptozotocin induced diabetic rats*" by D.-D. Zhao et al. deals with diabetes mellitus (DM), a kind of metabolic disease, which has been increasing over the last four decades in the world. The purpose of their study was to investigate the effect of Jiang Tang Xiao Ke (JTXK) granule, a naturally occurring ingredient from Chinese herbal medicines, on serum glucose, lipids, and oxidative stress in DM rats induced by high-fat diet and streptozotocin. In conclusion, they found that JTXK granule, at 9 g/kg (based on crude herbal material), treatment for 4 weeks reduced serum glucose via increasing insulin sensitivity and protection of pancreas islets in DM rats. In addition, the JTXK granule decreased serum total cholesterol, triglyceride, and low density lipoprotein (LDL) levels but increased high density lipoprotein (HDL) levels, compared with the drug-untreated DM rats. At the same time, JTXK granule showed improved antioxidant activity, which was manifested by decreased malondialdehyde and nitric oxide levels and with elevation in superoxide dismutase levels in DM rats. Islet morphology showed marked improvement in DM rats treated with JTXK granule. These findings suggested that JTXK granule may be an effective and safe alternative treatment for type 2 DM.

S. Telles et al. wrote the paper entitled "*Research on traditional medicine: what has been done, the difficulties, and possible solutions*." They stated that traditional medicine (TM) is being used more frequently all over the world. However, most often, these are choices made by the patient. Integrating TM into mainstream healthcare would require research to understand the efficacy, safety, and mechanism of action of TM systems. This paper describes research done on TM and difficulties encountered in researching TM, especially when an attempt is made to conform to the model for conventional medicine. The last part of the report provides suggestions to make research on TM more acceptable and useful, with the ultimate goal of integrating TM into mainstream healthcare with sufficient knowledge about the efficacy, safety, and mechanism of action of TM systems.

The contribution by H. Zhang et al. is entitled "*A disturbance rejection framework for the study of traditional Chinese medicine*." In this paper, traditional Chinese medicine (TCM) is explained in the language of engineering cybernetics (EC), an engineering science with the tradition of rigor and a long history of practice. The inherent connection is articulated between EC, as a science of interrelations, and the Chinese conception of Wuxing. The combined cybernetic model of Wuxing seems to have significant explaining power for the TCM and could potentially facilitate better communications of the insights of the TCM to the West. EC and TCM also share commitment and goal to attain and maintain a quality that is described as balance, equilibrium, or homeostasis; correspondingly, there is a great similarity in how such quality is maintained: by what is called "disturbance rejection" in EC. In disturbance rejection, a great metaphor is found to show how TCM is practiced, using a case study as illustration. The results from this six-year study seem to support this line of investigation and to provide a clear exposition of both the principles of the TCM and how they are practiced with herbal medicine. It is the authors' hope that what started in this paper could help future researchers to build the bridge from the general system qualities and behaviors to the properties of the system components.

X.-Y. Wang et al. dealt with "*Supplementation with the extract of Schisandrae Fructus pulp, seed, or their combination influences the metabolism of lipids and glucose in mice fed with normal and hypercholesterolemic diet*." Schisandrae Fructus (SF), which possesses five tastes, can produce a wide spectrum of biological activities in the body. In this paper, the authors investigated the effects of the ethanolic extract of SF pulp, seed, or their combination (namely, EtSF-P, EtSF-S, or EtSF-P/S, resp., collectively called EtSF) on the metabolism of lipids and glucose in normal diet- (ND-) and hypercholesterolemic diet- (HCLD-) fed mice. In conclusion, results showed that the supplementation with EtSF produced a significant influence on lipid/glucose metabolism in ND- and HCLD-fed mice, especially in hypercholesterolemic (HCL) mice. Dietary supplementation with EtSF-P or EtSF-S/P elevated serum lipid levels, except for that of serum triglyceride levels which was lowered, in HCL mice. Dietary supplementation with EtSF-S, EtSF-P, or EtSF-S/P reduced hepatic lipid and glucose concentrations in both normal and HCL mice. EtSF-S/P, but not EtSF-S or EtSF-P, supplementation increased fecal cholesterol excretion in HCLD-fed mice. EtSF-P and EtSF-S/P attenuated the HCLD-induced hepatotoxicity. Supplementation with fenofibrate decreased serum and hepatic lipid and glucose levels and increased serum ALT activity and liver weight in mice fed with ND and/or HCLD. The ensemble of results indicates a differential effect between SF seed and pulp on lipid and glucose metabolism, particularly in HCL mice. Thus, the authors concluded that supplementation with EtSF might ameliorate the lipid accumulation in liver cells and thus protect against liver injury in HCL mice.

The paper entitled "*Historical perspective of traditional indigenous medical practices: the current renaissance and conservation of herbal resources*" by S.-Y. Pan et al. takes a thorough look at Chinese, Indian, and Arabic herbal medicine over the centuries. It was found that, based on cultures and geographical regions, various kinds of herbal remedies have evolved. Herbal medicines are therefore an integral part of culture and geographical environment, and various kinds of herbal medicines have their own unique way of understanding and treating a disease. However, the globalization of trade and market has brought about an integration of different kinds of herbal medicines over the world. At present, herbal medications or related products in the global market are derived from Chinese herbs, Indian herbs, Arabic herbs, and Western herbs. Herbal remedies may be also classified into three categories, namely, modern herbs, theoretical herbs, and empirical herbs, in accordance with their nature/characteristics and the nature of current usage. In general, most herbal remedies/formulae are considered

to be safe and are well tolerated because they have been successfully used for thousands of years as foods to promote health and as medicines to treat diseases. To date, herbal products are widely available to consumers and have become increasingly popular throughout the world. For the authors, there is no doubt that herbal products will continue to play a crucial role in the healthcare system of human societies, not to mention that secondary metabolites of plants are economically important as drugs, fragrances, cosmetics, food additives, and pesticides.

I. O. Lawal et al. obtained "*Phytotherapeutic information on plants used for the treatment of tuberculosis in Eastern Cape province, South Africa*," because the current rate of deforestation in Africa constitutes a serious danger to the future of medicinal plants on this continent. Conservation of these medicinal plants in the field and the scientific documentation of our knowledge about them are therefore crucial. An ethnobotanical survey of plants used for the treatment of tuberculosis (TB) was carried out in selected areas of the Eastern Cape, South Africa. The documented medicinal plants used by the Xhosa herbalist reflect a rich ethnomedicinal knowledge in the municipality. These results strengthen the firm belief that traditional medicines are readily accessible and still play an important role in meeting the basic healthcare of many people in African communities. Phytomedicinal information on the treatment of TB in this region is well established. Thirty plants belonging to 21 families were mentioned to be used for the treatment of TB and associated diseases. Other diseases treated using these plants were respiratory infections. The commonly mentioned species are *Clausena anisata*, *Haemanthus albiflos, Artemisia afra, Carpobrotus edulis, Ptaeroxylon obliquum,* and *Tulbaghia violacea.* The most frequently mentioned species was *Clausena anisata,* known locally as *Iperepes*. Many studies have revealed some bioactive chemical compositions in these plants which probably justify their pharmacological properties. The following five species were recorded for the first time for the management of TB in Eastern Cape province, South Africa: *Clausena anisata, Haemanthus albiflos, Araujia sericifera, Scabiosa albanensis, and Silene undulata*. This study has contributed to the scientific documentation of medicinal plants used for the treatment of TB. This is necessary in the rural communities to avert the erosion of traditional medicine knowledge. The larger percentage of the traditional healers are old people; therefore, this legacy needs to be conserved. Finally, the authors announced that further studies are in progress on the antituberculosis assay to validate ethnopharmacology relevance of the most mentioned plants in the study area.

The paper by X.-Q. Wang et al. is entitled "*New perspectives on specific immune-depletion technique using monoclonal antibodies against small active molecules in herbs*." One of the main focuses in Chinese medicine research is the identification of efficacious components in Chinese herbal medicine (CHM). According to the authors, current methods to track and separate active components are not adequate to meet the needs of revealing effects and identify substances and pharmacological mechanisms, which directly restrict the modernization and globalization of CHM. Thus, they introduced a new methodology to deplete a single active component via immunoassay. The specific active component in a CHM mixture can then be identified and studied through comparative analyses of the pharmacological effects before and after immune-depletion. These tools allow researchers to have a clear understanding on how to conduct a more in-depth study in CHM research. *Radix puerariae* was used as an example for demonstrating monoclonal antibody technology using mAb deletion. By comparing the pharmacological properties and pharmacodynamics of *Pueraria* extract before and after undergoing puerarin immunodepletion, the main contribution of puerarin to the overall medicinal property of *Pueraria* can then be easily evaluated, in vivo and in vitro, static and dynamic. As a result, puerarin can then be better utilized in QC, compatibility studies, and pharmacokinetic studies of *Pueraria* roots, which expands the usage of *Pueraria* roots in clinical medicine in the future. And also as a result, the experimental foundation for any future CHM studies using target specific deletion techniques was established. However, the methodology introduced in this paper still has its limitations. Taking everything into account, Wang et al. conclude that the new research strategy still holds many advantages and has proven to provide more effective and convenient ways of pursuing CHM research and help the modernization of Chinese medicine.

The paper entitled "*Ethyl acetate extract of Artemisia anomala S. Moore displays potent anti-inflammatory effect*" was authored by X. Tan et al. They state that *Artemisia anomala* S. Moore has been widely used in China to treat inflammatory diseases for hundreds of years. However, mechanisms associated with its anti-inflammatory effect are not clear. In this study, they prepared ethyl acetate, petroleum ether, *n*-BuOH, and aqueous extracts from ethanol extract of *Artemisia anomala* S. Moore. Comparing anti-inflammatory effects of these extracts, they found that ethyl acetate extract of this herb (EAFA) exhibited the strongest inhibitory effect on nitric oxide (NO) production. EAFA suppressed the production of NO in a time- and dose-dependent manner without eliciting cytotoxicity. To understand the molecular mechanism underlying EAFA's anti-inflammatory effect, the authors showed that EAFA increased total cellular antioxidant capacity while reducing the amount of inducible nitric oxide synthase (iNOS) in stimulated RAW264.7 cells. EAFA also suppressed the expression of IL-1β and IL-6, whereas elevating the level of heme oxygenase-1(HO-1). These EAFA-induced events were apparently associated with NF-κB and MAPK signaling pathways because the DNA binding activity of p50/p65 was impaired and the activities of both ERK and JNK were decreased in EAFA-treated cells comparing to untreated cells. Their findings suggest that EAFA exerts its anti-inflammatory effect by inhibiting the expression of iNOS. In conclusion, this study indicates that EAFA can potently suppress inflammatory responses.

In their paper entitled "*New insights into the chemical and biochemical basis of the "Yang-invigorating" action of Chinese Yang-tonic herbs*," J. Chen et al. propose a biochemical mechanism of "Yang-invigorating" action produced by Yang-tonic herbs. Triterpenes or phytosterols, the active components in Yang-tonic herbs with similar chemical structure,

can stimulate mitochondrial electron transport through the increase in the fluidity of the inner mitochondrial membrane, with a resultant increase in ATP generation capacity. The stimulation of electron transport is accompanied by an increase in ROS production, which triggers a retrograde response to upregulate cellular/mitochondrial antioxidant defense mechanisms through the Nrf2 pathway. In addition, they found that mitochondrial ROS can also stimulate the activity of uncoupling protein and thereby lower the membrane potential through the dissipation of proton gradient. With a recurring "Yang-invigorating" action produced by the active components of Yang-tonic herbs, an intermittent stimulation of mitochondrial ROS production at low levels results in the prolonged activation of mitochondrial uncoupling and the upregulation of antioxidant defense components characteristic of mitohormesis, which is beneficial in retarding the decline in body function and delaying the onset of age-related diseases.

Acknowledgments

We would like to express our sincere gratitude to the reviewers whose professional comments and suggestions guaranteed the high quality of these selected papers. Also, we are grateful to the authors for their contributions. Some work is supported in some parts by the National Natural Science Foundation of China (Grant no. 31071989). Some work has also been supported by Sino-Austrian Projects (Austrian Federal Ministries of Science, Research and Economy and of Health) and the European Academy of TCM. The statements made herein are solely the responsibility of the editors.

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Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2014, Article ID 856273, 13 pages
http://dx.doi.org/10.1155/2014/856273

Review Article

New Insights into the Chemical and Biochemical Basis of the "Yang-Invigorating" Action of Chinese Yang-Tonic Herbs

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Received 24 February 2014; Revised 7 June 2014; Accepted 21 June 2014; Published 29 December 2014

Academic Editor: Kelvin Chan

In the practice of traditional Chinese medicine, many Yang-tonic herbs have been used for retarding the decline in bodily function and delaying the onset of age-related diseases. Our earlier studies have demonstrated that Yang-invigorating herbs/formulations protect against oxidative injury in various organs and also extend the median lifespan in mice. This lifespan extension was associated with an upregulation of cellular antioxidant status including that of mitochondria whose functional capacity is also increased by "Yang-invigorating" herbs/formulations. In this paper, we propose that triterpenes and phytosterols, which are ubiquitously found in Yang-tonic herbs, may be the chemical entities responsible for enhancing mitochondrial functional and antioxidant capacity and thus the "Yang-invigorating" action. The biochemical mechanism underlying this "Yang-invigorating" action may involve a sustained production of low levels of mitochondrial reactive oxygen species (ROS) secondary to an increased activity of the electron transport chain, with the possible involvement of mitochondrial uncoupling. The increase in mitochondrial functional capacity can retard the decline in bodily function during aging, whereas the mitochondrial ROS production is instrumental in eliciting a glutathione antioxidant response via redox-sensitive signaling pathways, which can delay the onset of age-related diseases.

1. The Role of Mitochondrial Decay in Aging-Related Diseases

Population aging, resulting from both longer life expectancy and declining fertility rates, has become one of the most distinctive demographic events in the 21st century in most developing and developed countries. According to the World Health Organization, between 2000 and 2050, the proportion of the world's population over 60 years of age will double from about 11 to 22% [1]. There is no doubt that aging can exert financial pressures on publicly funded healthcare systems and on long-term and income support programs in various countries.

From a biological perspective, aging is defined as an inevitable degenerative process in physiological functions and metabolic processes, ultimately leading to morbidity and mortality [2]. The incidence of neurodegenerative diseases, cardiovascular disorders, metabolic diseases, and cancers increases exponentially with age [3]. While the complex biochemical pathways leading to pathological consequences of aging remain relatively unclear, a consensus is emerging on the theory that describes a gradual decline in mitochondrial function with age, which eventually leads to progressive tissue damage resulting from oxidative stress [4–6]. The mitochondrial electron transport chain (ETC), in which numerous one-electron transfer reactions occur, has been recognized as quantitatively the most important source of reactive oxygen species (ROS) in the majority of eukaryotic cell types [7]. The mitochondrial ETC reduces oxygen molecules to water by tetravalent reduction that occurs in one-electron transfers in complex IV, with reactive oxygen intermediates being tightly bound in the complex. However, electrons leaking out from the ETC can result in the formation of partially reduced reactive oxygen species in both the mitochondrial matrix and the cytoplasm [8–10]. A large body of evidence has accumulated showing that, with advancing age, mitochondrial ROS production significantly increases in various organs, including the heart, liver, and brain,

indicative of increasing oxidative stress [11–13]. However, the mitochondrial free radical theory of aging (MFRTA) has been criticized over the last decades. Inconsistent with the MFRTA, a growing number of genetic studies indicated that longevity was not affected by increasing or decreasing the level of endogenous antioxidant enzymes [14–16]. To provide a unifying view of aging and diseases, the "Double-Agent Theory" postulated that mitochondrial ROS generation produces a genetic response mimicking that triggered by infection associated increase in intracellular oxidative stress. However, the continuous mitochondrial ROS production would lead to a persistent shift in gene expression, with resultant chronic inflammation which is commonly involved in age-related diseases [17]. The abnormal mitochondrial ROS production and detoxification is accompanied by self-inflicted mitochondrial oxidative damage, which results in a significant decline in respiratory complex (I–V) activities and mitochondrial respiratory efficiency [18]. The extent of oxidative damage to key metabolic enzymes increases with age, with consequent decreases in substrate binding affinity and mitochondrial ATP generating capacity [19, 20]. Given that decay in mitochondrial structure and function is likely one of the primary causal factors in the process of aging and in the development of age-related diseases, results obtained from a large number of studies over the past two decades have suggested that the maintenance of mitochondrial functional integrity, particularly oxidative phosphorylation capacity, is important for mitigating the adverse effects of aging, as evidenced by rodent models of neurodegenerative diseases [21], heart disease [22], and aging skeletal muscle [23].

In addition, the decline in electron transport function may be directly related to the increased level of electron leakage, thereby triggering a vicious cycle of mitochondrial ROS generation [24]. To cope with an array of potentially damaging ROS, cells of aerobic organisms are believed to have developed sophisticated antioxidant mechanisms to maintain intracellular ROS at low levels within a narrow range [25]. As reduced glutathione (GSH) is the most abundant intracellular nonprotein thiol, the glutathione redox system, which involves the redox cycling of GSH and oxidized glutathione (GSSG), can be regarded as the first line of defense against ROS. The GSH/GSSG ratio has been used to assess redox status in biological systems. A reduced state of the GSH/GSSG couple appears to correlate with the proliferating state of the cell, whereas an increasingly more oxidized state is characteristic of cells during differentiation and apoptosis. The GSH/GSSG ratio decreases with aging in various organs, such as the liver, kidney, heart, and brain [26], so that mitochondrial GSH becomes more oxidized predisposing to the development of age-related diseases such as type 2 diabetes, Alzheimer's disease, and cardiovascular diseases [27]. Therefore, the maintenance of glutathione redox status is crucial for the prevention of many age-related diseases.

2. Yang-Invigorating Herbs and Mitochondrial Function

The practice of traditional Chinese medicine (TCM) always emphasizes the prolongation of a healthy lifespan. In this regard, many Chinese tonic herbs have long been used in the hope of retarding the age-related decline in body function and delaying the onset of age-related diseases. According to TCM theory, there are four categories of tonic herbs, namely, "Yang-invigorating"; "Yin-nourishing"; "Qi-invigorating"; and "Blood-enriching" herbs. Herbs from each category (namely, Yin, Yang, Qi, and Blood) are used for the treatment of particular types of deficiencies in body function. Holistically, the "Yang-invigorating" action involves the general upregulation of cellular activities, which is in turn critically dependent on the generation of mitochondrial ATP via the mitochondrial respiratory chain at the cellular level [28]. In this regard, some Yang-tonic herbs have been found to increase mitochondrial energy metabolism in various tissues under certain experimental conditions. For instance, treatment with *Epimedium brevicornum* increased respiratory complex IV activity and the rate of ATP synthesis in heart, brain, and skeletal muscle, thereby protecting against mitochondrial oxidative damage in aged rats [29]. Long-term treatment with an *Eucommiae ulmoides* extract produced an increase in oxidative metabolism in skeletal muscle and increased the endurance of rats to running exercise [30]. Pretreatment with a water extract of *Cynomorium songaricum* was found to restore the normal structure and function of liver mitochondria in D-galactose-challenged rats [31]. A detailed study in our laboratory has demonstrated that pretreatment with ethanol extracts of eleven Yang-tonic herbs, including *Eucommiae ulmoides*, *Cistanche deserticola*, *Cynomorium songaricum*, *Curculigo orchioides, Epimedium brevicornum*, *Dipsacus asper*, *Drynaria fortune*, *Psoralea corylifolia*, *Cuscuta australis*, *Morindae officinalis,* and *Allium tuberosum*, consistently increased mitochondrial ATP generation capacity in isolated heart mitochondria *ex vivo* and H9c2 cells *in vitro*, in association with a significant stimulatory action on the mitochondrial electron transport system [28].

In addition to upregulating mitochondrial functional capacity, Yang-tonic herbs/formulations have also been shown to enhance cellular/mitochondrial antioxidant status, thereby protecting against oxidant injury in various tissues of rats. Previous studies in our laboratory have demonstrated that pretreatment with an ethanol extract of *Cistanche deserticola* protected against myocardial ischemia/reperfusion injury *ex vivo* in rats [32], possibly through the enhancement of mitochondrial glutathione status and functional capacity [33]. Long-term treatment with a Yang-invigorating Chinese herbal formula (VI-28; composed of *Panax ginseng, Cervus nippon, Cordyceps sinensis, Salvia miltiorrhiza, Allium tuberosum, Cnidium monnieri, and Euodia rutaecarpa*) was found to increase the levels/activities of mitochondrial antioxidant components such as GSH, α-tocopherol (α-TOC), and manganese-superoxide dismutase (Mn-SOD) in various tissues, indicative of upregulation of mitochondrial redox status as a result of "Yang-invigoration," thereby protecting against oxidative tissue damage in rat models of cerebral/myocardial ischemia-reperfusion injury, carbon tetrachloride hepatotoxicity, and gentamicin nephrotoxicity [34, 35]. More interestingly, long-term dietary supplementation with VI-28 could extend the median lifespan and mitigate

age-associated declines in mitochondrial antioxidant status and functional status of various tissues in male and female C57BL/6J mice [36]. Conceivably, the retardation of decay in mitochondrial structural and functional integrity by "Yang invigoration" may have clinical applicability in delaying the onset of age-related diseases and thereby promote a healthy lifespan (i.e., "healthspan") of humans.

3. Chemical Basis of "Yang-Invigorating" Action: Triterpene or Steroid-Like Compounds

Although the ability of Yang-tonic herbs to enhance functional capacity and antioxidant status of mitochondria as well as to delay the onset of aging-related diseases has been demonstrated, the chemical components responsible for the "Yang-invigorating" action of tonic herbs remain largely unknown. As a "Yang-invigorating" action involves the general upregulation of cellular activities, the enhancement of mitochondrial ATP generation can serve as a pharmacological activity marker for the activity [37]. To define the chemical basis underlying the "Yang-invigorating" action, an activity-directed fractionation of an ethanol extract of *Cynomorium songaricum* revealed that a relatively nonpolar semipurified fraction, HCY2, possessed the most potent activity in stimulating ATP generation capacity in H9c2 cells. This fraction was chemically characterized by LC-UV-MS spectrometry, and ursolic acid was found to be its major constituent. Further pharmacological studies showed that both HCY2 and ursolic acid pretreatments significantly protected against myocardial ischemia/reperfusion (I/R) injury, carbon tetrachloride hepatotoxicity, and gentamicin nephrotoxicity in both male and female rats, and this tissue protection was accompanied by an upregulation of cellular/mitochondrial antioxidant status and functional capacity [38, 39]. Another study in our laboratory using animal and cell models showed that a phytosterol-enriched fraction of *Cistanche deserticola* and its active ingredient, β-sitosterol, protected against hypoxia/reoxygenation-induced apoptosis in H9c2 cells and myocardial I/R injury in rats. It is likely that both of these effects were mediated by an upregulation of cellular/mitochondrial glutathione redox cycling [40]. Furthermore, asperosaponin VI, a major saponin of *Dipsacus asper* (a Yang-tonic herb), was found to protect against acute myocardial infarction in rats, an effect which might be attributed to the detoxification of lipid peroxidation products and ROS, and increases in antioxidant enzyme activities, which would result in the prevention of mitochondrial damage [41]. The aforementioned findings suggested that triterpenes or steroid-like compounds derived from Yang-tonic herbs may be the chemical components responsible for producing the "Yang-invigorating" action. The triterpenes belonging to ursane, oleanane, cycloartane, chiratane, and hopane groups and triterpene saponins as well as the steroid-like constituents in Yang-tonic herbs are summarized in (Table 1 and Figure 1).

As shown in Table 1, the ubiquitous triterpenes, like ursolic acid and oleanolic acid, and steroids, such as β-sitosterol and β-sitosteryl glucoside, were found in most of the Yang-tonic herbs. In addition, triterpene saponins were identified as major constituents in several Yang-tonic herbs. For instance, a series of triterpene saponins, which are based on the chemical structure of hederagenin and oleanolic acid, were isolated from *Dipsacus asper* [42]. Several methyl ester derivatives of olanene glycosides from seeds of *Astragalus complanatus* R.Br were reported [43]. A series of spirostanol and furostane-type oligoglycosides were also isolated from *Allium tuberosum* Rottl. ex Spreng. [44–46]. In summary, triterpenes and steroids are the major types of phytochemicals identified in Yang-tonic herbs, and this information is crucial in elucidating the biochemical mechanism(s) underlying "Yang-invigorating" activity.

4. Biochemical Mechanism of "Yang-Invigorating" Activity

Triterpenes and phytosterols have been reported to possess a wide spectrum of biological activities, including but not limited to antioxidant, antiatherosclerotic, antiviral, anti-inflammatory, and anticancer actions [47–51]. Although they are not usually considered as classical antioxidants, a large volume of studies have demonstrated that triterpenes such as ursane, lupane, and oleanane-types produce protective effects against oxidant injury in various organs, including heart, kidney, liver, and brain [52–54]. The tissue protection was paralleled by an improvement in antioxidant status, as manifested by increases in activities of SOD, catalase, glutathione transferases, and glutathione peroxidase [55–57]. However, the biochemical mechanism(s) underlying the antioxidant action of triterpenes and phytosterols has remained unclear.

As mentioned earlier, preliminary studies in our laboratory showed that an ursolic acid-enriched fraction derived from *Cynomorium songaricum* Rupr. and a phytosterol-enriched fraction derived from *Cistanche deserticola* Y. C. Ma invariably protected against oxidative injury, which was associated with the upregulation of cellular/mitochondrial antioxidant status as well as the functional activity of various organs [38, 39]. In these studies, both ursolic acid and β-sitosterol were found to increase mitochondrial electron transport chain activity. In the experiment, the electron transport in isolated mitochondria, which is driven by pyruvate and malate, was assessed by the reduction of MTT. The enhancement in mitochondrial electron transport likely contributes to the stimulatory effect of ursolic acid and β-sitosterol on mitochondrial ATP generating capacity, which is an indirect measure of oxidative phosphorylation [40]. A study using high throughput drug screening, whose aim was to identify disease-modifying compounds for Parkinson's disease, also showed that a series of natural triterpenes, such as ursocholanic acid and ursolic acid, significantly increased the activity of all four complexes of the mitochondrial respiratory chain and preserved intracellular ATP levels in parkin (PARK2) mutant fibroblasts [58]. In addition, a recent study demonstrated that β-sitosterol can become incorporated into the mitochondrial inner membrane and increase its

Table 1: Triterpenes and phytosterols in Chinese Yang-tonic herbs.

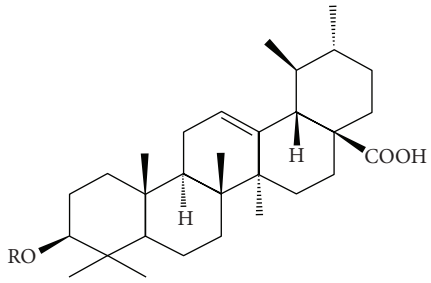
Compounds	Herbs	Medicinal parts	Reference
Ursane-type triterpenes			
Ursolic acid (1)	*Cynomorium songaricum* Rupr.	Stem	[69]
	Morindae officinalis How	Root	[70]
	Eucommiae ulmoides Oliv.	Bark	[71]
		Leaf	[72]
Acetyl ursolic acid (2)	*Cynomorium songaricum* Rupr.	Stem	[69]
Malonyl ursolic acid hemiester (3)	*Cynomorium songaricum* Rupr.	Stem	[69]
Rotungenic acid (6)	*Morindae officinalis* How	Root	[73]
Ulmoidol A (20)	*Eucommiae ulmoides* Oliv.	Leaf	[72]
Ulmoidol (21)	*Eucommiae ulmoides* Oliv.	Leaf	[72]
Corosolic acid (22)	*Eucommiae ulmoides* Oliv.	Leaf	[72]
2α,3α-Dihydroxy-24-nor-4(23)-12-oleanadien-28-oic acid (23)	*Eucommiae ulmoides* Oliv.	Leaf	[72]
Oleanane-type triterpenes			
Oleanolic acid (4)	*Morindae officinalis* How	Root	[70]
	Eucommiae ulmoides Oliv.	Leaf	[72]
	Epimedium brevicornum Maxim.	Leaf	[74]
Malonyl oleanolic acid hemiester (5)	*Cynomorium songaricum* Rupr.	Stem	[69]
Oleanolic acid-derived triterpene saponins (7–19)	*Dipsacus asper* Wall. Ex Henry	Root	[42, 75]
Methyl ester derivatives of oleanane glycosides (37–42)	*Astragalus complanatus* R. Br	Seed	[43]
Chiratane-type triterpene			
20β-Hydroxychiratan-22-one (25)	*Drynaria fortune* J. Sm.	Root and stem	[76]
Hopane-type triterpenes			
Fern-9(11)-ene (26)	*Drynaria fortune* J. Sm	Root and stem	[76]
Dryocrassol acetate (27)	*Drynaria fortune* J. Sm	Root and stem	[76]
Dryocrassol (28)	*Drynaria fortune* J. Sm	Root and stem	[76]
Hop-22(29)-ene (29)	*Drynaria fortune* J. Sm	Root and stem	[76]
Isoglaucanone (30)	*Drynaria fortune* J. Sm	Root and stem	[76]
Diploptene (31)	*Drynaria fortune* J. Sm	Root and stem	[77]
Hop-21-ene (32)	*Drynaria fortune* J. Sm	Root and stem	[77]
Diplopterol (33)	*Drynaria fortune* J. Sm	Root and stem	[77]
Cycloartane-type triterpenes			
Cycloart-3β, 25-diol (24)	*Eucommiae ulmoides* Oliv.	Leaf	[72]
Cyclolaudenol (34)	*Drynaria fortune* J. Sm	Root and stem	[77]
Cyclomargenol (35)	*Drynaria fortune* J. Sm	Root and stem	[77]
Cyclolaudenone (36)	*Drynaria fortune* J. Sm	Root and stem	[77]
Curculigenin A, B, D (45, 56, 43)	*Curculigo orchioides* Gaertn.	Root and stem	[78]
Curculigosaponin A–J (46–55)	*Curculigo orchioides* Gaertn.	Root and stem	[78]
Curculigosaponin K, L (57, 58)	*Curculigo orchioides* Gaertn.	Root and stem	[78]
Curculigosaponin M (44)	*Curculigo orchioides* Gaertn.	Root and stem	[78]
Furostane-type saponins			
Tuberoside A–C (59–61)	*Allium tuberosum* Rottl. ex Spreng.	Seed	[44]
Tuberoside F–I (65–68)	*Allium tuberosum* Rottl. ex Spreng.	Seed	[79]
Furostane-type oligoglycosides (69, 70)	*Allium tuberosum* Rottl. ex Spreng.	Seed	[80]
Spirostane-type saponins			
Tuberoside M (62)	*Allium tuberosum* Rottl. ex Spreng.	Seed	[46]
Tuberoside D, E (63, 64)	*Allium tuberosum* Rottl. ex Spreng.	Seed	[81]
Neogitogenin (71)	*Allium tuberosum* Rottl. ex Spreng.	Seed	[82]
Protodioscin (72)	*Allium tuberosum* Rottl. ex Spreng.	Seed	[82]

Table 1: Continued.

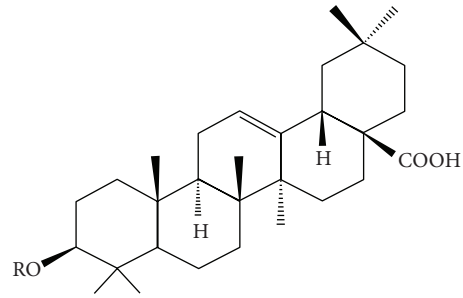
Compounds	Herbs	Medicinal parts	Reference
Steroids			
	Cynomorium songaricum Rupr.	Stem	[83]
	Cistanche deserticola Y. C. Ma	Stem	[84]
β-Sitosterol (73)	*Morindae officinalis* How	Root	[85]
	Drynaria fortune J. Sm	Root and stem	[75]
	Epimedium brevicornum Maxim.	Leaf	[71]
	Astragalus complanatus R. Br	Seed	[77]
β-Sitosteryl oleate (74)	*Cynomorium songaricum* Rupr.	Stem	[74]
	Cynomorium songaricum Rupr.	Stem	[83]
	Cistanche deserticola Y. C. Ma	Stem	[84]
	Morindae officinalis How	Root	[86]
β-Sitosteryl glucoside (75)	*Dipsacus asper* Wall. Ex Henry	Root	[75]
	Eucommiae ulmoides Oliv.	Bark	[71]
	Epimedium brevicornum Maxim.	Leaf	[74]
	Psoralea coryli folia L.	Seed	[85]
	Cuscuta australis R. Br.	Seed	[87]
β-Sitosteryl glucoside 6′-O-aliphatate (76)	*Cynomorium songaricum* Rupr.	Stem	[83]
β-Sitosterol palmitate (77)	*Cynomorium songaricum* Rupr.	Stem	[83]
5α-Stigmast-9(11)-en-3β-ol (78)	*Cynomorium songaricum* Rupr.	Stem	[83]
5α-Stigmast-9(11)-en-3β-ol tetracosantrienoic acid ester (79)	*Cynomorium songaricum* Rupr.	Stem	[83]
β-Sitosterol-3-O-acetic acid (80)	*Cynomorium songaricum* Rupr.	Stem	[83]
5α-Stigmast-9(11)-en-3β-ol (81)	*Cynomorium songaricum* Rupr.	Stem	[83]
5α-Stigmast-9(11)-en-3β-ol tetracosantrienoic acid ester (82)	*Cynomorium songaricum* Rupr.	Stem	[83]
Campesterol (83)	*Cistanche deserticola* Y. C. Ma	Stem	[88]
	Cuscuta australis R. Br.	Seed	[87]
Stigmastan-2, 5, 22-triene (84)	*Cistanche deserticola* Y. C. Ma	Stem	[88]
Stigmasterol (85)	*Morindae officinalis* How	Root	[86]
	Psoralea coryli folia L	Seed	[85]
Delta 5-avenasterol (86)	*Cuscuta australis* R. Br.	Seed	[87]
Gamma-sitosterol (87)	*Epimedium brevicornum* Maxim.	Leaf	[74]

fluidity, without affecting the fluidity of the outer mitochondrial membrane, with a consequent increase in mitochondrial membrane potential and mitochondrial ATP content [59]. The mitochondrial inner membrane fluidity was measured using fluorescence polarization probe trimethylamine-diphenylhexatriene (TMA-DPH) in that study [59]. Given that the mitochondrial inner membrane is mainly composed of unsaturated phospholipids molecules, the ability of β-sitosterol to increase mitochondrial inner membrane fluidity may be due to the interaction between β-sitosterol and the double bond of unsaturated phospholipids, which promotes membrane disorder and fluidity. In this connection, given the structural similarity between ursolic acid and β-sitosterol, the ability of both ursolic acid and β-sitosterol to stimulate mitochondrial electron transport may well be related to the increase in mitochondrial membrane fluidity.

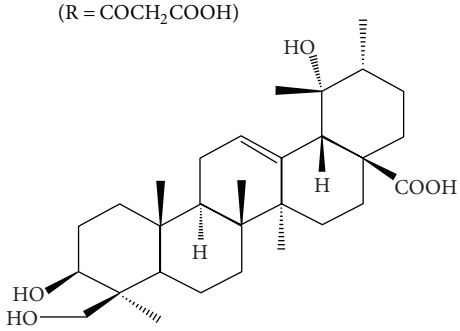
Given that ROS are unavoidably generated during the process of oxidative phosphorylation, particularly under conditions of increased electron transport activity, the stimulation of mitochondrial electron transport by ursolic acid and β-sitosterol is accompanied by a small amount of mitochondrial ROS production [40, 60]. Although ROS are usually considered as culprits in the development of a number of diseases, a compelling body of evidence has demonstrated that low levels (higher than basal level but within physiological limit) of ROS also play a major physiological role in intracellular redox homeostasis and signal transduction under normal conditions [61]. The low level mitochondrial ROS-induced oxidative stress produced by prooxidant exposure, caloric restriction, hypothermia, or physical exercise elicits adaptive cellular signaling/responses that can activate various signaling pathways/transcription factors in nuclear and mitochondrial genomes, including SIRT/FOXO, electrophile response element (EpRE)/nuclear factor erythroid 2-related factor 2 (Nrf2), cAMP responsive element-binding protein (CREB), or nuclear factor (NF)-κB, leading to the maintenance of homeostasis and longevity [62–65]. In addition, ROS can induce mitochondrial uncoupling that has been shown to reduce oxidative stress [66]. In our studies, we have found that the induction of mitochondrial ROS production by ursolic acid and β-sitosterol can elicit mitochondrial uncoupling and



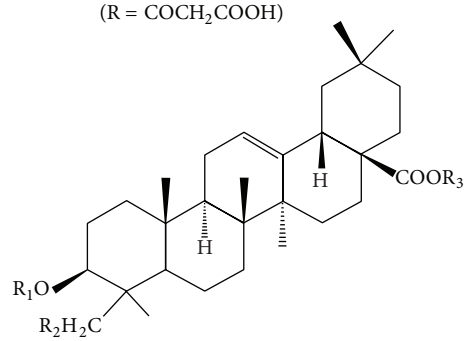
(1) Ursolic acid (R = H)
(2) Acetyl ursolic acid (R = $COCH_3$)
(3) Malonyl ursolic acid hemiester
(R = $COCH_2COOH$)



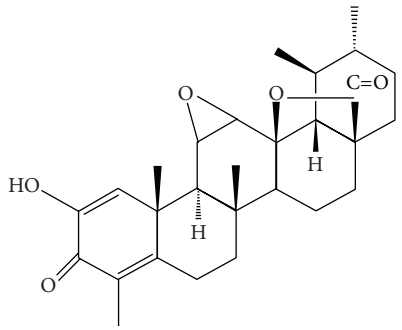
(4) Oleanolic acid (R = H)
(5) Malonyl oleanolic acid hemiester
(R = $COCH_2COOH$)



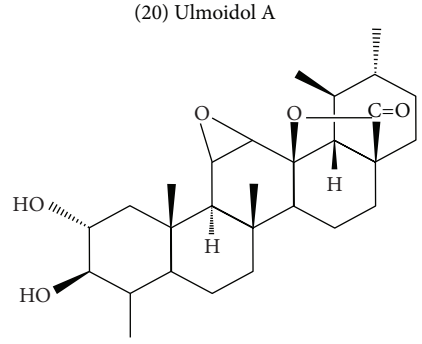
(6) Rotungenic acid



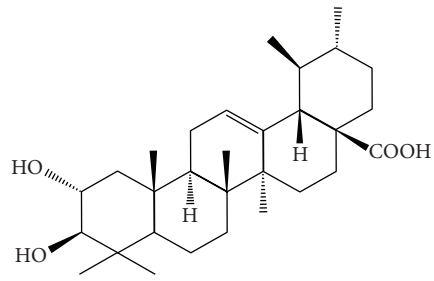
(7) R_1 = Ara, R_2 = H, R_3 = H
(8) R_1 = Xyl(1 → 4)Glc(1 → 4)[Rha(1 → 3)]Glc(1 → 3)Rha(1 → 2)Ara
R_2 = OH, R_3 = Glc(1 → 6)GLc
(9) R_1 = Glc(1 → 3)Rha(1 → 2)Ara, R_2 = OH, R_3 = Glc(1 → 6)GLc
(10) R_1 = Ara, R_2 = OH, R_3 = Glc(1 → 6)GLc
(11) R_1 = H, R_2 = OH, R_3 = Glc(1 → 6)GLc
(12) R_1 = Ara, R_2 = OH, R_3 = Glc
(13) R_1 = Ara, R_2 = H, R_3 = Glc(1 → 6)GLc
(14) R_1 = Ara, R_2 = OH, R_3 = H
(15) R_1 = Rha(1 → 3)Glc(1 → 3)Rha(1 → 2)Ara, R_2 = OH, R_3 = H
(16) R_1 = 4-O-acetyl-L-Ara, R_2 = OH, R_3 = D-Glc(1 → 6)D-Glc
(17) R_1 = Ara, R_2 = OH, R_3 = Glc(1 → 6)-GLc
(18) R_1 = Ara, R_2 = H, R_3 = Glc-(1 → 6)GLc
(19) R_1 = Ara, R_2 = OH, R_3 = Glc(1 → 6)GLc



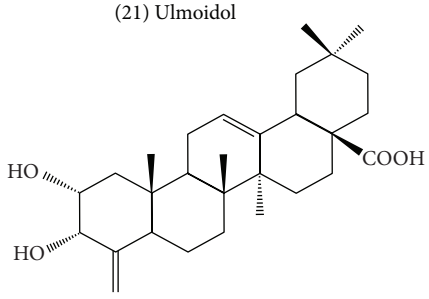
(20) Ulmoidol A



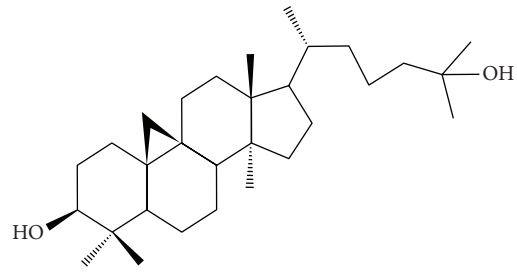
(21) Ulmoidol



(22) Corosolic acid



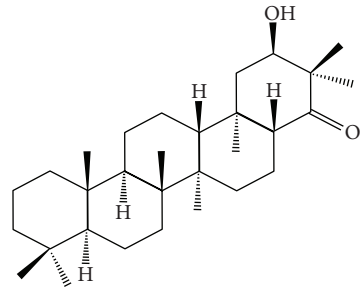
(23)2α,3α-Dihydroxy-24-nor-
4(23),12-oleanadien-28-oic acid



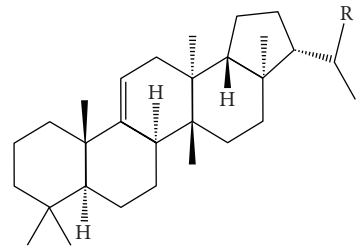
(24) Cycloart-3β,25-diol

(a)

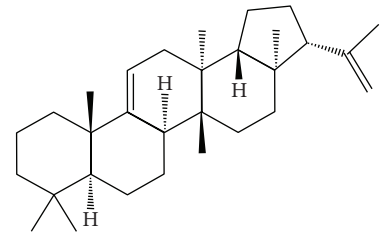
Figure 1: Continued.



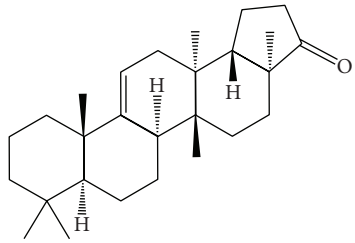
(25) 20β-Hydroxychiratan-22-one



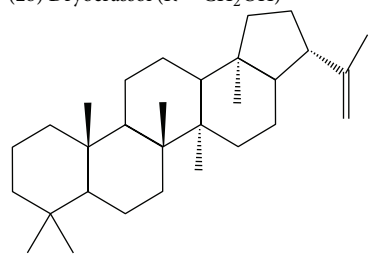
(26) Fern-9(11)-ene (R = CH_3)
(27) Dryocrassol acetate (R = CH_2OAC)
(28) Dryocrassol (R = CH_2OH)



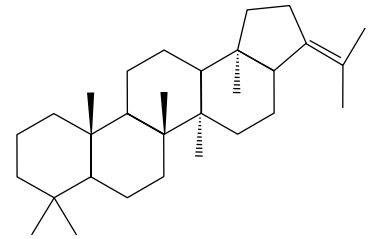
(29) Hop-22(29)-ene



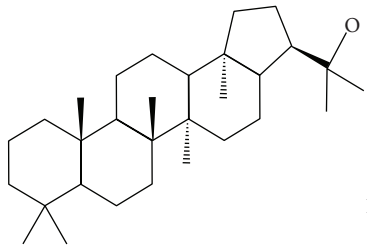
(30) Isoglaucanone



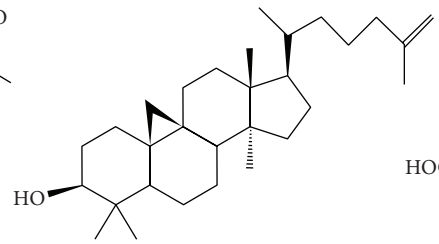
(31) Diploptene



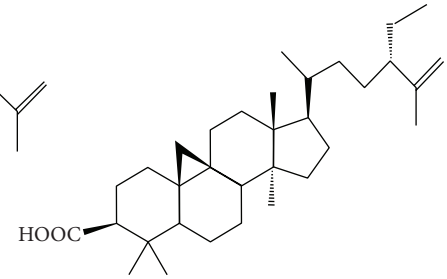
(32) Hop-21-ene



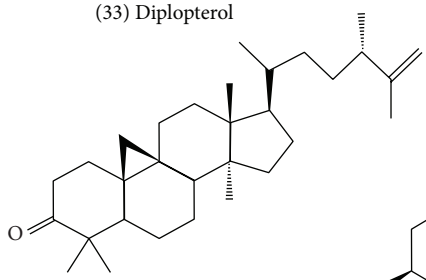
(33) Diplopterol



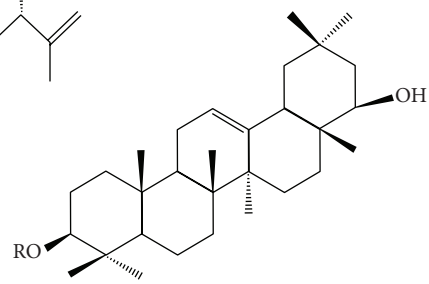
(34) Cyclolaudenol



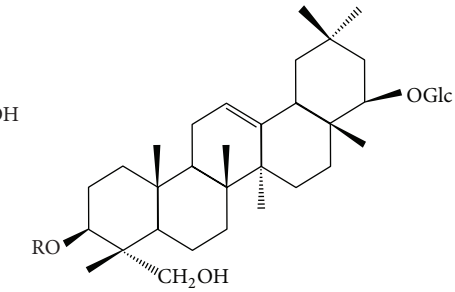
(35) Cyclomargenol



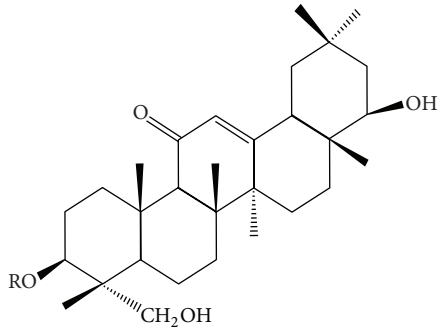
(36) Cyclolaudenone



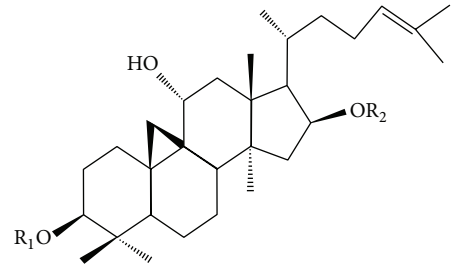
(37) Astragaloside VIII methyl ester
[R = RHa(1 → 2)Xyl(1 → 2)-6-O-methyl-Glc]
(38) Soyasaponin I methyl ester
[R = RHa(1 → 2)Gal(1 → 2)-6-O-methyl-Glc]



(39) 3-O-Rha(1 → 2)-Xyl(1 → 2)-6-O-methyl-Glc-soyasapogenol B 22-O-Glu
[R = RHa(1 → 2)Xyl(1 → 2)-6-O-methyl-Glc]
(40) 3-O-Rha(1 → 2)-Gal(1 → 2)-6-O-methyl-Glc-soyasapogenol B 22-O-Glu
[R = RHa(1 → 2)Gal(1 → 2)-6-O-methyl-Glc]



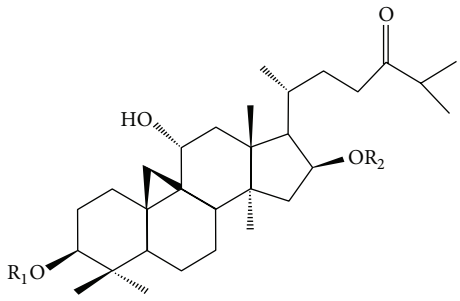
(41) 3-O-Rha(1 → 2)-Xyl(1 → 2)-6-O-methyl-Glc-3β,22β,24-trihdroxy-11-oxo-olean-12-ene
[R = RHa(1 → 2)Xyl(1 → 2)-6-O-methyl-Glc]
(42) 3-O-Rha(1 → 2)-Gal(1 → 2)-6-O-methyl-Glc-3β,22β,24-trihdroxy-11-oxo-olean-12-ene
[R = RHa(1 → 2)Gal(1 → 2)-6-O-methyl-Glc]



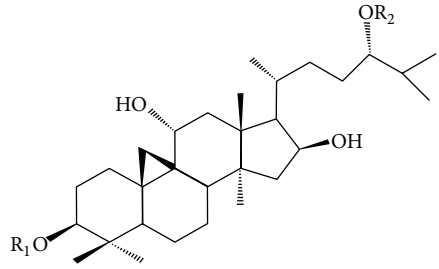
(43) Curculigenin D [R_1 = H, R_2 = H]
(44) Curculigosaponin M
[R_1 = Glc(2 → 1)Glc(3 → 1)Glc, R_2 = Ara]

(b)

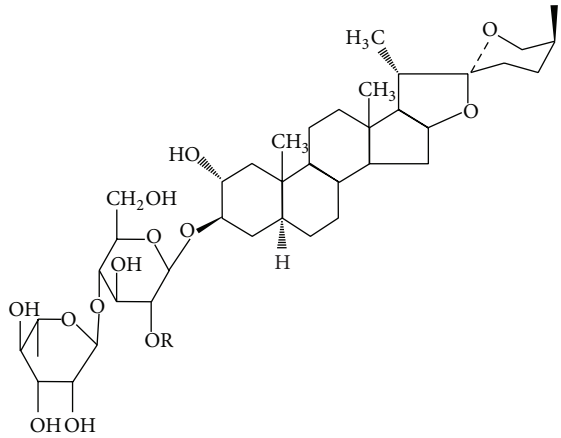
Figure 1: Continued.



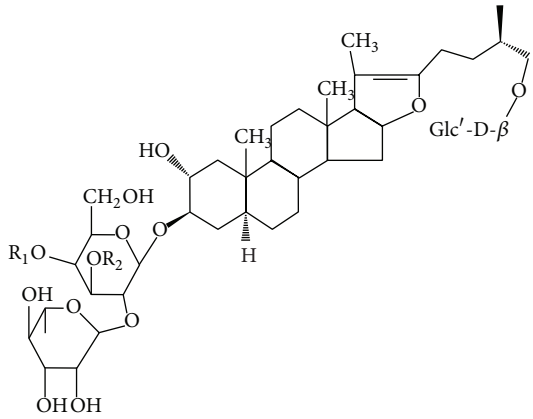
(45) Curculigenin A (R_1 = H, R_2 = H)
(46) Curculigosaponin A (R_1 = Glc, R_2 = H)
(47) Curculigosaponin B (R_1 = H, R_2 = Ara)
(48) Curculigosaponin C (R_1 = Ara, R_2 = Glc)
(49) Curculigosaponin D [R_1 = H, R_2 = Glc(1 → 2)Glc]
(50) Curculigosaponin E [R_1 = Glc(1 → 2)Glc, R_2 = Ara]
(51) Curculigosaponin F [R_1 = Glc(1 → 2)Glc(1 → 3)Glc, R_2 = H]
(52) Curculigosaponin G [R_1 = Rha(1 → 2)Glc, R_2 = H]
(53) Curculigosaponin H [R_1 = Rha(1 → 2)Glc, R_2 = Ara]
(54) Curculigosaponin I [R_1 = Glc(1 → 3)Rha(1 → 2)Glc, R_2 = H]
(55) Curculigosaponin J [R_1 = Glc(1 → 3)Rha(1 → 2)Glc, R_2 = Ara]



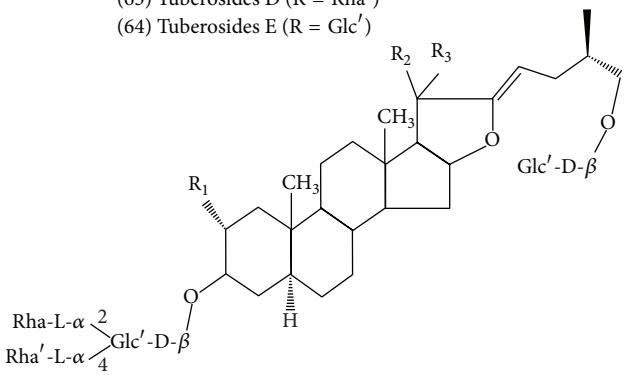
(56) Curculigenin B (R_1 = H, R_2 = H)
(57) Curculigosaponin K [R_1 = Glc(1 → 3)Glc(1 → 2)Glc, R_2 = H]
(58) Curculigosaponin L [R_1 = Glc(1 → 3)Glc(1 → 2)Glc, R_2 = H]



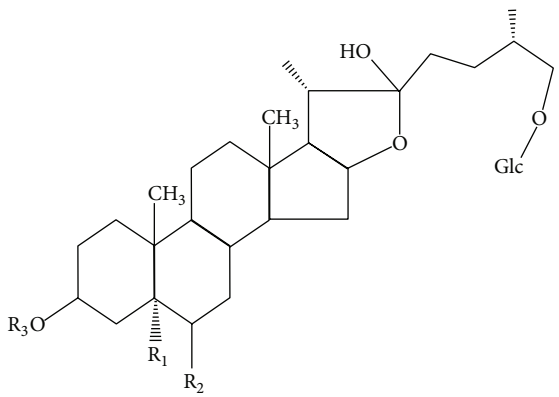
(62) Tuberosides M (R = H)
(63) Tuberosides D (R = Rha′)
(64) Tuberosides E (R = Glc′)



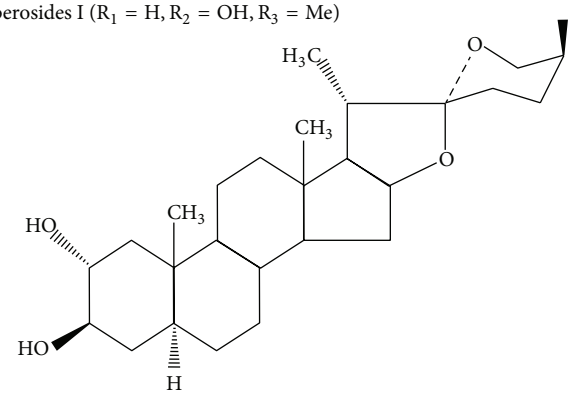
(59) Tuberoside A (R_1 = R_2 = H)
(60) Tuberoside B (R_1 = Rha′, R_2 = H)
(61) Tuberoside C (R_1 = H, R_2 = Glc′)



(65) Tuberosides F (R_1 = OH, R_2 = Me, R_3 = OMe)
(66) Tuberosides G (R_1 = OH, R_2 = Me, R_3 = OH)
(67) Tuberosides H (R_1 = OH, R_2 = OH, R_3 = Me)
(68) Tuberosides I (R_1 = H, R_2 = OH, R_3 = Me)



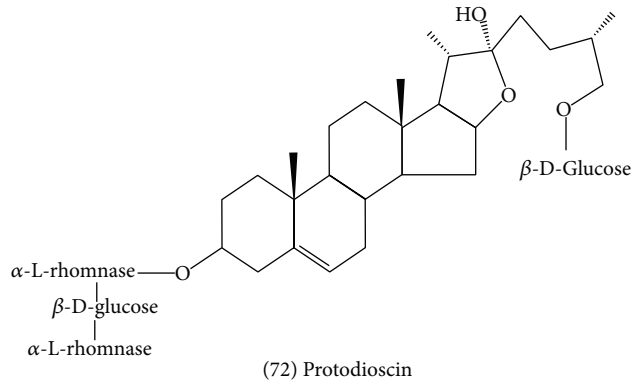
(69) R_1 = R_2 = H, R_3 = Rha(1 → 4)-[Rha-(1 → 2)]-Glc
(70) R_1 = R_2 = OH, R_3 = Glc-4′-Rha



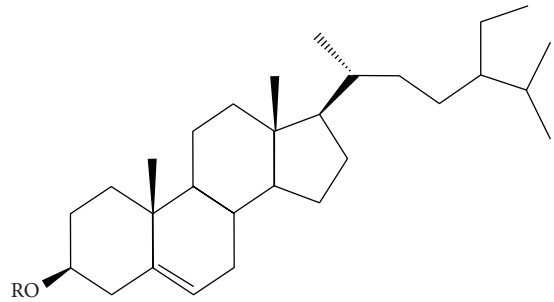
(71) Neogitogenin

(c)

Figure 1: Continued.



(72) Protodioscin



(73) β-Sitosterol (R = H)

(74) β-Sitosteryl oleate (R = oleyl)

(75) β-Sitosteryl glucoside (R = Glc)

(76) β-Sitosteryl glucoside 6′-O-aliphatate

[R =6′-aliphatyl glc (44% oleic acid, 37% palmitic acid, 15% linoleic acid and 3% stearic acid)]

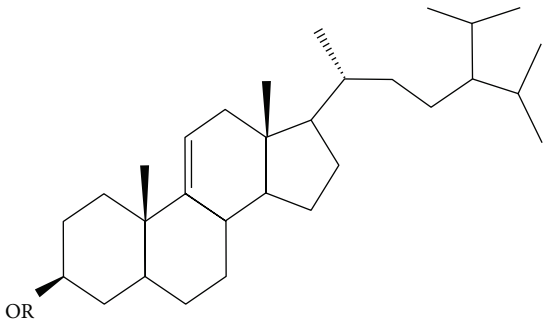
(77) β-Sitosterol palmitate (R = $CH_3(CH_2)_{14}CO$)

(78) β-Sitosteryl 3-O-β-D-xylopyranoside (R = Xyl)

(79) β-Sitosterol-3-O-β-D-glucoside tetraacetate

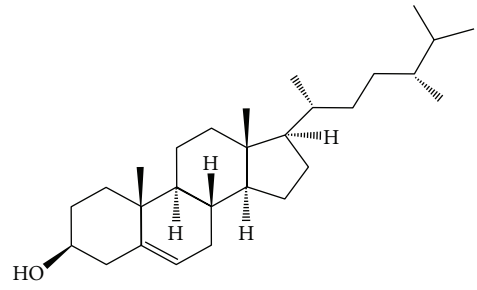
(R = Glc-tetraacetate)

(80) β-Sitosterol-3-O-acetic acid (R = COOH)

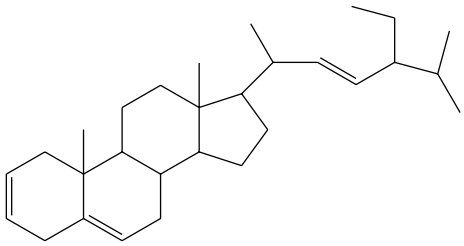


(81) 5α-Stigmast-9(11)-en-3β-ol (R = H)

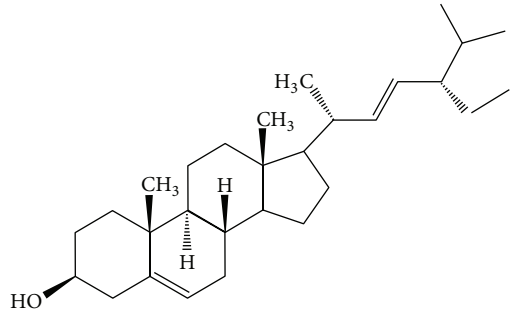
(82) 5α-Stigmast-9(11)-en-3β-ol tetracosantrienoic acid ester (R = $OC_{24}H_{41}$)



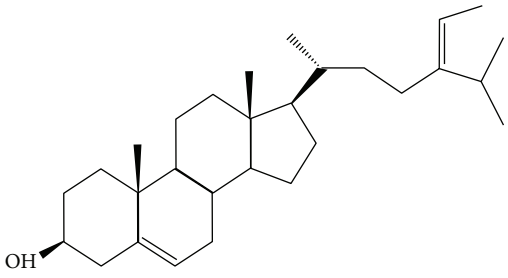
(83) Campesterol



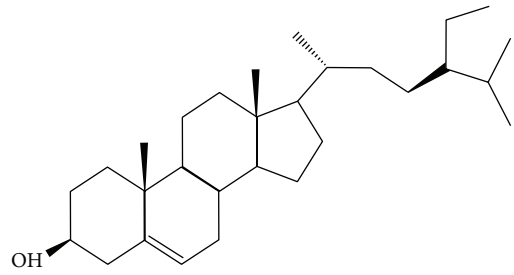
(84) Stigmastan-2,5,22-triene



(85) Stigmasterol



(86) Delta 5-avenasterol



(87) Gamma-sitosterol

(d)

Figure 1: Chemical structures of triterpenes and phytosterols in Chinese Yang-tonic herbs.

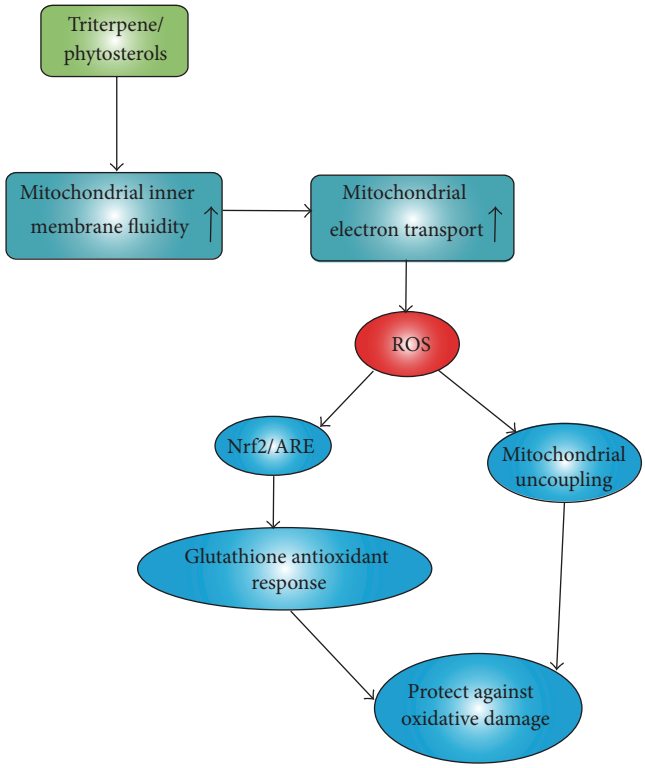


Figure 2: Biochemical basis of "Yang-invigorating" action.

glutathione reductase-catalyzed glutathione redox cycling, which offer protection against oxidant injury. Ursolic acid and β-sitosterol not only stimulated state-3 respiration, but also caused an increase in state-4 respiration in rat mitochondria and H9c2 cardiomyocytes, with the latter being reversed by guanosine diphosphate, an uncoupling protein inhibitor. The experimental findings suggested the involvement of mitochondrial uncoupling protein activation in the "Yang-invigoration" produced by triterpene or steroid-like compounds from Yang-tonic herbs [40]. Studies by other researchers have demonstrated that both ursolic acid and oleanolic acid treatments activated the Nrf2 pathway through ROS generation, with resultant protection against cerebral ischemia and hepatotoxicity *in vivo* [54, 67]. Oleanolic acid treatment was found to dramatically increase expression of Nrf2 and its dependent-genes, such as NAD(P)H:quinone oxidoreductase 1 (Nqo1), heme oxygenase-1 (Hmox1), and glutamate-cysteine ligases (Gclc and Gclm) in rat and mouse livers [67]. Given that the activation of Nrf2 and the subsequent induction of antioxidant response genes play an important role in eliciting an adaptive response to oxidative stress [68], the ursolic acid and β-sitosterol-induced enhancements in glutathione redox cycling, as observed in cultured H9c2 cells [39, 40], may involve the activation of the Nrf2 pathway.

Taken together, we therefore propose a biochemical mechanism of "Yang-invigorating" action produced by Yang-tonic herbs (Figure 2). Triterpenes or phytosterols, the active components in Yang-tonic herbs with similar chemical structure, can stimulate mitochondrial electron transport through the increase in the fluidity of the inner mitochondrial membrane, with a resultant increase in ATP generation capacity. The stimulation of electron transport is accompanied by an increase in ROS production, which triggers a retrograde response to upregulate cellular/mitochondrial antioxidant defense mechanisms through the Nrf2 pathway. In addition, mitochondrial ROS can also stimulate the activity of uncoupling protein and thereby lower the membrane potential through the dissipation of proton gradient. With a recurring "Yang-invigorating" action produced by the active components of Yang-tonic herbs, an intermittent stimulation of mitochondrial ROS production at low levels results in the prolonged activation of mitochondrial uncoupling and the upregulation of antioxidant defense components characteristic of mitohormesis, which is beneficial in retarding the decline in body function and delaying the onset of age-related diseases. To test the hypothesis, we will conduct thorough chemical analyses of biologically active extracts of Yang-tonic herbs and identify the presence of triterpenes or phytosterols. The effects of these putative active compounds on the fluidity of mitochondrial inner membrane will be investigated in association with *in vitro* and *ex vivo* bioassays on ATP generation capacity and glutathione redox cycling.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2014, Article ID 402718, 7 pages
http://dx.doi.org/10.1155/2014/402718

Review Article

Potential Therapeutic Effects of Meditation for Treating Affective Dysregulation

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Received 25 March 2014; Revised 30 June 2014; Accepted 1 July 2014; Published 12 August 2014

Academic Editor: Si-Yuan Pan

Affective dysregulation is at the root of many psychopathologies, including stress induced disorders, anxiety disorders, and depression. The root of these disorders appears to be an attenuated, top-down cognitive control from the prefrontal cortices over the maladaptive subcortical emotional processing. A form of mental training, long-term meditation practice can trigger meditation-specific neuroplastic changes in the brain regions underlying cognitive control and affective regulation, suggesting that meditation can act as a kind of mental exercise to foster affective regulation and possibly a cost-effective intervention in mood disorders. Increasing research has suggested that the cultivation of awareness and acceptance along with a nonjudgmental attitude via meditation promotes adaptive affective regulation. This review examined the concepts of affective regulation and meditation and discussed behavioral and neural evidence of the potential clinical application of meditation. Lately, there has been a growing trend toward incorporating the "mindfulness" component into existing psychotherapeutic treatment. Promising results have been observed thus far. Future studies may consider exploring the possibility of integrating the element of "compassion" into current psychotherapeutic approaches.

1. Introduction

Affective regulation is fundamental to adaptive social and psychological functioning, and affective dysregulation is the root of a number of mental disorders, including maladaptive stress reactions, anxiety, and depression. Associated with these disorders is significant maladaptive functioning, which creates heavy socioeconomic costs for societies worldwide. For example, major depressive disorder (MDD) ranks second in the global burden of disease [1]. Lifetime prevalence rate of MDD is relatively higher for women than men, suggesting a sex-related difference in affective regulation [2]. Hence, there is a timely demand for cost-effective intervention in disorders stemming from affective dysregulation. Recently, in addition to the pharmaceutical advancement in Eastern and Western medicine in treating affective disorders, people are yearning for cost-effective behavioral strategies that help prevent and intervene in affective dysregulation and can be incorporated into large-scale public health programs. One promising candidate that has been well received by people across ethnic and cultural groups is meditation. A literature review reveals reports of the therapeutic effects of meditation on depression [3, 4] and grieving [5].

This review covers the existing empirical evidence of the neural effects of mindfulness and compassion meditation. While mindfulness meditation has received most of the empirical attention [6, 7], there has been growing interest in compassion meditation, which emphasizes positive affective states. To the best of our knowledge, reviews of the empirical evidence of compassion meditation, especially its neural effects, are scarce. We intend to bridge this gap by discussing the effects of compassion meditation and mindfulness meditation on the brain and behaviors, as well as their potential therapeutic value in treating some commonly identified disorders stemming from affective dysregulation (e.g., stress induced disorders, anxiety, and depression).

This review will first lay the theoretical groundwork for affective regulation and meditation. It will then present evidence from behavioral and neuroimaging studies to corroborate the important role of meditation—specifically, mindfulness meditation and compassion meditation—in affective regulation in both healthy and clinical populations.

2. Affective Regulation

A spectrum of affective-regulation strategies for downregulating negative emotional responses has been identified. One that has received much research attention is the reappraisal of affective stimuli in the brain. This involves reappraising the affective value via reinterpreting the meanings of emotion-evocative stimulus in order to mitigate its affective impact [8–11]. For example, when viewing disgust-eliciting film clips, cognitive reappraisal downregulated the negative emotional experience to a greater extent when compared to expressive suppression, a response-focused strategy that targets inhibiting behaviors (e.g., facial expression) associated with emotional response [12]. Neuroimaging findings have reported that cognitive reappraisal is associated with reduced neural responses in the amygdala and insula but enhanced neural activation in the prefrontal cortices that involve cognitive control of emotion. Expressive suppression demonstrated an opposite pattern [12]. Stemming from the concept of experience-induced neuroplastic change [13, 14], an experience specifically designed and induced to the brain to facilitate adaptive cognitive reappraisal may promote affective regulation by downregulating the negative emotional experience of affective stimuli. The human brain is a malleable organ whose structure and functions are changeable in response to experience and learning [15, 16].

3. Meditation and Affective Regulation

Empirical findings demonstrated that a regular practice of meditation induces significant changes in brain structure and functions; therefore, behaviors change. Meditation is a mental exercise of regulatory practices intending to attain certain psychological states that involve mind and body interaction of being in the present moment with a nonjudgmental attitude [17]. The aim is to cultivate specific psychological states or mental capacities, such as quiescence and equanimity [6, 18]. Quiescence is to be tranquil at rest—a completely relaxed bodily state with full concentration on the present moment [19]. Equanimity, on the other hand, is the ability to accept things as they are, with a balanced state of mind that is calm and spacious. This enables one to be present, fully and nonjudgmentally [18, 20, 21].

There is a vast array of contemplative practices and techniques within the Buddhist tradition. Our review intends to focus exclusively on two specific forms of meditation which, from the perspective of neuroscience, engage in different cognitive processes. Mindfulness meditation is a form of meditation that emphasizes on monitoring the present experience from moment to moment while acknowledging the nature or content of the experience without spontaneously reacting to it [6]. For this reason, the practice of mindfulness meditation highly hinges on the ability to control attention, which is essential for the practitioners to openly monitor all the experience in the present moment without focusing their attention on an explicit object. On the contrary, the ability to regulate emotions is more fundamental to compassion meditation or loving-kindness meditation (LKM) in general. When viewing others' suffering, it is not uncommon for us to feel distress or upset about others' painful experience. The practice of LKM, which involves cultivation of a state of universal love and compassion towards self and other beings with a desire to relieve their pain and suffering [20, 22], can attenuate the emotional impact by sharpening one's ability to downregulate the negative emotions (e.g., sadness) triggered by distressful experience and restore the emotional balance.

Researchers have made significant progress in understanding how meditation promotes positive emotions. Using a high-resolution EEG to measure neural oscillations, researchers have reported patterns of brain electrical activity associated with a positive, "blissful" experience [23]. Moreover, functional neuroimaging findings have suggested that meditation could increase neural activity associated with positive emotions [24]. Researchers proposed that the affect-labeling technique that meditators use is the neurocognitive mechanism that reduces negative affect [25].

While meditation emphasizes nonjudgmental attention to a present moment experience, cognitive reappraisal focuses primarily on reinterpreting the meaning of an emotion-eliciting stimulus. A growing body of research has shown that these two theoretical constructs are interrelated to exercise their effects on affective regulation at both neural and behavioral levels.

3.1. Behavioral Evidence. Several behavioral studies reported significantly better affective regulation for meditators than nonmeditators [2, 26]. For instance, Lykins and Baer [26] compared the differences between experienced meditators in mindfulness meditation and demographically similar nonmeditators and found that the two groups significantly differ on several aspects, including higher scores of several adaptive psychological functions and lower scores of several maladaptive emotion traits and cognitive-related variables on experienced meditators over nonmeditators. This observation suggests that individuals who practice mindfulness meditation regularly are more ready to observe thoughts and feelings nonjudgmentally, ruminate less on emotion, and are more likely to function adaptively in emotionally arousing situations. They concluded that the practice of mindfulness meditation cultivates mindfulness in daily life, therefore promoting psychological well-being.

Kemeny et al. [27] studied the relationship between mindfulness meditation and psychological functioning in a sample of 82 healthy females randomly assigned to the training group or wait-list control group. This 42-hour training program (4 full days and 4 evening sessions over 8 weeks) combined mindfulness meditation practices with affective regulation strategies. They found significantly decreased negative affect

(specifically, ruminative thoughts), reduced destructive emotions and emotional behavior, and higher acceptance and awareness of emotions and feelings in the training group as compared to the control group. The psychological benefits were maintained for 5 months after completion of the training program, which demonstrated the long-term effects of intensive meditation training in healthy individuals. Arch and Craske [28] found that participants, even after a brief mindfulness induction, displayed more adaptive affective regulation when faced with negative stimuli. The experimental group showed a greater willingness to maintain visual contact with the pictures depicting strong negative affect. This group was also relatively more stable and less emotionally volatile across affective slide types when compared to the control groups.

A number of behavioral studies attempted to investigate the effects of LKM training [29, 30]. In a recent study, 105 undergraduate women were randomized into self-compassion, attention control, or no intervention groups. After brief self-compassionate training, the self-compassion group showed greater resilience during a social threat than the other two groups [29]. A longitudinal study conducted by Fredrickson et al. [30] found that a 7-week LKM training for novice meditators significantly increased positive emotion (e.g., love, pride, hope, gratitude, and contentment). In turn, these changes enhanced life satisfaction when compared to the waitlist group. After completing a brief guided LKM and focus-attention meditation (FAM) training once a week for 12 weeks, participants in both groups showed decreased anxiety and negative affect and increased hope [31].

3.2. Neural Evidence. Evidence from neuroimaging studies provided further support for the beneficial role of long-term meditation practice in emotional processing. For example, long-term meditators did not show any significant increase in late positive potentials (LPP) (LPP is known to be evoked by emotionally arousing stimuli, and its amplitude increases with the emotional potency of the stimuli [32]) when viewing the negative images, as opposed to their matched controls, which had no prior experience in meditation. It appears that meditation practice can possibly enhance emotional processing and hence mitigate the affective impact of emotion-eliciting stimuli. On the other hand, Taylor et al. [33] revealed that mediators perceived pictures portraying positive and negative emotions to be less intense during a mindful (rather than nonmindful) state. Their functional neuroimaging findings further indicated that meditators recruit different neural networks when processing emotional stimuli. Farb et al. [34] revealed that mindfulness-based stress reduction (MBSR) [35] training could modulate neural reactivity in response to sadness provocation. MBSR is an 8-week intensive and well-structured program, in which mindfulness meditation is a core element, that intends to promote mindfulness or present moment awareness as well as well-being in all individuals, especially those with stress, pain, and other medical conditions [17, 35]. Similarly, Hölzel et al. [36] also documented the longitudinal neural effects of MBSR in terms of significant increases in gray matter concentration in the left hippocampus, posterior cingulate cortex, temporoparietal junction, and the cerebellum after 8 weeks of MBSR training. It indicated that mindfulness meditation could benefit memory and learning and affective regulation, as well as social cognition.

Relatively few studies have shed light on the neural effect of LKM or compassion meditation when compared to mindfulness meditation. During voluntary cultivation of compassion states, expert LKM meditators manifested stronger neural activity in the anterior insula when responding to negative sounds relative to positive sounds, suggesting that LKM practice can sharpen an individual's ability to identify others' emotional states based on their voice [20]. Compassion meditation training can also alleviate the emotional impact of witnessing others' distress. Instead of eliciting activations in the neural network associated with empathy for pain, Klimecki et al. [37] revealed that participants, after receiving compassion meditation training, reported an increase in positive affect and engagement in the neural regions, implicating a positive affect and affiliation in response to others' suffering. Findings from longitudinal studies also found that short-term compassion meditation training can promote altruistic behavior and increase the tendency to approach, rather than avoid, the suffering of others [38]. After receiving two weeks of compassion or reappraisal training, participants' altruistic behavior, as measured by greater redistribution of funds toward victims of unfair treatment, was positively associated with neural activation in regions implicating social cognition and affective regulation, including the inferior parietal cortex and dorsolateral prefrontal cortex. This significant correlation was specific to the participants who underwent compassion training but not their counterparts from the reappraisal training group, suggesting a training-specific change [38]. Another study also reported that 8 weeks of cognitive-based compassion training could significantly enhance the accuracy in inferring others' mental states in the Reading the Mind in the Eyes Test [39] and increased the neural activation in the inferior frontal gyrus and dorsomedial prefrontal cortex, which are important for the theory of mind [40]. Taken together, these findings suggest that functional neuroplasticity is not unique to cognitive or motor domains, which raises the possibility of inducing neural plastic changes in the affective regulation system, assuaging the impact of affective dysregulation. Further research will be needed to explore this plausibility.

4. Potential Clinical Applications of Meditation

Findings from recent studies suggest that different forms of meditation induce meditation-specific effects on the brain. Lee et al. [22] compared the neural effects of FAM and LKM. Their findings revealed that only FAM is associated with different patterns of activation in the attention-related region. Furthermore, FAM and LKM practitioners engage in different neural networks when processing affective stimuli. The practices of FAM and LKM are associated with different

patterns of neural activity in regions involved in attention-related processing and in emotion processing and regulation, respectively. Meditation-induced neuroplastic changes are not limited to functional activity. Meditation may modify brain morphometry. For example, Leung et al. [41] observed that long-term LKM practitioners are associated with enlarged right angular and posterior parahippocampal gyri, regions heavily involved in empathy and affective regulation. These promising findings shed light on the plausibility of designing a specific mental exercise (e.g., meditation) to enhance a particular cognitive or affective functioning, which can have consequential implications for the clinical population, especially those suffering from affective disorders. There is a growing trend toward integrating the core elements of mindfulness meditation with the current psychotherapeutic approach. In subsequent sections, we will review the empirical evidence of the effectiveness of meditation as a form of treatment in the clinical population.

4.1. Stress Induced Disorder. Prolonged exposure to stress increases the risk of developing physical and mental illnesses. Substantial evidence has shown that stress-induced responses can be ameliorated by mindfulness meditation. Following the MBSR program, the level of self-perceived stress dropped significantly among the individuals who initially reported a high level of stress right before participating in the MBSR program. Changes in self-perceived stress level induced by MBSR were also found to be positively correlated with the morphological changes in the right amygdala. In other words, the more the stress level decreased, the greater the gray matter density of the right amygdala decreased, suggesting that mindfulness meditation can induce changes in both behavioral and neural levels [42].

4.2. Anxiety Disorders. In another line of research, focus has shifted to investigating the potential value of MBSR in alleviating anxiety symptoms among patients with social phobia/social anxiety disorder (SAD). A few studies have examined the neural effect of MBSR in patients with SAD. Patients with SAD, after completing MBSR, demonstrated a reduction in neural activity in the amygdala and an increase in neural activity in attention-related regions when performing a breath-focused attention task [43]. In a later study, Goldin et al. [44] included a control group (aerobic exercise stress reduction program (AE)) and compared its effectiveness with that of MBSR among patients with SAD. Their findings showed that self-reported emotional reactivity to negative self-beliefs decreases to a similar extent in both MBSR and AE groups. They also identified a dissociable neural pattern between MBSR and AE groups during an affective regulation task. Specifically, neural activations in the attention-related regions increased in the MBSR but decreased in the AE group, reflecting greater engagement in neural regions involved in attention upon completion of the MBSR program.

Building on the core concept of cognitive behavioral therapy and mindfulness meditation, mindfulness-based cognitive therapy (MBCT) has been developed to treat depression (discussed below) and clinical patients with generalized anxiety disorder (GAD). Preliminary findings revealed a reduction in self-reported depressive and anxiety symptoms in GAD patients following MBCT [45]. However, this pilot study lacks a control group for comparison, so their results should be interpreted with caution. Further empirical investigation will be needed to verify the effectiveness of MBCT in treating GAD patients.

4.3. Major Depressive Disorder. Because of the biased cognitive processes in people suffering from MDD, MBCT tackles the cognitive processes that create the vulnerability to relapse in MDD patients. As one prominent feature of MDD is an inability to disengage from negative stimuli due to heightened emotional reactivity and attenuated cognitive control [46], the "mindfulness" component in the MBCT can afford the MDD patients an opportunity to cultivate awareness of their own thoughts or emotions and to approach them from a wider and decentered perspective (e.g., "I am not my thoughts") [47]. The MBCT advocates the detached observation of thoughts or emotions without responding to the emotional stimuli spontaneously. The MBCT capitalizes on the nonjudgmental awareness cultivated by mindfulness meditation, which allows the MDD patients to observe and disengage from their negative thoughts or emotions as they arise, rather than trying to change or react to them spontaneously. The MBCT is conducted on a group basis and is currently only applicable to MDD patients who are in remission or recovery, whereas the MBSR targets individuals suffering from stress, pain, and other medical conditions. A number of studies have provided initial support for the effectiveness of MBCT in reducing the relapse rate of depression, especially for MDD patients with more than 3 previous episodes of depression [47]. Britton et al. [48] found that individuals with partially remitted depression in the MBCT group reported an overall attenuation in emotional reactivity (in terms of anxiety) after performing the Trier Social Stress Test. In contrast, similar differences were not observed in their counterparts in the waitlist control group after undergoing the same stress test. These findings suggested that MBCT could accelerate the process of recovering from negative emotion-provocative stressors and mitigate the emotional impact induced by stressful events. Thus far, the effectiveness of MBCT has been found to be comparable to that of antidepressant medication [49, 50]. Self-reported symptoms of depression were also found to be significantly reduced after receiving MBCT, whereas a similar reduction was not identified in patients who only received their usual pharmacological treatment [51]. It remains unclear whether MBCT can also benefit other clinical populations, but recent efforts have examined the potential therapeutic value of MBCT in treating patients in the acute phase of MDD and patients with bipolar disorder [52]. Its effectiveness is yet to be validated.

5. Discussion

Considerable evidence has documented experience-dependent neuroplastic changes in the brain structure [15, 22, 53], which raises the possibility of designing a specific kind

of training directed at a particular function. Meditation, a mental exercise inducing a specific experience in the brain, is the focus of this review. A substantial body of research has evidenced that mindfulness and compassion meditation can have a positive impact on attention and affective regulation [54, 55] and can induce long-lasting morphological changes in the corresponding neural regions—specifically, the prefrontal cortices and amygdala [56–58]. These findings indicate that mindfulness or compassion meditation, as a kind of mental training in cognitive control and affective regulation, can be a potential form of intervention for mood disorders. This review explored the potential clinical application of meditation for disorders stemming from affective dysregulation by assessing behavioral and neural evidence of the relationship between meditation and emotion functioning. The core effect of meditation on promoting adaptive affective regulation is its effect on halting maladaptive automatic thoughts while allowing the opportunity to rectify cognitive and emotional biases associated with specific emotional memory and/or affective stimuli. Furthermore, through voluntary cultivation of positive emotional states [59], people suffering from MDD may learn to foster self-compassion. The outcome could be prevention of future relapse in terms of offsetting the detrimental effects brought on by negative self-referential thoughts.

To date, most research in the field of meditation has focused specifically on investigating the trait effects of meditation practice (experienced meditators versus meditation novices) and the longitudinal effects of meditation training in a normal, healthy population. Comparatively less empirical attention has been paid to the clinical application of meditation. Even though the existing studies provided initial support for the effectiveness of MBCT in relieving depressive and anxiety symptoms and preventing recurrence of depressive episodes, their interpretation is inevitably obscured by the lack of a comparison group, concurrent antidepressant medication, and a relatively short follow-up period. Future research shall direct its focus toward verifying whether MBCT can act as an alternative treatment in mood disorders, elucidating the mechanism of action initiated by meditation.

This review only probes the behavioral and neural evidence that addresses the effect of mindfulness and compassion meditation on affective regulation among healthy adults as well as people suffering from stress-induced disorders, MDD, and SAD/GAD. Although a number of studies have examined the potential value of applying meditation to patients with other psychiatric disorders (e.g., schizophrenia), this area of research is not included here as dysregulation of the affective system does not seem to contribute to the positive and negative symptoms observed in schizophrenia patients. This review will be more condensed if the discussion is restricted to affective disorders resulting directly from dysfunction in the affective regulation system.

Future studies will shed light on the possible role of meditation in assuaging grief. Thus far, only one study has examined the relationship between mindfulness meditation training and the grieving process in patients diagnosed with chronic pain [5]. It might be worthwhile for future research to explore the healing power of meditation in bereavement.

6. Conclusions

Adaptive affective regulation hinges on the top-down cognitive control of the prefrontal regions over the bottom-up emotional processing that is carried out in the subcortical regions. If there is an aberrant activation in the limbic system (specifically, the amygdala) and cognitive control is weakened, there will be heightened responses to emotional stimuli, which might in turn lead to the onset of mood disorders (e.g., MDD) [46]. The mainstream treatment approaches for MDD are antidepressant medication and cognitive behavioral therapy. The latter tackles the maladaptive thinking pattern (biased attention toward sad stimuli) that pervades MDD patients. In recent decades, substantial evidence from behavioral and neuroimaging studies has documented the beneficial role of meditation in promoting adaptive affective regulation. Meditation advocates a cultivation of awareness and acceptance of our thoughts and emotions with a nonjudgmental attitude, which encourages an individual to observe, rather than avoid, positive or negative thoughts and emotions from a detached view. Because of its emphasis on present-moment awareness and acceptance, a growing body of research has explored the potential clinical applications for disorders stemming from affective dysregulation. Current evidence reveals that meditation, a form of mental training and exercise, may prevent and intervene in mood and other affective disorders.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper. There is no conflict of interests including any financial, personal, or other relationships with persons or organizations for any author related to the work described in this paper.

Authors' Contribution

Natalie T. Y. Leung and Mandy M. Lo contributed equally to this work.

Acknowledgments

This work was supported by the Research Grants Council General Research Fund (HKU747612H to Tatia M. C. Lee) and the Research Grants Council Humanities and Social Sciences Prestigious Fellowship Scheme (HSSPFS) (Ref: HKU703-HSS-13 to Tatia M. C. Lee).

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Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2014, Article ID 871217, 5 pages
http://dx.doi.org/10.1155/2014/871217

Review Article

Acupuncture and Depth: Future Direction for Acupuncture Research

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Received 19 May 2014; Accepted 8 June 2014; Published 8 July 2014

Academic Editor: Gerhard Litscher

The research on acupuncture has increased steadily over the years and regular review and revision of the direction of future acupuncture research are necessary. This paper aims to review and explore the significance of acupuncture depth in modern acupuncture research. Searches conducted in Science Direct and China National Knowledge Infrastructure (CNKI) databases reflected a lack of focus on depth of acupuncture. We propose that the research trends of acupuncture should progress to the depth of insertion. It is suggested that future acupuncture research, especially randomized controlled trials (RCTs), should take into consideration the depth of insertion. Comparison between databases using different language of medium suggests the need for international collaboration of researchers from the same field. It is also crucial to inherit and innovate traditional medicine (TM) through modern technology. The use of bibliometric method is also suitable for development of TM research trends. Acupuncture and depth should be considered as one of the future directions of acupuncture research.

1. Introduction

The research of traditional medicine (TM) and acupuncture has increased steadily in the recent years. Globalization and the World Wide Web have allowed researchers from different countries to obtain the latest development of all research studies. It is appropriate to review and analyze the trends of the research focus methodology by all researchers in the same fie.

Acupuncture depth is an area of study left unexplored by many researchers. Deep or superficial insertion of needle through the skin would directly affect the type and amount of tissues that it excites. Numerous randomized controlled trials (RCTs) have been conducted to test the efficacy of acupuncture and the presence of placebo effect. On the contrary, relatively less emphasis has been placed on acupuncture depth. Researchers using functional magnetic resonance (fMRI) to test mild cognitive impairment found that deep muscle insertion of acupuncture is necessary to achieve appreciable clinical effects [1–3]. Others have used ultrasound scans to guide the specific depth for achieving acupuncture sensation [4]. Such findings correspond to the Layer Analysis as described in the Yellow Emperor's Inner Classic [5]. Hence, we hypothesize that there could be a relationship between the efficacy of acupuncture treatment and the depth of insertion.

We believe that acupuncture depth should be considered an area of research in the future. The use of bibliometric indicators is gaining popularity to evaluate clinical research [6]. In order to determine the consideration of depth in recent acupuncture researches, this review also explores the bibliometric data of acupuncture depth.

2. Lack of Acupuncture Depth Research

A search in databases Science Direct and China National Knowledge Infrastructure (CNKI) using the follow strategies was conducted. For Science Direct, "acupuncture" or "moxibustion" or "acupuncture-moxibustion" or "needling" and "depth" were used. For search in CNKI, "acupuncture"

(针刺) or "moxibustion" (灸) or "acupuncture-moxibustion" (针灸) and "depth" (深度) or "layer" (层次) (not "Layer Analysis method" (层次分析法)) were used. Similar search strategies were employed for publications on efficacy using the terms "efficacy," "效应," or "有效率". Only articles from the recent ten years (2004–2013) were included. All languages available were included. Microsoft Excel was used to collect the data for analysis as described by previous studies [7].

The search yielded a total of 16517 and 79786 publications in Science Direct and CNKI, respectively. The search also found that 14.58% of publications in Science Direct and 1.32% in CNKI on acupuncture are depth related, while that of clinical efficacy research is 43.06% and 23.65%, respectively (Figure 1). This highlights the lack of study in the acupuncture depth arena, warranting more focus on acupuncture and depth research.

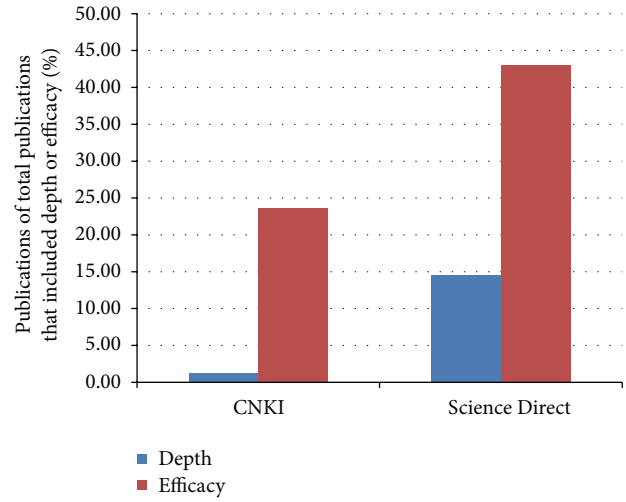

Figure 1: Percentage of total publications in CNKI and Science Direct on acupuncture, acupuncture-moxibustion, moxibustion, and needling from 2004 to 2013 that included depth (blue) or efficacy (red).

3. Proposed Progression of Research Trends

The research on TM has reached a point whereby researchers should pause and reflect on its current directions. The research on acupuncture started off focusing on the meridian effects [8], which was one of the essential building blocks of traditional Chinese medicine (TCM) theory and acupuncture. In the recent years, specificity of meridian acupoint has also become a research hotspot [9–13]. As we progress from meridian studies to specificity of a meridian acupoint, we propose more research to study the variability of effects caused by different insertion depth on a selected point (Figure 2). Such a progression of research trends is in line with the development of modern technology such as ultrasound, fMRI, and laser, which were not available in the past.

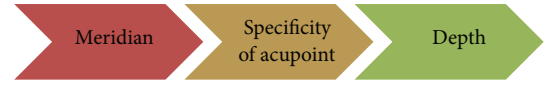

Figure 2: A proposed possible progression of research trends of acupuncture.

4. Depth of Insertion

The depth of insertion, invasion of needles, and other techniques are always varying among different practitioners. Acupuncture textbooks stating recommended ranges for the depth of insertion are mainly for safety purpose [14]. This review emphasizes the importance of depth of insertion and puts forward the consideration of depth in future acupuncture research.

4.1. Factor for Guideline of Acupuncture Control Procedure. Numerous acupuncture researches had focused on the placebo effect of acupuncture [15–18] and used minimal or sham acupuncture as control to real acupuncture [19–23]. Studies had questioned the accuracy of the definition of placebo [24]. Others also questioned whether minimal, superficial, or sham acupuncture procedures are acceptable as inert placebo controls [25, 26]. Another review concluded that clear guideline to assess acupuncture control procedures will improve the quality of RCTs and systematic reviews [27]. Such studies are indicators that the research on depth of acupuncture is crucial for acupuncture research since the common discrepancy is the lack of understanding on effect of depth of insertion on efficacy of acupuncture. It is also evident that acupuncture control procedures guideline should consider the depth of real and control acupuncture.

4.2. Modern Development of Acupuncture. Developments of acupuncture have evolved from the traditional methods and depth of insertion is crucial in some, such as Fu's subcutaneous needling (FSN). FSN is a modern innovative style of acupuncture which focused on the subcutaneous depth [28, 29]. Studies have been done on FSN, such as clinical trial of FSN on low back pain which suggested its immediate and safe effects [30]. Such innovation suggests the clinical effect of acupuncture apart from the traditional insertion and signifies the importance of depth in modern acupuncture research.

4.3. An Example of Inheriting and Innovating TM through Modern Technology. Effective clinical effects produced by renowned acupuncturists are significantly related to the depth they needle for different diseases or point. It is crucial to inherit and explore these experiences by research in the fields of acupuncture depth. We believe that the key to developing TM is to inherit the traditional methods and innovate with the modern technology in order to achieve better healthcare for mankind.

Depth of insertion could only be estimated by acupuncturists in the past due to limitations of expertise. However, with modern technological advancements, such as laser technology, the depth of stimulation can be examined. There are

researchers who have investigated specifically on the violet laser acupuncture (405 nm) which has a penetration depth of 1-2 mm [31–35]. The same team also carried out investigations to compare differences between violet laser acupuncture and red laser acupuncture (685 nm; 3-4 mm) and found different effects on heart rate variability [36]. Such findings showing two specific depths of penetration producing different effects are a breakthrough on research on TM. Clinical trials were also conducted to test the effectiveness and efficacy of laser acupuncture [37–39]. Research on acupuncture should be in tandem with modern technology. In addition to innovation, one has to keep in mind the importance of inheritance. In this case, the principle of "point location according to proportional bone measurement" [40] (骨度分寸) has to be abided. The ratio or proportion of the depth of insertion relative to the part of the body being needled should be considered. This is a display of how we can both inherit and innovate TM for its development.

It is crucial to inherit from experienced mentors in order to find a focus for the research. The authors' mentor, Professor Yang Jia-san [41], had always emphasized the depth of insertion of the needle and hence the authors hope to inherit from and innovate his experiences. TM places immense emphasis on the inheritance of experiences from mentors and such experiences are deemed important treasures of TM. Such experiences left behind by mentors and practitioners before us are crucial for the inheritance and innovation of TM.

4.4. Proposal to Determine the Precise Point of Insertion. Through the abovementioned method, we can propose determining which tissues or levels would create optimal treatment effect. Taking the acupuncture point on the meridian and collateral as the horizontal axis of the point of insertion, the precise acupoint, X, can be determined by finding the depth of insertion, which serves as the vertical axis (Figure 3). The meridian and collateral theory would be the guide for choice of acupoint on the horizontal axis, while more research is warranted to study the depth of the chosen acupoint.

5. Research Methodology

The amount of research done on alternative, complementary, and traditional medicine has increased steadily in the recent years. It is appropriate to review and analyze the trends of the research methodology by all researchers in the same field. This review serves as an example of comparing the research from different parts of the world through two databases which use two different main languages of medium.

5.1. Comparison between CNKI and Science Direct. Comparison between CNKI and Science Direct provides researchers with an apt opportunity to pause and reflect on the trend of future direction of research. Comparison between databases from different countries could be worthwhile. The number of acupuncture-related publications in CNKI is about five times that in Science Direct (Figure 4). Interestingly, the percentage of research in Science Direct done on depth was

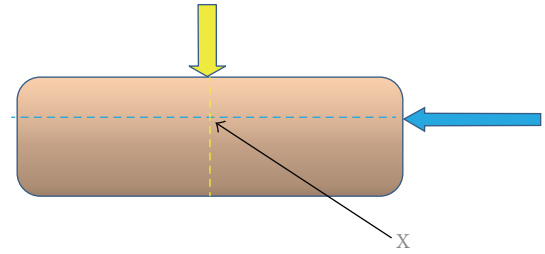

Figure 3: A simulated diagram of acupoint chosen guided by the traditional meridian and collateral theory which forms the horizontal axis (yellow), while depth of chosen acupoint would form the vertical axis (blue) of Point X. Point X is the proposed precise point of insertion.

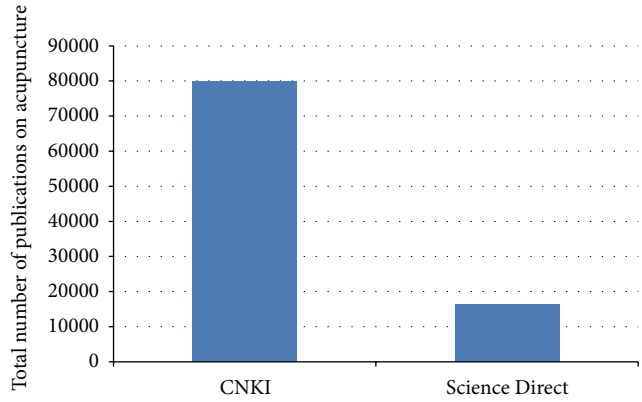

Figure 4: The total publications on acupuncture, acupuncture-moxibustion, moxibustion, and needling that were indexed in Science Direct and CNKI from 2004 to 2013.

more than ten times that of CNKI (Figure 5). 1.32% of CNKI reports involved depth while Science Direct had 14.58%. This result is inversed as compared to the total publications on acupuncture from both databases. This suggests the difference in focus of research from both databases, with respect to depth of acupuncture.

Paradoxically, although acupuncture and the Layer Analysis method [5] originate from China, the percentage of research indexed in the area of acupuncture depth in Science Direct was ten times as compared to CNKI, the biggest published research database in China (Figure 5). In addition, the search from both databases also points to the lack of research focus on depth generally. The authors believe that the amount of research that considers depth in both CNKI and Science Direct should be and would be increased in the future.

This result indicates that studies on acupuncture from the two databases had relatively different focus. Such comparisons are crucial for researchers to determine the focus of their research. It also indicates that international collaborations are warranted for research in acupuncture to build dynamic and innovative research.

Future studies are necessary to compare and analyze the directions of research teams from different parts of the world,

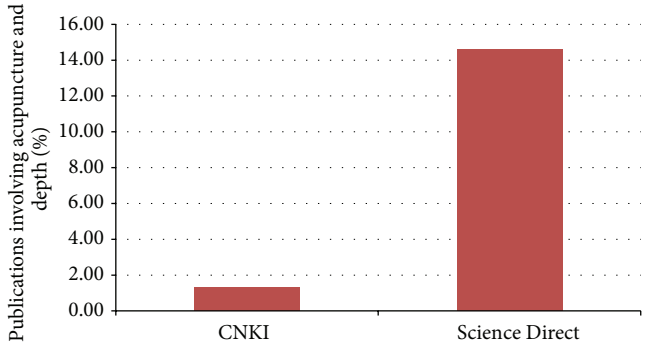

Figure 5: Percentage of total publications in CNKI and Science Direct on acupuncture, acupuncture-moxibustion, moxibustion, and needling from 2004 to 2013 that included depth.

in order to work hand in hand to propel the development of TM. This review shows that such comparison between different databases could produce worthwhile results.

5.2. Bibliometric Method. Bibliometric studies are gaining popularity since the amount of research done increases every year. Bibliometric analysis is an important tool for TM researchers in order to consider the trend of research of TM. More bibliometric studies are warranted in the field of TM in order to determine the future trends. Based on the holism concept of TCM, researchers are familiar with the importance of consideration of the big picture. Hence, this paper proposed the importance of bibliometric data and studies on future TM research.

This paper aims to consider all kinds of research in order to obtain information from all possible research teams. Future studies are warranted to consider more information in order to propel more detailed research in this field.

6. Conclusion

The plentiful variables that exist in acupuncture research are the reason for the lack of scrutiny on the depth of acupuncture. However, as research progresses, one should also focus on the amount of tissue invaded and types of tissues excited. Along with the advancements in imaging, laser technologies, and so forth, research on acupuncture depth could also progress. We suggest that future acupuncture research, especially RCTs, should take into consideration the depth of insertion. In addition, the use of bibliometric method is crucial for future development of TM research trends too.

7. Future Direction

Acupuncture depth could possibly be an important aspect in future research arena of traditional medicine (TM). The use of bibliometric indicators to analyze and compare the existing research from all parts of the world is warranted too.

Conflict of Interests

The authors declare that they have no conflict of interests.

Acknowledgments

The research was supported by the National Natural Science Foundation of China (no. 81373730) and International S & T Cooperation Program of China (no. 2011DFA31370).

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Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2014, Article ID 475192, 8 pages
http://dx.doi.org/10.1155/2014/475192

Research Article

Antidiabetic and Antioxidative Effect of Jiang Tang Xiao Ke Granule in High-Fat Diet and Low-Dose Streptozotocin Induced Diabetic Rats

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Received 28 March 2014; Revised 18 May 2014; Accepted 19 May 2014; Published 25 June 2014

Academic Editor: Si-Yuan Pan

Diabetes mellitus (DM), a kind of metabolic disease, is increasing over the last four decades in the world. The purpose of this study was to investigate the effect of Jiang Tang Xiao Ke (JTXK) granule, a naturally occurring ingredient from Chinese herbal medicines, on serum glucose, lipids, and oxidative stress in DM rats induced by high-fat diet and streptozotocin. JTXK granule 9 g/kg (based on crude herb equivalent) and pioglitazone 1.5 mg/kg (as a positive control for comparison) were orally administrated to DM rats for 4 weeks. Results showed that administration of JTXK granule reduced serum glucose, total cholesterol, triglyceride, and low density lipoprotein levels (by 12%, 33%, 57%, and 44%, resp.) but increased high-density lipoprotein level by 69%, compared with the drug-untreated DM rats. Serum malondialdehyde and nitric oxide levels were lowered (by 34% and 52%, resp.) associated with the elevation in serum superoxide dismutase levels (by 60%) after JTXK granule treatment. In addition, JTXK granule suppressed serum alanine aminotransferase activity (up to 50%) and alleviated pathological changes of pancreas and liver tissues in DM rats. The beneficial changes of pioglitazone on biomarkers were also found in DM rats. These findings suggested that JTXK granule may be an alternative medicine for the management of DM.

1. Introduction

Diabetes mellitus (DM) is a multifactorial metabolic disorder characterized by chronic hyperglycemia with disturbances of carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion and/or insulin action. In 2013, according to International Diabetes Federation, 381 million people suffered from diabetes, which was estimated to almost double by 2030 [1]. DM has become a major worldwide health problem given to its multiple complications [2]. Scholars and physicians have been probed to research and develop the effective drugs or methods to control DM. Different kinds of oral drugs and insulin injection methods are gradually improved in modern medicine [3, 4]. Although western drugs are effective in reducing the glucose level, they are poor in relieving clinical symptoms and controlling diabetic complications. In addition, the drug resistance and side effects of western drugs are also important reasons why special emphasis has been put on Chinese medicine these years [5]. Traditional Chinese medicine (TCM) has shown the advantages of universal adjustment in the treatment of DM reflecting in the aspects of not only lowering blood glucose but also regulating other related aspects of DM, such as lipid metabolic disorders [6, 7]. The herbal remedies can provide a simpler, more natural way of controlling DM without any unpleasant side effects, so people use the herbal remedies in addition to their medication. Some of the herbs have shown promise of useful antidiabetic effect, along with their known mechanism of action [8], For example *Ginseng* and *Rhizoma coptidis* are famous not only for their wide application in treatment of DM but also for the profound research of their antidiabetic mechanisms [9].

Up to now, various studies have been carried out to identify the underlying mechanism of DM [10, 11]. Increasing

evidence in both experimental and clinical studies suggests that oxidative stress (OS) was actively involved in the development of diabetes as well as diabetes-related complications [12, 13]. OS may cause a serious imbalance between reactive species (RS) production and antioxidant defense, which occurs due to an increased generation and/or reduced elimination of RS by the antioxidant defense system. Some of the consequences of an oxidative environment are the development of mitochondrial dysfunction, insulin resistance, and β-cell dysfunction, which can lead ultimately to diabetes [14]. Jiang Tang Xiao Ke (JTXK) granule is a specific formula created by Professor Si-Hua Gao based on his experience in the clinical management of DM. It has been used clinically for several years and the satisfactory result of hypoglycemic effect has been observed [15]. Current study was designed to explore the effect of JTXK granule on the serum glucose level and lipid profiles in DM rats. The changes of oxidative stress parameters were also studied to reveal the possible regulatory mechanism of glucose and lipid metabolism after JTXK granule treatment.

2. Materials and Methods

2.1. JTXK Granule Preparation Procedure. JTXK granule mainly consists of *Radix rehmanniae* (Di Huang), *Fructus corni* (Shan yu rou), *Ginseng* (Ren Shen), *Radix salviae miltiorrhizae* (Dan Shen), and *Rhizoma coptidis* (Huang Lian) with a proportion of 3 : 1 : 1 : 3 : 1. The raw herbs were purchased from Beijing Tong Ren Tang medicinal materials Co., Ltd. (Beijing, China) and authenticated by Professor Chun-Sheng Liu in the Beijing University of Chinese Medicine. For the preparation of the aqueous extract of JTXK granule, the herbs (*Radix rehmanniae* and *Radix salviae miltiorrhizae*, etc.) were boiled in twelve volumes of distilled water for 1 h. The procedure was repeated three times. The pooled aqueous extract was filtered through gauze cloth and the filtrate was evaporated by heating until the relative density reached 1.15. For the preparation of the ethanolic extract of JTXK granule, the herbs (*Fructus corni*, *Ginseng*, and *Rhizoma coptidis*) were extracted three times with twelve volumes of 60% (v/v, in H_2O) ethanol under reflux. A final yield of 20% (w/w) (i.e., 5 g of herbs for every 1 g of extract) was obtained. JTXK granule was made from the pooled aqueous and ethanolic extracts and then stored at 4°C until use.

2.2. Drugs and Reagents. Pioglitazone pills, the positive control drug used in this study, were purchased from Beijing Taiyang pharmacy Co. Ltd. (Beijing, China). Streptozotocin (STZ, Cat number S0-130) was bought from Sigma-Aldrich Chemical Co., Ltd. (St. Louis, USA). STZ was dissolved into 0.1 mol/L sodium citrate-hydrochloric acid buffer when used. Insulin ELISA assay kits were purchased from Beijing north biotechnology research institute (Beijing, China). Total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C), alanine aminotransferase (ALT), superoxide dismutase (SOD), malondialdehyde (MDA), and nitric oxide (NO) kits were purchased from Nan Jing Jian Cheng biological research institute (Nanjing, China).

2.3. Animal Care and Treatment. Male Sprague Dawley rats, weighing 180–200 g, were purchased from Beijing Wei Tong Li Hua experimental animal center (certification number SCXK (Jing) 2012-0001). The animals were housed under the clean level conditions (certification number SCXK (Jing) 2011-0024) with the temperature of 22 ± 1°C, humidity of 55 ± 5%, and 12 : 12 h light/dark cycle in Beijing University of Chinese medicine. All rats were allowed free access to tap water and food. The high-fat diet (HFD) containing 20% sucrose, 2.5% cholesterol, 10% lard, 0.3% sodium cholic acid, and 66.5% (w/w) in standard feed was provided by Ke'ao xieli feed Co., Ltd. (Beijing, China).

The rats subjected to the experiments were allowed to adapt to the environment for a week. Ten animals were chosen and fed with standard diet as the normal control group. The other 35 rats were fed with HFD for four weeks, and then a single intraperitoneal injection of a prepared solution of STZ (30 mg/kg suspended in 0.1 mol/L citrate buffer at pH 4.5) was applied to induce diabetic models. If the volume of fasting blood glucose (FBG) was not less than 16.7 mmol/L after 72 hours of STZ injection, the diabetic models were successful. One week late, DM rats were randomly divided into 3 groups of 10 animals in each: (1) drug-untreated DM rats and (2) and (3) DM rats treated with pioglitazone 1.5 mg/kg and JTXK granule 9 g/kg, respectively. Both drugs were dissolved in distilled water and given to DM rats via gastro gavage once a day. The normal and drug-untreated DM rats were administrated with the same volume of vehicle. The study protocol was approved by the animal ethics committee of Beijing University of Chinese medicine, (Beijing, China).

2.4. Serum Biochemical Analysis. Before and after the drug administration, the fasting blood glucose (FBG) and random blood glucose (RBG) levels in the tail vein were monitored using a glucometer (Johnson & Johnson). At the end of the experimental period, rats were anesthetized with ether after 12 h of fasting. Serum samples were prepared by centrifuging the clotted blood collected from the abdominal aorta and then centrifuged at 3,000 rpm/min for 15 min. The serum fasting insulin (FINS) levels were determined according to the manufacturer's instruction, and insulin sensitivity index (ISI) was calculated according to the formula as follow:

$$\mathrm{ISI} = \ln\left(\frac{1}{\mathrm{FBG} \times \mathrm{FINS}}\right). \quad (1)$$

Serum TC, TG, HDL, and LDL levels, as well as ALT activity, were determined with automatic biochemistry analyzer (BECKMAN Company, America). Serum SOD activity and the volume of MDA and NO were measured using commercially available kits.

2.5. Oral Glucose Tolerance Test (OGTT). Rats were deprived of food overnight and a baseline (0 min) blood glucose level was measured. Then a single dose of glucose (2 g/kg) was

Table 1: Effect of JTXK granule on glucose levels in DM rats.

Groups	Dose (g/kg)	FBG (mmol/L)		RBG (mmol/L)	
		Before treatment	After treatment	Before treatment	After treatment
Normal	—	6.0 ± 0.31	5.43 ± 0.29	7.04 ± 0.20	6.79 ± 0.21
DM	—	30.6 ± 0.91**	27.82 ± 0.46**	30.88 ± 1.09**	30.56 ± 1.04**
DM/pioglitazone	0.0015	29.68 ± 0.96	23.94 ± 0.79#	29.93 ± 1.26	25.24 ± 2.09#
DM/JTXK	9	29.19 ± 1.02	24.51 ± 0.80#	30.05 ± 1.37	25.62 ± 1.42#

Diabetes mellitus (DM) rats were induced by combination of high-fat diet and streptozotocin described in Section 2. Jiang Tang Xiao Ke (JTXK) granule 9 g/kg (based on crude herbal material) and pioglitazone dissolved in distilled water were orally administrated to DM rats for 4 consecutive weeks. Normal and drug-untreated DM rats were treated with the vehicle. After that, fasting blood glucose (FBG) and random blood glucose (RBG) levels were measured using a glucometer. Values are expressed by means ± SE, with $n = 10$. $^{**}P < 0.01$ versus normal rats; $^{\#}P < 0.05$ versus DM rats. Statistically significant differences were determined using a one-way ANOVA followed by Dunnett's post hoc analysis.

dissolved in 1 mL of water and administered by gavages. Over the following 30 min, 60 min, and 120 min, the blood samples were taken from the tail vein and used to detect the glucose levels, respectively [16].

2.6. Examination of Pancreas and Liver Histology. After 4 weeks of drug treatment, the pancreas and liver were removed and fixed in 10% neutral buffered formalin. The organs were routinely processed and sectioned at 4-5 mm thickness. Sections of pancreas and liver were stained with hematoxylin and eosin (HE) and examined by light microscopy in order to demonstrate the histopathological changes of DM rats. The photomicrographs of each tissue section were taken on laboratory microscopy (Olympus, Tokyo, Japan).

2.7. Statistical Analysis. SPSS version 17.0 software (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. All data were presented as Mean ± SE. Statistical significance among groups was determined by one-way analysis of variance (ANOVA) followed by Duncan's analysis to compare various groups with each other. $P < 0.05$ was considered to be statistically significant.

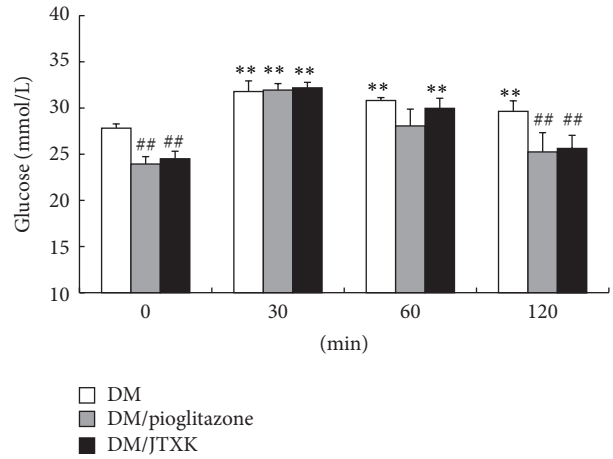

Figure 1: Effect of JTXK granule on OGTT in DM rats. Experimental details were described in Table 1. Following a fast, a glucose load (2 g/kg) is intragastrically administered and blood glucose was measured over a span of 2 h (0, 30, 60, and 120 min after glucose intake). Values are expressed by means ± SE, with $n = 10$. $^{**}P < 0.01$ versus 0 min; $^{\#\#}P < 0.01$ versus DM rats. Statistically significant differences were determined using a one-way ANOVA followed by Dunnett's post hoc analysis.

3. Results

3.1. Effect of JTXK Granule on Serum Glucose Levels in DM Rats. To evaluate the effect of JTXK granule on glucose homeostasis, the FBG and RBG were measured as routine protocols. FBG and RBG levels in DM rats were significantly higher (4 folds higher) than the normal rats, which indicated that the rat model of DM was successfully established. JTXK granule and pioglitazone treatment for 4 weeks reduced both FBG (by 16% and 19%, resp.) and RBG (by 15% and 16%, resp.), compared with before medications. JTXK granule decreased FBG and RBG (by 12% and 16%, resp.) compared with untreated DM rats, while pioglitazone decreased FBG and RBG (by 14% and 17%, resp.) (Table 1).

OGTT is a more physiological method of assessing the glucose induced insulin secretion and glycemic control. After treatment with JTXK granule and pioglitazone for 4 weeks, blood glucose levels significantly decreased (by 14% and 15%, resp.) at 120 min of glucose load, compared with the drug-untreated DM rats (Figure 1).

3.2. Effect of JTXK Granule on Insulin Sensitivity in DM Rats. Although there were no differences in serum FINS levels between normal and DM rats, ISI was markedly reduced in DM rats (by 23%), compared with the normal rats. Pioglitazone and JTXK granule treatment did not alter the serum FINS levels in DM rats. However administration of pioglitazone and JTXK granule increased the ISI by 7% and 5% in DM rats, respectively (Table 2).

3.3. Effect of JTXK Granule on Serum Lipids in DM Rats. As shown in Table 3, it were observed that serum HDL level deceased (39%), and serum TC, TG and LDL levels markedly increased (12 folds, 13 folds, 34 folds, resp.) in DM rats compared with the normal rats. Pioglitazone reduced serum TC (by 34%), TG (by 73%), LDL (by 46%) but increased HDL (by 31%) in DM rats. In the same situation, JTXK granule treatment decreased serum TC, TG, and LDL levels by 33%,

Table 2: Effect of JTXK granule on levels of serum FINS and ISI in DM rats.

Groups	Dose (g/kg)	FINS (μ/L)	ISI
Normal	—	45.89 ± 5.40	−5.49 ± 0.10
DM	—	41.90 ± 6.28	$-7.10 \pm 0.14^{**}$
DM/pioglitazone	0.0015	38.70 ± 5.99	$-6.63 \pm 0.10^{\#\#}$
DM/JTXK	9	40.30 ± 6.13	$-6.72 \pm 0.14^{\#}$

Experimental details were described in Table 1. Fasting insulin (FINS). Insulin sensitivity index (ISI) was calculated according to the formula ISI = Ln (1/FBG × FINS). Values are expressed by means ± SE, with $n = 10$. $^{**}P < 0.01$ versus rats in normal group; $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ versus DM rats. Statistically significant differences were determined using a one-way ANOVA followed by Dunnett's post hoc analysis.

57%, and 44%, respectively. Moreover, it elevated serum HDL by 69% in DM rats.

3.4. Effects of JTXK Granule on Oxidative Stress and Hepatic Function in DM Rats. As shown in Table 4, serum MDA, NO, and ALT activities were increased by 56%, 119%, and 163% in DM rats, respectively, compared with the normal control rats. At the same time, serum SOD activity was reduced by 65%. Pioglitazone treatment increased the serum SOD activity by 81% but reduced serum MDA and NO level (up to 17% and 32%, resp.) in DM rats. Four weeks of JTXK granule administration significantly enhanced serum SOD activity and reduced MDA and NO levels (by 60%, 34%, and 52%, resp.). JTXK granule and pioglitazone administration reduced serum ALT actively (up to 50%).

3.5. Effect of JTXK Granule on Pancreas and Liver Histology in DM Rats. In light microscopy, the normal rats showed typical histological structure with normal islet (Figure 2(a)A). The sizes of islet were smaller than the normal and the dilated acini were found in DM rats. The islets showed necrotic cells with pyknotic nuclei and dense eosinophilic cytoplasm (Figure 2(a)B). Pioglitazone (Figure 2(a)C) and JTXK granule (Figure 2(a)D) treatment improved the structure of islet in DM rats. The liver sections of normal rats showed normal cell structure with distinct hepatic cells, sinusoidal spaces, and a central vein (Figure 2(b)A). Histological examination revealed that long-term HFD feeding induced massive hepatic steatosis. DM rats showed lymphocyte in filtration and liver cell hypertrophy (Figure 2(b)B). Pioglitazone treatment reversed HFD induced adverse changes of DM rat's liver to some extent, and it showed slight lymphocyte infiltration (Figure 2(b)C). JTXK granule treatment relieved the hepatic steatosis in DM rats (Figure 2(b)D).

3.6. Effect of JTXK Granule on Body Weight in DM Rats. Compared with the normal rats, DM rats lost their body weight (up to 30%) at the end of the experiment. Administration of JTXK granule and pioglitazone for four weeks improved the body weight loss in DM rats (Table 5).

4. Discussion

Among all patients with DM, type-2 diabetes mellitus (T2DM) makes up about 90% of the cases. Immense amounts of research on mechanisms and control of T2DM have been launched considering its increased levels of incidence and associated mortality. Several DM models were constructed and explored in these researches [17]. Among the various models, HFD fed animals with exposure to low dose of STZ are commonly used. It has been reported that HFD results in insulin resistance, which leads to adipocyte dysfunction and decreased inhibition of released free fatty acids into the blood [18]. STZ is the most commonly used diabetogenic agent. It is used in medical research to produce an animal model for diabetes by selectively destroying pancreatic β-cells, which associates strictly with the induction of oxidative stress, both systemically and locally.

According to traditional Chinese medicine (TCM) theory, it is considered that the main pathogenesis of T2DM is due to the malfunction of liver, spleen, and kidneys organ systems. The treating principle of JTXK granule is to restore the function of the organs in TCM point of view. Accumulating evidence suggests that the main ingredients of JTXK granule, such as berberine, tanshinone, and catalpol, can not only reduce the FBS and lipid levels but also regulate the oxidative stress in the body according to the pharmacological study of modern medicine. Therefore, JTXK granule has potency to control glucose and lipid metabolism and relieve symptoms of diabetes. However, mechanism of the formulated JTXK granule for preventing and controlling the development of DM remains to be investigated.

In the present study, DM rats induced by HFD and STZ showed impaired glucose tolerance and stable fasting and random hyperglycemia compared with the normal rats. Four weeks administration of pioglitazone and JTXK granule improved oral glucose tolerance and reduced FBG and RBG levels in DM rats. Some researchers found that the serum fasting insulin level elevated [19] in diabetic rats. However, the other papers reported decreased serum fasting insulin level [20]. This may relate to the different diabetic models or stages. But the ISI universally reduces as the insulin resistance is the common pathological changes of T2DM [21]. It was observed in the study that ISI in DM rats was reduced, compared with the normal rats, but the serum insulin level did not change significantly. Pioglitazone improves glycaemic control in people with T2DM by improving insulin sensitivity through its action at PPARγ. It can increase glucose uptake and utilization in the peripheral organs and decrease gluconeogenesis in the liver through increasing glucose transporters 1 and 4, lowering free fatty acids and remodeling of adipose tissue [22]. In accordance with the report, administration with pioglitazone markedly increased the ISI. The results also suggested that JTXK granule enhanced the ISI in DM rats. It was reported that some of the active ingredients of JTXK granule, such as berberine and *Ginseng*, were efficacious for treating hyperglycaemia [23, 24], which might result from their antioxidant and anti-inflammatory properties as well as improvement of insulin resistance [25]. In the present study it was also found that JTXK granule treatment

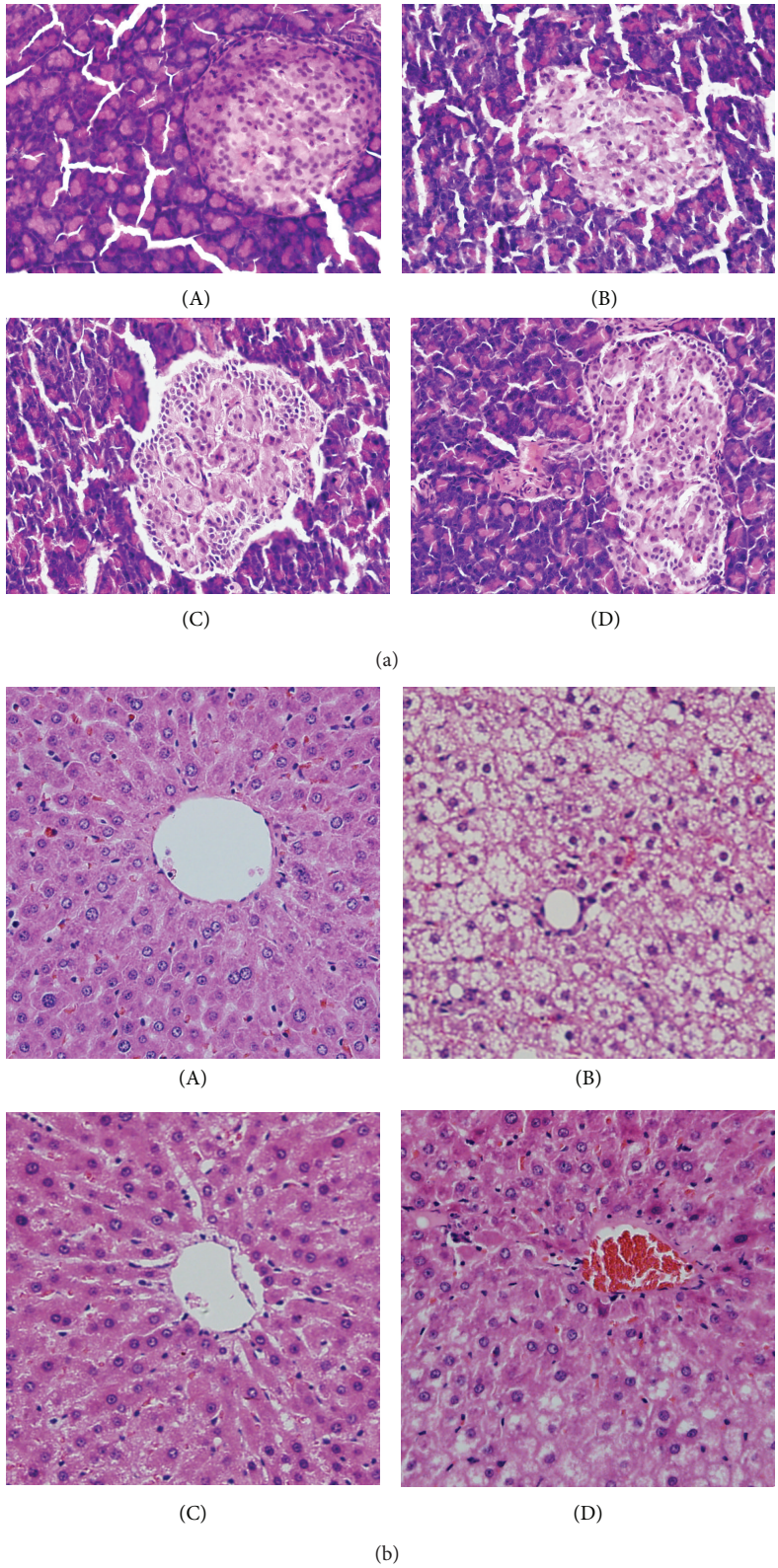

Figure 2: Effect of JTXK granule on pancreas and liver histology in DM rats. Experimental details were described in Table 1. Photomicrographs of histological changes of hematoxylin-eosin stained pancreatic (a) and liver (b) section at magnification of 200x. (A) normal rats; (B) DM rats; (C) DM/pioglitazone; and (D) DM/JTXK granule.

Table 3: Effect of JTXK granule on serum lipid profiles in DM rats.

Groups	Dose (g/kg)	TC (mmol/L)	TG (mmol/L)	HDL (mmol/L)	LDL (mmol/L)
Normal	—	1.77 ± 0.10	0.52 ± 0.05	0.57 ± 0.03	0.27 ± 0.03
DM	—	22.78 ± 3.68**	7.33 ± 2.07**	0.35 ± 0.33**	9.33 ± 1.62**
DM/pioglitazone	0.0015	14.93 ± 2.98	1.95 ± 0.33$^{\#\#}$	0.46 ± 0.05	5.04 ± 1.09$^{\#}$
DM/JTXK	9	15.27 ± 3.40	3.14 ± 0.93$^{\#}$	0.59 ± 0.09$^{\#}$	5.23 ± 1.27$^{\#}$

Experimental details were described in Table 1. Rats were treated with either pioglitazone or JTXK granule for 4 weeks. After that, serum triglycerides (TG), total cholesterol (TC), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) were measured. Values are expressed by means ± SE, with $n = 10$. $^{**}P < 0.01$ versus normal rats; $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ versus DM rats. Statistically significant differences were determined using a one-way ANOVA followed by Dunnett's post hoc analysis.

Table 4: Effect of JTXK granule on oxidative stress and hepatic function in DM rats.

Groups	Dose (g/kg)	Serum NO (umol/L)	Serum SOD (U/mL)	Serum MDA (mmol/L)	Serum ALT activity (U/L)
Normal	—	22.43 ± 2.83	53.43 ± 8.41	5.21 ± 0.35	60.00 ± 1.93
DM	—	49.10 ± 6.51*	18.71 ± 6.64*	8.12 ± 1.30**	157.89 ± 26.75**
DM/pioglitazone	0.0015	40.78 ± 4.24	33.83 ± 8.30$^{\#}$	5.56 ± 0.61$^{\#\#}$	85.90 ± 8.79$^{\#\#}$
DM/JTXK	9	23.43 ± 3.89$^{\#\#}$	29.97 ± 3.66$^{\#}$	5.38 ± 0.68$^{\#\#}$	79.50 ± 10.04$^{\#\#}$

Experimental details were described in Table 1. Four weeks after drug treatment serum nitric oxide (NO), superoxide dismutase (SOD), malondialdehyde (MDA), and alanine aminotransferase (ALT) were determined. Values are expressed by means ± SE, with $n = 10$. $^{*}P < 0.05$ and $^{**}P < 0.01$ versus normal rats; $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ versus DM rats. Statistically significant differences were determined using a one-way ANOVA followed by Dunnett's post hoc analysis.

relieved the impairment of pancreas cells in DM rats. It may be one of the reasons for JTXK granule antidiabetic property.

Hyperlipidemia is an important contributor to insulin resistance, and hence reduction of lipid profiles is helpful in the remission of DM [26]. The hypolipidemic effect of many herbs was also demonstrated by previous studies [27], which means JTXK granule had good foundation for treating dyslipidemia. Compared with the normal rats, TC, TG, and LDL levels in DM rats were elevated, and the serum HDL level reduced significantly. Pioglitazone and JTXK granule treatment restored the abnormal changes of TG, LDL, and HDL in DM rats. It has been known that pioglitazone affects lipid metabolism through action at PPARα [28], but the mechanism of action of JTXK granule on hyperlipidemia needs to be further studied. Furthermore, fatty changes have been found in centrilobular portions of the liver in DM rats, which is consistent with the literature [28]. ALT, which mediates conversion of alanine to pyruvate and glutamate, is a suitable indicator of hepatic injuries. In the current study, it showed that ALT level of DM rats significantly reduced after JTXK granule treatment. The liver shows pathological changes of histological section indicating that JTXK granule can adjust steatosis of liver structure in DM rats.

Increasing evidence implicates the role of oxidative stress in the different stages of the development of DM, starting from the prediabetes state, impaired glucose tolerance, and overt diabetes mellitus to diabetic complications states [14]. As it is shown, oxidative stress plays an important role in the pathogenesis of both beta cell dysfunction and insulin resistance [29]. As the typical production of lipid peroxidation, MDA affects the fluidity and permeability of cell membrane, inducing dysfunction or even death of the cells. So plasma MDA may serve as a good and sensitive marker of oxidative stress in the pathological process [30]. SOD as one of the main antioxidant enzymes maintains the cellular levels of O^{2-} within the physiological concentrations by converting superoxide anion radicals produced in the body to hydrogen peroxide [31, 32]. Its activity can reflect the reactive oxygen species' elimination ability of the body indirectly. The present study showed that, compared with normal rats, MDA levels in DM rats were significantly increased, while SOD activity was significantly decreased at the end of the study. And compared with untreated DM rats, JTXK granule treatment was observed to demonstrate recovery from the decreased levels of SOD associated with the suppressed MDA content.

Generally, NO at physiological levels produces many benefits to the body. Metabolic disorders of diabetes influenced the content and the activity of NO through NO/cGMP pathway, implicating the elevation and following decrease of NO level in the early and late stage of diabetes [33]. Hyperglycemia promotes the expression of nitric oxide synthase by activating numbers of stress sensitive signaling pathways (NFkB, P38 mitogen activated protein kinase, NH2 terminal junk kinase, etc.), which therefore stimulates the overproduction of NO, a cytotoxic molecules that directly damage the cells and tissues [34]. The results of the current study showed an obvious increase on NO levels in diabetic rats, while JTXK granule reduced NO level after 4 weeks administration. In this study, we provide evidence that protection from the development of diabetes by JTXK granule treatment involves changes in antioxidation. These findings are consistent with some reports [35].

Table 5: Effect of JTXK granule on body weight in DM rats.

Groups	Dose (g/kg)	Body weight (g)				
		Week 0	Week 1	Week 2	Week 3	Week 4
Normal	—	431 ± 17	451 ± 18	474 ± 19	486 ± 20	494 ± 19
DM	—	342 ± 12*	341 ± 12*	362 ± 13*	360 ± 16*	348 ± 12*
DM/pioglitazone	0.0015	347 ± 10	349 ± 14	366 ± 16	363 ± 15	371 ± 15
DM/JTXK	9	348 ± 13	344 ± 12	368 ± 14	372 ± 13	386 ± 12#

Experimental details were described in Table 1. Rats were weighed every week for a period of 4 consecutive weeks after drug treatment. Values are expressed by means ± SE, with $n = 10$. $^{*}P < 0.05$ versus normal rats; $^{\#}P < 0.05$ versus DM rats. Statistically significant differences were determined using a one-way ANOVA followed by Dunnett's post hoc analysis.

In conclusion, JTXK granule, a Chinese medicinal formula, at 9 g/kg (based on crude herbal material) treatment for 4 weeks reduced serum glucose via increasing insulin sensitivity and protection of pancreas islets in DM rats. In addition, the JTXK granule decreased serum TC, TG, and LDL levels but increased HDL levels, compared with the drug-untreated DM rats. At the same time, JTXK granule showed improved antioxidant activity, which was manifested by decreased MDA and NO levels and with elevation in SOD levels in DM rats. Islet morphology showed marked improvement in DM rats treated with JTXK granule. These findings suggested that JTXK granule may be an effective and safe alternative treatment for T2DM.

Conflict of Interests

The authors declared no conflict of interests with respect to the authorship and/or publication of this paper.

Authors' Contribution

Dan-Dan Zhao and Na Yu contributed equally to the work.

Acknowledgments

This paper was supported by New Drug Development Program (Grant no. 2012ZX09103201-005) and National Natural Science Foundation of China (Grant nos. NSFC81274041 and NSFC81273995).

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Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2014, Article ID 495635, 5 pages
http://dx.doi.org/10.1155/2014/495635

Research Article

Research on Traditional Medicine: What Has Been Done, the Difficulties, and Possible Solutions

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Received 28 March 2014; Accepted 26 May 2014; Published 12 June 2014

Academic Editor: Si-Yuan Pan

Traditional medicine (TM) is being used more frequently all over the world. However most often these are choices made by the patient. Integrating TM into mainstream health care would require research to understand the efficacy, safety, and mechanism of action of TM systems. This paper describes research done on TM and difficulties encountered in researching TM, especially when an attempt is made to conform to the model for conventional medicine. The research articles were PubMed searched and categorized as experimental, quasiexperimental, reviews, descriptive, historical, interviews, case histories, and abstract not available. The last part of the report provides suggestions to make research on TM more acceptable and useful, with the ultimate goal of integrating TM into mainstream healthcare with sufficient knowledge about the efficacy, safety, and mechanism of action of TM systems.

1. Traditional Medicine: The Existing Knowledge and Research

According to the World Health Organization atlas (2002), "traditional medicine (TM)" refers to health practices, approaches, knowledge, and beliefs incorporating plant, animal, and mineral based medicines, spiritual therapies, and manual techniques applied individually or in combination to treat, diagnose, and prevent illnesses or maintain wellbeing. It is worth noting that the description of TM given by the WHO in 2002 may have altered in some respects since then.

TM can be considered to belong to three main categories [1]. These are (i) codified medical systems, (ii) folk medicine, and (iii) allied forms of health knowledge [1]. Codified medical systems include great traditions which have evolved over 3-4 millennia and include Ayurveda, Siddha, and Unani in the Indian subcontinent and traditional Chinese medicine and acupuncture in China. These medical traditions have a unique understanding of physiology, pathogenesis, pharmacology, and pharmaceuticals which are different from Western biomedicine [2]. Perhaps because of this systematic approach these medical systems have been professionalized within the last millennia. Folk medicine is those traditional knowledge systems which are more often orally transmitted, have been generated by communities over centuries, and use components of the ecosystem which are locally available and accessible [1]. Folk medicine has not been formalized and is diverse and adaptable based on changing contexts. There are several similarities in the folk/indigenous medicine of widely differing, geographically distinct, communities. Allied forms of health knowledge include techniques which are related to wellbeing though they are not purely medical systems, such as yoga, tai-chi, qi-gong, and different meditations and breathing techniques [1]. The WHO published a global atlas to compile information on TM globally, in terms of policy, regulations, financing, education, research, practice, and use [3]. This provides a regional overview of TM, whether the systems are codified medical systems, folk medicine, or allied forms of knowledge. The description includes the use of TM in the African region, the Americas, the South East Asian region, the Western Pacific region (including Japan and the Republic of Korea), the European region, Eastern Mediterranean region, and Australian region. Globally the interest in TM, specific to that region as well as of other geographic areas, has increased due to easy accessibility, flexibility, relatively low cost, low levels of technological input,

and relatively low side effects (WHO, 2002). Hence there is a definite need to mainstream TM into public health care. According to the WHO some of the major policy challenges include safety, efficacy, quality, and rational use of TM. Various policy measures have been and are being applied to the use of TM, in order to increase its acceptability, safety, and efficacy [4].

According to the WHO, the quantity and quality of safety and efficacy data on TM are not sufficient to meet the criteria needed to support its use worldwide.

There is no paucity of research on TM. A search in February 2014 of the bibliographic database PubMed, leads to 73,704 responses to "TM" as the search words, and the number has increased since then. An attempt was made to determine the number of papers published for different systems of TM such as Aboriginal, African, Alaskan, Ayurveda, Bhutanese, Caribbean, Inca, Maori, Mexican, Native American, Naturopathy, Persian, Siddha, South American, Tibetan, and Unani. It must be emphasized that this sample does not include all TM systems but attempted to cover those used often in different geographical locations.

In the present paper, the 300 most recent publications were categorized as (i) experimental (which included randomized controlled trials/nonrandomized trials, and detailed analysis of the active ingredients in herbal medicines and epidemiological studies); (ii) quasiexperimental which included observational studies; (iii) descriptive reports which mentioned the principles underlying the method of treatment; (iv) historical descriptions which detail the origins of the system; and (v) case histories or narratives. This is an arbitrary description, but the results, given in Table 1, are intended to give an approximation of the amount of research in each system of TM and the type of research being conducted.

2. Difficulties in Research in Traditional Medicine (TM)

While there is an increase in the use of TM worldwide, research in this area is inadequate, with serious difficulties in accepting the studies conducted [5].

Some of the main reasons why the studies conducted are considered flawed and inadequate are small sample sizes, variable or inconsistent results, and inadequate research designs [5]. Other problems include insufficient statistical power (possibly related to small sample sizes), poor controls, inconsistency of descriptions of the treatment or product, and lack of comparisons with other treatments or with a placebo or with both.

Most TM interventions use complex treatment methods which include botanical medications; individualized diagnosis and treatment; an emphasis on maximizing the body's innate ability to heal itself and a "whole systems" approach, wherein the physical, mental, and spiritual attributes of a patient are emphasized, rather than a focus on the disease as in conventional medicine (CM) [5].

Another difficulty encountered when designing a research study on a traditional healing method is that there are often differences in the forms, approaches, and nature (duration and intensity) of treatment, making it difficult to describe any TM in a single sentence, which would be understood to mean the same method by all people, everywhere. In the absence of such standardization, research on TM requires detailed descriptions of the interventions.

The criteria for including and excluding persons in a randomized control trial, RCT, differ between CM and TM; for example, having chosen TM as a therapy could be a criterion for exclusion, to reduce bias. If these exclusions are not observed the value of the RCT would be lowered. Other difficulties encountered are in randomizing patients, selecting a suitable placebo, and/or alternate intervention, as well as in masking and blinding. Randomization is very often difficult as patients have strong beliefs for or against TM and hence most often patients select to receive TM as a modality of treatment or alternatively choose to reject it. Another difficulty is that many of the treatments are carried out in specialized residential setting, under the supervision of a person trained in TM [6]. Quite often the residential center is in quiet surroundings, which have their own healing effects [7]. If the comparison group receives conventional treatment in their homes it is questionable whether the comparison between the two has any meaning, as the very fact that TM is carried out in a different setting [8] and with the personal attention of a TM healer could have a positive impact on the way the person feels and influences their subjective reports [9] and even possibly the outcome of the disease.

Another problem encountered with TM is selecting a suitable placebo. To begin with, interaction between the healer and the patient, which is usual in TM, can have a placebo effect [10, 11]. Apart from this when the participant receives an intervention such as chiropractic, massage, or acupuncture a sham treatment or placebo would be difficult to devise. This is all the more difficult if the patient is actively involved in the intervention, as in the case of yoga, practiced as therapy. A single study did attempt to use a device to simulate yoga breathing, as breathing through the device resulted in inhalation and exhalation being in a ratio of 1 : 2 automatically [12]. The sham device was identical to the active device but did not change respiration and hence was the placebo. Breathing through the active device did have a favorable effect in mild bronchial asthmatics, reducing their responsivity to histamine [12]. However practitioners of yoga may well question whether yoga breathing involves a change in the inhalation to exhalation ratio alone. Most of these techniques require subtle mental changes as well [13]; hence attempting to find a placebo for a TM intervention may actually result in evaluating limited components of the intervention.

3. Future Directions for Research in Traditional Medicine (TM)

The sections which precede this have demonstrated convincingly that there is the necessity for a new way to plan and conduct research on TM. The reasons why different guidelines are required for TM are due to the differences between TM and CM which are mentioned in Table 2.

Table 1: Details of articles on TM found in the PubMed search mentioned in the paper.

Serial number	Name of some TM systems	Articles found in PubMed on February 2014		Category							
		Total articles	Relevant articles	EX	QS	RV	DS	DS/HS	IN	CH	NV
1	Aboriginal	136	135	57	2	14	52	7	0	3	0
2	African*	2661	216	131	18	16	18	7	10	6	10
3	Alaskan	9	4	0	0	0	3	1	0	0	0
4	Ayurveda*	3514	300	172	17	24	43	16	0	13	15
5	Bhutan	20	16	8	1	0	3	0	3	0	1
6	Caribbean	306	106	56	8	7	9	21	0	2	3
7	Inca	5	4	2	0	0	2	0	0	0	0
8	Maori	30	21	6	0	0	8	0	1	1	5
9	Mexican	376	249	154	24	14	17	8	16	5	11
10	Native American	536	198	22	13	6	82	9	9	5	52
11	Naturopathy	1002	461	23	24	6	42	5	6	8	44
12	Persian	69	66	16	0	12	1	35	0	1	1
13	Siddha	203	113	59	17	0	15	3	2	0	17
14	South American	281	205	29	14	6	62	8	0	3	83
15	Tibetan	530	237	118	0	12	86	1	0	1	9
16	Unani	303	189	99	8	9	20	7	0	2	44

Note: *where total articles exceeded 1500, the 300 most recent articles were categorized.
EX = experimental.
QS = quasiexperimental.
RV = review.
DS = descriptive.
HS = historical.
IN = interviews.
CH = case history.
NV = abstract not available.

Future directions include (i) policy making and standardization, (ii) training of researchers in TM with a combination of conventional research methods and those relevant exclusively to TM, (iii) financing research in TM and guidelines for writing and reviewing research grant proposals, and (iv) planning and designing studies in TM.

(i) Policy making and standardization are perhaps the most difficult challenge in TM systems (even those described as codified medical systems) [1]. There are vast differences in the methods used for any intervention and also in the way the healers are trained. Some courses, for example, may emphasize the physical aspects of the healing system, whereas other courses conducted elsewhere may emphasize mental and spiritual aspects. To make it possible for TM to be integrated into mainstream medical care it is essential that there be an attempt to standardize the healing method and courses involved in training those who deliver it. This would require having policies and specific nodal agencies to control and provide guidelines for this to be done properly.

(ii) Training of researchers in TM in conventional research methods and those relevant to TM is an essential step to increasing research in TM. It is important to realize that many persons trained in using TM have a deep and abiding belief in the system of healing. This fact and the fact that they may not be trained in conventional physiology and anatomy may make them less suitable to carry out unbiased research on TM. Hence an important step is to select motivated yet unbiased persons who preferably have a basic knowledge of human anatomy and physiology. Many researchers in CM are trained to practice CM. Similarly if motivated persons trained in TM receive training in conventional research methods with the adaptations needed for TM [14], these trained persons would be ideal to conduct research on TM.

(iii) Obtaining funds for research in TM is another essential step. Just as the NC-CAM of the NIH has allocated separate funds for research in TM, this is true for other countries as well. For example the Department of AYUSH, Government of India, India, has separate funds allocated for research in Ayurveda, Yoga, Unani, Siddha, and Homeopathy (AYUSH). However the format for research proposals is often more suited to research in CM. Apart from this the reviewers often have a distinguished career in CM with a partial or peripheral interest in TM. Hence only those research projects which investigate TM using the standards and norms set for CM are considered worth financing. Many areas related to understanding the mechanism underlying the benefits of TM may be considered "unscientific" or "dubious" by conventional researchers, as they involve concepts such as the subtle energy (*prana* in Indian medicine and *chi* in

Table 2: Differences between CM and TM.

Areas which differ	Conventional medicine (CM)	Traditional medicine (TM)
(1) Mode of treatment	Primarily through medicine or surgery with additional information about precautions and side effects.	Includes polyherbal and mineral preparations, surgery, and guidelines encompassing the whole lifestyle (diet, mental attitude, physical activity, and even spiritual beliefs).
(2) Standardization	Well standardized so that it can be comprehended all over the world.	TM remains unstandardized. There are differences within a healing method; hence detailed descriptions are essential.
(3) Training of the practitioners	A well-defined system has been developed in each country.	There are differences in training program with respect to their content and duration.
(4) Quality of medicines	The medicines undergo rigorous testing and have to meet predetermined standards for safety which are set in each country.	Some of the codified medical systems, such as Ayurveda, do undergo testing for quality control and component analysis. However this is not rigorous and also it is not uniform within a country.
(5) Involvement of the healer	The healer who would be a trained physician or surgeon would need to know the detailed medical history of the patient and other details relevant to the disease before deciding and completing a course of treatment.	A healer of TM most often has to be involved closely with the patient's case history including the physical, mental, and even spiritual aspects. Diagnosis also involves interacting with the patient as do the treatments, which require the healer to participate in the treatment.
(6) Involvement of the patient	The patient has to be cooperative in the diagnosis, treatment, and follow up. Most often this involves taking specified medicines at specified times.	The patient actively participates in TM healing systems during the diagnosis, treatment, and follow up. While some TM methods such as massage require passive cooperation of the patient, others, such as yoga practiced as therapy, require the patient's active participation.
(7) Safety	The safety of CM is based on rigorous drug trials which go through several levels, from trials on experimental animals to final trials after approval on human subjects.	A few systems such as Ayurveda and TCM have had rigorous trials. However most TM preparations are not scrutinized with rigor.
(8) Adverse effects	Adverse effects for all medicines and surgical procedures are reported and made available to the medical community globally.	Adverse effects of TM systems are often not systematically documental or reported. This is an area in which considerable work remains to be done so that TM systems can have adequate legitimacy and be used widely.
(9) Efficacy and dosage	CM has details of the efficacy of the medicines and surgical procedures. Also, the dosages have been worked out taking into account factors such as age, body weight, and liver and kidney functions.	TM systems often decide the type and quantum of treatment based on individual factors. In some cases trying to apply the CM model to TM may reduce the usefulness of the TM system. Nonetheless there has to be a definite description of the factors which could determine TM efficacy and dosage.
(10) Mechanisms of action	The mechanisms of action of many CM methods of treatment are known.	Many TM are effective in healing but little is known about their mechanism of action. Research in this area is often made difficult by the fact that TM systems include subtle concepts such as "spiritual wellbeing," "energy medicine," and others which are not described in conventional medicine.

Chinese medicine). Nonetheless these concepts are a part of TM and if they are disregarded on the basis of being scientifically unacceptable, the risk is that TM would not be understood in its entirety. Hence an effort should be made to review all research grant proposals on TM by a panel comprising of experts in research on CM, researchers in TM, and persons with an in-depth knowledge of TM, but who are not biased in their approach to investigating TM.

(iv) Planning and designing a research study on TM are challenging and require a change in the way research in this area is viewed. When planning efficacy trials it is necessary to accept that randomization and finding the proper controls are difficulties peculiar to TM and not found in efficacy trials on CM. Hence instead of randomization and attempting to have placebo controlled trials research on TM has to take into account various issues. For example, (i) the patient selecting TM with a belief in it could have its own placebo effect, (ii) the complexity of the interventions in TM often does not allow for a placebo, and (iii) the basic difficulty of comparing a whole life style changes with the approach of specific prescribed medicines in CM. A possible and indeed probably the only way forward in efficacy trials of TM is to adopt a "whole systems approach" where the entire set of practices which make up a TM healing system are compared with

the conventional treatment, without any attempt to consider different aspects of the treatment, separately. Research on the mechanisms underlying the effects of TM also requires a shift in the way of thinking, so as to include complex concepts not used in CM such as "subtle energy" and "psychological and even spiritual benefits."

Other research in TM, particularly related to herbology and plants used in healing, already follows conventional methods. Additional studies are required to understand the safety of herbomineral compounds and determine whether they have a risk of heavy metal toxicity or not [15].

Hence this brief report has attempted to provide an idea of the research which has been done in TM, the difficulties in applying CM research guidelines to TM, and possible guidelines for future directions which could make research in the area of TM worldwide more authentic as well as more scientifically rigorous. The ultimate goal would be to arrive at standardized systems of TM which can be integrated into mainstream healthcare, after having sufficient research-based information about their efficacy, safety, and mechanisms of action.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Evidence-Based Complementary and Alternative Medicine
Volume 2014, Article ID 787529, 8 pages
http://dx.doi.org/10.1155/2014/787529

Research Article

A Disturbance Rejection Framework for the Study of Traditional Chinese Medicine

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Received 28 March 2014; Accepted 9 May 2014; Published 4 June 2014

Academic Editor: Si-Yuan Pan

The traditional Chinese medicine (TCM) is explained in the language of engineering cybernetics (EC), an engineering science with the tradition of rigor and long history of practice. The inherent connection is articulated between EC, as a science of interrelations, and the Chinese conception of Wuxing. The combined cybernetic model of Wuxing seems to have significant explaining power for the TCM and could potentially facilitate better communications of the insights of the TCM to the West. In disturbance rejection, an engineering concept, a great metaphor, is found to show how the TCM is practiced, using the liver cancer pathogenesis and treatment as a case study. The results from a series of experimental studies seem to lend support to the cybernetic model of Wuxing and the principles of disturbance rejection.

1. Introduction

Holistic, self-contained, and independent developed over millenniums, traditional Chinese medicine (TCM) is the embodiment of a cosmic view, rooted in Yin-Yang and Wuxing, in the inner working of human bodies. The TCM traces its origin to the Yellow Emperor's Canon of Medicine, which systematically builds the basic tenants of Chinese medicine in the language of Yin-Yang and Wuxing. Westerners may be familiar with the term Yin-Yang but not with the term Wuxing, whose standard translation is "five elements," which is, unfortunately, a bit misleading. In Wuxing the Chinese try to describe the characteristics of changes in matters, rather than the matters themselves, using the oldest tool of the Chinese thinkers: analogy. In philosophy and in medicine, the Chinese are more interested in how things change, rather than the things in themselves. The Yin and Yang are two opposing forces behind all changes in nature and in human body. Likewise, in Wuxing, water, fire, wood, metal, and earth are used as analogies to symbolize the mutual relationships of promotion and restriction. Take, for example, water, in nature it nourishes the wood and restricts fire; in human body it mirrors kidney, promotes liver (wood), and restricts heart (fire). It is through such analogies Wuxing is systematically used to explain human physiology and pathology [1, 2]. Starting with the Yellow Emperor's Canon of Medicine, Wuxing has become the cornerstone of the TCM that has evolved over the millenniums and is still the basis of the TCM, that is, widely practiced today in China and beyond.

There is little doubt in the effectiveness of some of the TCM practices even in the West, as the wide acceptance of acupuncture, for example, attests. It is also widely recognized, however, that due to the historical and cultural differences, the tenants of TCM are often considered rather abstract and vague and are prone to misinterpretations, in the context of Western science. The values of TCM and the desire to understand it in the language of Western science have occupied the minds of scholars of many generations, East and West.

Much work has been done to quantify the TCM using the methodologies of modern science, including those from anatomy, physiology, pathology, pharmacology, biochemistry, cell biology, and molecular biology. Much progress has been made that gives us new insight into the workings of human body, disease, and treatments, all based on the TCM. The limitations of such line of investigation, however,

are also evident in dissecting the holistic TCM using the reductive approach of the modern science. Physiologically and pathologically, a living body is not just a collection of molecules, cells, tissues, and organs, governed, but it is a complex whole governed by the laws of interrelationships among them and between them and their environments Precisely such interrelationships are front and central in the conceptions of the TCM and have been largely beyond the grasp of quantitative studies of the Western science. The missing link, it seems, is the science of interrelationships, to which we now turn.

It is well known in cell biology that a cell, the basic building block of life, constantly communicates with its environment and other cells in what is called cell signaling, which describes the generation, transmission, and reception of biological signals as well as the sequence of actions they trigger. Such "signaling" is commonly seen in engineering systems and is the subject of study in separate engineering disciplines such as signal processing, control, and communication. Such commonalities behind the biological and engineering mechanisms did not escape the mind of the American mathematician Wiener, who in 1948 wrote the landmark book titled "Cybernetics: or Control and Communication in the Animal and the Machine" [3], followed by Tsien's book of "Engineering Cybernetics" in 1954 based on which a whole new engineering science of mutual relationships was born [4].

Unlike other natural sciences of matter, energy, heat, and so forth, cybernetics, according to Tsien, is a general science of "the qualitative aspects of the interrelations among various components of a system and the synthetic behavior of the complete mechanism." Sounds like Wuxing? For example, in Wuxing one is concerned with the balance of the whole system, natural or human; likewise, in cybernetics one is mesmerized by the quality called stability, which is found not in individual components of a system but in how they are connected, or related, to each other. This shared concern by Wuxing and cybernetics on the holistic behavior rather than the material parts of system gives rise to the hope that, perhaps, the ideas of the TCM can be exposited through the language of cybernetics, a basic tenant of this paper. Instead of the five elements, we use the phase the cybernetic model of Wuxing in this paper to emphasize the connection and commonality in Wuxing and cybernetics.

Engineering cybernetics (EC), like the TCM, is guided by the general principles but is also very pragmatic. It aims at, according to Tsien, "those parts of the broad science of cybernetics which have direct engineering applications in designing controlled or guided systems" [4]. After sixty years of furious developments, engineering cybernetics has become a well-established field of scientific study with rigorous mathematical foundation and a set of extremely effective tools to guide the engineering practice.

Similar to the concept of Wuxing and its embodiments in the practice of TCM, EC has as primary goal the establishment and retention of balance in a dynamic system in the presence of internal and external disturbances. A particular example is the principle of active disturbance rejection control (ADRC) and its various embodiments in different domains of engineering [5–7]. The concepts and tools like ADRC give researchers a much needed vocabulary and methodology to study the TCM, where the diseases are caused by internal and external disturbances and the treatments can be generally seen as various means of disturbance rejection.

The human body is both a wonder and a mystery. This paper takes a small step to clarify the complexity by explaining the principle of Wuxing, which is central to the TCM, in the language of EC and ADRC. Interestingly perhaps to the Western scholars, Wuxing gives a holistic view of the complex system of human body, particularly in how the vital systems interact with each other. By understanding such interrelationship, described in the language of EC, the ADRC framework is then borrowed to explain how the disturbance rejection is accomplished in the TCM. The congenial connection between the system concepts and tools of cybernetics and the Ying-Yang and Wuxing in the TCM gives us hope to quantify and standardize the teachings of the TCM on a rigorous scientific foundation. To this end, the paper is organized as follows: the disturbance rejection paradigm in engineering and its connection to the TCM are explored in Section 2; the cybernetic model of Wuxing and disturbance rejection in the context of TCM are discussed in Section 3, followed by a case study in Section 4, using the liver cancer treatment as an example. Finally, some concluding remarks are given in Section 5.

2. Disturbance Rejection in Engineering Cybernetics

Biological organisms are large and complex systems that interact with each other and with the environment. In the context of the TCM, it is the same dynamic balance that one seeks among the organs in the body and between the body and nature in which it resides. Such balance is the foundation of any bio-organisms and it is explained in the vocabulary of the Yin-Yang and Wuxing. It was recognized universally that an organism always possesses internally a self-regulation mechanism, whether it is called the Yin-Yang balance in the East or homeostasis in the West. It is also evident to all that the destruction of such balance, regardless of the causes, invariably leads to the destruction of bodily functions and therefore health. The restoration of such balance is the aim of both Western medicine and the TCM. What makes the communication between the two difficult is the incompatibility in the languages that reflects the incompatibility in the way of thinking. The science of cybernetics offers a new path by which a quantitative study of the TCM can be explored.

2.1. Cybernetics. The engineering practice of control and communication long proceeded Wiener's book of 1948. The name cybernetics was chosen because of Wiener's wish "to recognize that the first significant paper on feedback mechanism is an article on governors, which was published by Clerk Maxwell in 1868 and that governor is derived from a Latin corruption of *κυβερνήτης*" [3]. In cybernetics,

Wiener initiated a new science that was previously in "a no-man's land between the established fields;" it overlaps with "pure mathematics, statistics, electrical engineering, and neurophysiology." By the time the second edition was published in 1961, the field has become "an existing science" and "a whole discipline for the engineer, for the physiologist, for the psychologist, and for the sociologist" [8]. This is because all these various kinds of specialties are concerned with interrelations among components within a system. These interrelations are important in studying and modifying system behaviors, particularly the balanced behaviors that are the most desirable.

The study of interrelations took a drastic turn, with increasingly more significant practicality, after the invention of feedback control in servomechanisms and communication engineering, where interrelations among process variables are modified intentionally to achieve desired system characteristics. Causality is seen as linear in Western science for centuries. But now, with cybernetics, scholars began to contemplate a new kind of causality, that is, "circular," much like Wuxing in the TCM where the key entities in a system, whether in nature or human body, are mutually dependent. But unlike Wuxing and Yin-Yang, the study of cybernetics led to quantitative sciences such as information theory and control theory, which are known for their mathematical rigor and clarity in thinking, the qualities solely lacked in the texts of the TCM.

2.2. Engineering Cybernetics. Our research is therefore largely motivated by combining the methodology of cybernetics and the TCM in order to make the transition in the study of the TCM from qualitative to quantitative, along the line of EC suggested by Tsien six decades ago.

Engineering cybernetics is a branch of engineering science founded by H.S. Tsien in his ground breaking book "Engineering Cybernetics" of 1954. Its aim is "to study those parts of the broad science of cybernetics which have direct engineering applications in designing controlled or guided systems." In EC the study of cybernetics takes a turn from qualitative to quantitative and the book of Tsien "anticipated much of the development after 1954" [9]. After furious developments in the cold war era, fueled by the space and arms race, a great deal of resources have been put into use to further develop the science of cybernetics, giving it a well-established mathematical foundation and the tradition of rigor. Today, feedback control theory, in particular, is highly developed in separate areas of linear and nonlinear system theory, multivariable control, adaptive control, estimating, system identification, and so forth. The paradigm of modeling and model-based control design has firmly established itself as the dominant paradigm in the modern era.

Another line of investigation, completely different from the model-based paradigm, in EC is perhaps a better fit for the study of the TCM. With foresight, Tsien pointed out in 1954 that control design cannot be solely dependent on the accurate mathematical model of the physical systems, because of the complexity and uncertainties involved in trying to capture the system dynamics, which constantly changes during operation. Such challenge is especially daunting in human physiology where accurate information of system dynamics is difficult to obtain and, even when it is obtained, it is unreliable. This leaves with us but one alternative, to which we now turn.

2.3. The Disturbance Rejection Paradigm. The disturbance rejection paradigm in EC originated in the landmark paper of Professor Han of 1989 where he carefully distinguishes the theory of engineering cybernetics from that of modern control theory [10]. He argues that a good control design is possible in the absence of global mathematical model because the information needed can be obtained locally in time and space. Han subsequently spend the next twenty years devising such a new mechanism of control where a simplistic dynamic model is assumed and the rest of dynamics, internal and external, is treated as disturbance to be estimated and cancelled in real time [5, 10]. The new paradigm successfully combined the ancient idea of the South-Pointing Chariot with the modern concepts of state and state observers, leading to the ground breaking principles and technologies of ADRC [6, 7, 11]. ADRC as a disruptive technology has been successfully validated in many industrial applications such as motion control, process control, robotics, automobile, and high energy physics [5, 12–16]. It has also been adopted by the industry giant, Texas Instruments, in a new line of DSP chips.

In ADRC, engineering scientists finally find a powerful weapon to systematically deal with vast amount of uncertainties in the physical world. This is of crucial importance in engineering cybernetics and, by extension, to the study of the TCM, because, as argued in [6], most problems of feedback can be seen as the problem of disturbance rejection. By exploring this common ground behind all dynamic systems, engineering or otherwise, we seek to connect the grand cosmic view and the heuristic practice in the TCM with the rigor and clarity of engineering cybernetics.

In the following section, the problem of the TCM will be explored in the framework of disturbance rejection where the engineering cybernetics approach is applicable.

3. Disturbance Rejection and the TCM

The life science of the East, to which the TCM belongs, exhibits its unique perspective on many fronts: the ecological view of medicine where the inner workings of nature and man mirror each other; the view of life as self-adjusting and self-stabilizing; the view of medical treatment, that is, seasonal, local and individual; and, finally, the goal of maintaining the Yin-Yang balance and the means for its restoration in the treatments of ailments. It is a great challenge to see such a distinct cosmic view through the lenses of modern science where rigorous analysis described in the language of mathematics is the gold standard, modeled after physics. In physics, the laws of natures are couched in the mathematical descriptions in the form of differential equations, known as the mathematical models. Much work has been done in the West to extend such methods of physics to the study of medicine. One such example is the science of system biology

where the tools of systems and control theory, which grew out of cybernetics, are *applied* to study bio-organisms. There are, however, inherent limitations.

The dynamics of bio-organisms are, by nature, complex, uncertain, always in flux, and individually unique. A mathematical model is, at best, a snapshot of a particular organism at a particular time, under a particular set of circumstances. Even so, it is still a rough approximation. It is therefore evident, even beyond doubt, that the modeling and model-based approach to bio-organisms are fundamentally limited. The solution in quantifying the TCM appears to lie elsewhere, as discussed in this section.

3.1. The Cybernetic Model of Wuxing. The TCM is consistent with the unique cosmic view of Yin-Yang and Wuxing. Out of the primordial soup of uniformity, of the one (Yuanqi), of the indistinguishable, came the Yin and Yang for which Wuxing is the further delineation and manifestation, as in nature and as in human body. The TCM, rooted in Wuxing, holds human body as an organic whole that consists of five Zang viscera and six Fu viscera; the *qi*-blood and the bodily fluid; the meridian; the body constituents, sense organs and ortifices; and the limbs and other parts. Each viscus has its own outside signs and is spatially related to certain body constituents and orifices. The five Zang viscera, including the liver, heart, spleen, lung, and kidney, are different in their physiological functions and pathological changes, but they are interrelated, that is, interpromoting, interrestricting,and interaffecting. It is these relationships among the five Zang viscera that are crucial to the working of the human body as a whole.

Physiologically, the TCM further stipulates that the functions of various visceral tissues and organs can be classified into the systems of five Zang organs, respectively, by the analogy to the five elements in Wuxing. Specifically, anchored in the five Zang organs are five distinct physiological systems that are interconnected through the meridian and extended to the six Fu organs of the gallbladder, stomach, small intestine, large intestine, urinary bladder, and Tri-Jiao. These five systems based on the five Zang organs in human body mirror the inner working of nature as seen in the cybernetic model of Wuxing, reflecting the mutuality between human and nature as seen in the Chinese philosophy.

In short, the manifestation of the Wuxing model of nature in human body gives us the five interconnected systems symbolized by the five Zang organs. The five elements in Wuxing may be seen as state variables in some sense; the medical treatments and medicines are control actions; the internal and external anomalies in the human body can be seen as disturbances, and the diagnosis based on the TCM produces the output measurements. And, just like in nature, each of the five systems of human body exerts influences on other systems and, in turn, is influenced by them; they are both controlling and are controlled at the same time, forming an intricate chain of causality that challenges the mind of the best of us. The impact of one system on the other can be seen as promoting (enabling) or restricting (inhibiting), directly or indirectly. In engineering terms, the "feedback" among the five systems can be seen as positive (reinforcing) or negative (limiting) and it is the collective whole of such relationships that determines the Yin-Yang balance in the human body.

When Tsien calls cybernetics a science of interrelations [4], perhaps he is influenced, knowingly or not, by Wuxing. When he described the feedback control of the turboalternator as a way to achieve the energy balance, perhaps he saw the connection of what N. Wiener described as control and communication in the animal and the machine. In any event, perhaps the time has finally come to adopt the thinking of engineering cybernetics of Tsien as a quantitative means to the holistic science of cybernetics and the TCM.

3.2. The Cause of Sickness: The Imbalance of the Body. The science of the causes and effects of diseases, that is, pathology, is the basis of medicine, Eastern or Western. The cybernetic model of Wuxing offers a distinctly different perspective, that is, complementary to the existing model based on modern science. The sickness, in view of Wuxing, is necessarily accompanied by certain imbalance in the body. In other words, the cause of disharmony leads to the ailment which can be seen as an undesirable *state* of the body. In the context of the theory of dynamic systems, the changes in the state are governed by the principle of causality [17], which tells us that the state is determined by the initial value and the external forces known as inputs. It is those harmful external forces, that is, disturbance, which is also known as *xie* or pathogenic *qi* in the TCM, that cause the imbalance in a body and the corresponding ailment, if any. The Western medicine focuses on the material basis of such disturbances, such as bacterial infection or dysfunctions of certain organs; the cybernetic model of Wuxing emphasizes the mutual relationships among the five systems in the body.

The ideal state of a body in the Western medicine is one that is disease-free; in the TCM, however, it is the perfect harmony among the five life systems symbolized by the five Zang organs of liver, heart, spleen, lung, and kidney. In other words, the ideal body in the TCM is one that the promotions and restrictions, or the positive and negative feedbacks, among the five life systems are perfectly balanced. The disturbances are powerless against such bodies. Such bodies may be seen in the Western medicine as having strong immunity against diseases, but it is the cybernetic model of Wuxing that lays the foundation for further scientific study and medical practice. Perhaps this is a place where the TCM and the Western medicine find the common goal, amid different philosophical orientations.

The imbalance in the body, according to Wuxing, is caused by the anomalies in the relationships, both promotional and restrictive. Those happened in the former are called mother-child involvement (MCI), where those who promote and those who are promoted are mutually affected; those happened in the latter are known as subjugation and violation. Subjugation refers to an abnormal condition in which one of the five elements excessively restricts another element; violation refers to an abnormal condition in which one of the five elements reversely restrains and bullies the

element that normally restricts it. Such anomalies caused by various small disturbances are not common, nor are they serious because, in the view of Wuxing, the mutual relationships among all five systems are self-balancing and self-repairing. In such cases, no external intervention, that is, adjustment or treatment, is needed.

In the presence of large and sustained disturbances, however, some or all of the five systems will be weakened and self-restoration becomes increasingly difficult. Li [18] from the Ming dynasty points out that "when the disease causing agent (*xie*) first entered the body, the positive *qi* is still strong and its opposite is weak, and the proper strategy is to attack the *xie*; if the *xie* persists and its effect deepens, the positive *qi* declines and the proper strategy is to both attack the *xie* and strengthen the body; finally, if and when the disease reaches the final stage where *xie* is ubiquitous and the positive *qi* is exhausted, the only remedy left is to invigorate the body. Therefore, the problem of ailment is reduced to that of balance, which is then linked to the problem of disturbance. It is here that the engineering principle of disturbance rejection can be used to clarify the principle and practice of the TCM.

3.3. Active Disturbance Rejection. The connection illustrated above between the TCM and the engineering principle of disturbance rejection makes relevant many engineering methods developed over the last few decades. In this section, a particular method of ADRC is used as an illustration.

Human body can be seen, in engineering terms, as a dynamic system, that is, bombarded constantly by disturbances of various kinds. Treatment, as external intervention, is deemed necessary when the body is out of balance and enters into an undesirable state, manifested in illness. The reduction or elimination of deviations from the ideal state is the common goal in medicine and in engineering.

ADRC, as introduced earlier, is a unique engineering solution to disturbance problem in that it seeks to actively reject the disturbance, as oppose to passively respond to its consequence. Its principle is strikingly similar to the basic tenant of the TCM: the supreme doctor treats the disease, that is, yet to occur. That is, a doctor with superior skills and understanding of the TCM will be able to detect and treat the subtle imbalances before they manifest themselves as ailments. The practice of the TCM medical acts as an outer loop to the control system, that is, already there in the body, helping the body to go back to the equilibrium through external assistance. In ADRC, such imbalance is detected early using the tools of the extended state observer, which extracts the disturbance information from the input-output data of the physical process. In the TCM, such information is obtained through four kinds of diagnosis means: watching, smelling, interrogating, and feeling of the pulse. In both ADRC and the TCM, the emphasis is on seeing early the cause of imbalance so as to minimize its effect. In other words, the disturbance rejection paradigm and its product in ADRC are the engineering equivalent of disease prevention and strengthening of the autoimmune system in human body.

In the cybernetic model of Wuxing and ADRC, we found a language to articulate the principles and methods of the TCM. The full exposition of it is of course beyond the scope of a single paper, but the main idea can be seen with an illustrative example, as shown below. It concerns with the treatment of liver cancer based on the interrelationships between the liver system and other systems of Wuxing. In particular, the promotion and restriction relationships around liver system provide the guideline for medical intervention, the effectiveness of which is demonstrated.

4. Case Study

In this section we demonstrate, by example, how the cybernetic model of Wuxing can be used to establish the therapeutic principles and methods in the treatment of certain liver cancer. A disease in any Zang organ, according to Wuxing, will propagate up and downstream in both the promotional and restrictive relationships to other organs, causing imbalance in this interconnected system of Wuxing. The treatment, therefore, must be holistic and not limited to the dysfunctional organ.

In particular, among the promotional relationships are the therapeutic principles of tonifying the mother and letting go of the child: the former rectifies the deficiency in the mother-child relationship in Wuxing and the latter reduces the excess. Typical treatments in the promotional relationships include replenishing water to nourish wood, mutual promotion of metal and water, reinforcing earth to strengthen metal, and fueling fire to strengthen earth. The corresponding material means will be explained shortly in the example.

Furthermore, among the restrictive relationships are the therapeutic principles of checking the strong and strengthening the weak: the former is a therapeutic principle to restrict the hyperactive viscus so as to benefit the recovery of the subjugated or violated viscus; the latter is to enrich the subjugated or violated viscus so as to balance the strengths of both sides. This applies to the case where the power for restriction becomes weak due to subjugation or violation. Typical treatments in the restrictive relationships include inhibiting wood to assist earth, banking up earth to treat water, assisting metal to subdue wood, reducing the south, and tonifying the north.

The application of these therapeutic principles and methods based on the cybernetic model of Wusing is shown below in an example based on the results from a sequence of liver cancer treatment studies.

4.1. Liver Cancer: Pathogenic Factors and Pathogenesis. Pathogenic factors of liver cancer are complex, in view of the TCM, and they are associated with stagnation of *qi* from sustained low mood and the lack of adequate flow; the dysfunction of the Zang and Fu organs resulting in inadequate cleansing of the turbidity; and the inadequate flow of *qi* and blood, leading to accumulation syndrome, that is, fiery and poisonous. Therefore, the TCM concludes the liver cancer is a disease of amassing in the abdomen, of tympanites, and of jaundice, and so forth.

As the liver encounters the pathogenic *qi*, it rises up and attacks. Both the healthy and the pathogenic *qi* are exuberant

at this stage and their struggle is violent and sustained. As the pathogenesis develops, the pathogenic *qi* takes hold and the healthy *qi* degrades, leading to the dysfunction of the liver and the symptoms of difficulty of dispersing, *qi* stagnation, phlegm obstruction, improper *qi* accumulation, lack of blood movement, and the gathering syndrome. As the pathogenic *qi* becomes increasingly stronger, the disease heightens, so does the loss of the healthy *qi*, until it is mostly gone, along with any resistance to the disease, which finally reaches the terminal stage.

In view of the cybernetic model of Wuxing, a human body is a dynamic system with mutually dependent five Zang systems. The above process of pathogenesis can be understood in the framework of disturbance rejection: the liver system is affected by disturbances and loses balance; the disturbance is compensated by the use of healthy *qi* which has limited supply. When such imbalance is not restored quickly, due to the severity of the disturbance and the weakened state of the body, the liver system runs out of energy and the disturbance rejection ability. At this point, only the external intervention, based on the understanding of the cybernetic model of Wuxing, can help the system recover its balance. The question is what Wuxing can tell us.

Wuxing tells us that even though the cancer is located at the liver, the treatment must include those organs that are mutually dependent on it, which in this case are the spleen and the kidney systems. The spleen has the restrictive and the kidney has the promotional relationships with the liver, respectively. The contents of these complicated relationships are explained as follows.

The cause of the liver cancer and its development are quite complicated. It could be the liver itself or the complications from other mutually dependent organs. The pathogenesis centers on the dysfunction of the liver, and it initially leads to the *qi* stagnation, ecchymosis, and fluid retention, and the interplay of the three takes hold in the liver and manifests itself in a variety of symptoms. Gradually, this condition propagates to the spleen and kidney and causes the spleen deficiency and the kidney impairment, while the liver itself loses the function of blood storage. At the later stage, the pathogenesis propagates to the heart system, leading to sudden unconsciousness, convulsive syncope, and hemorrhage [19, 20]. Based on such understanding of pathogenesis rooted in Wuxing, the treatment is devised accordingly, similar to the principle of active disturbance rejection in engineering [16].

4.2. The Wuxing-Based Therapeutic Approach. The cybernetic model of Wuxing provides the guideline, the principles, and the methods of a unique treatment of liver cancer. Specifically, the following herb medicines are used: the Jiang Huang (Rhizoma *Curcumae longae*) that enters the liver meridians and activates the blood and moves the *qi* and the Huang Qin (*Radix Scutellariae*) that enters the meridians of the lung, the gallbladder, and the large intestine for its functions of removing heat and dry dampness and purging the fire and toxicity. The main ingredient of Huang Qin is baicalin.

Because, according to Wuxing, the lung has the property of being mental and it restricts the liver. Lessening such restriction on the liver can be accomplished by using Huang Qin to first relieve lung and to dampen it a bit, as a precaution. That is, the Wuxing foretells the weakening of the liver by the restriction from the lung and this can be countered beforehand by first relieving the lung.

Heart, on the other hand, has the property of fire and is promoted by the liver. The dysfunction of the liver will likely to cause excessive fire and this can be, again, countered beforehand using the herbal medicine of Mu Dan Pi (Cortex Moutan), of which the main ingredient is Paeonol. It enters into the meridians of the heart, the liver, and the kidney, removing the heat, cooling and activating the blood, and resolving the stasis. Chuan Xiong (Rhizoma Chuanxiong) is another useful herbal medicine with the main ingredient of tetramethylpyrazine, which enters the meridians of the liver and the pericardium with the functions of activating the blood, moving the *qi*, suppressing the wind, and alleviating the pain. The preventive use of these two herbs is guided by the Wuxing's mother- (liver) child (heart) relationship discussed above. In anticipation of the liver's problematic behavior, the heart is first relieved before the excessive fiery effect from the liver arrives. In other words, to remove heat/fire generated by a malfunction liver, both the liver and the downstream heart are treated by the blood activating and *qi* moving medicine for better transport of the heat and fire.

The same principle of Wuxing and disturbance rejection also applies to spleen which is restricted by the liver. At the initial stage of the disease when the liver's activities are excessive, the spleen will likely be exceedingly suppressed. To counter such disturbance, reinforcement should be applied, again, beforehand, such as the use of Shan Yao (*Rhizoma dioscoreae*), which enters into the spleen, lung, and kidney meridians; fortifies the spleen and stomach; promotes the production of bodily fluid to nourish the lung; and strengthens the kidney to arrest spermatorrhea.

Note that the promotion of the liver and strengthening of the spleen can be carried out simultaneously by using the combination of Huang Qin and Shanyao.

Finally we arrive at the kidney, the most important organ in Wuxing, which has the property of water and promotes the liver. The corresponding treatment method is called replenishing the water to nourish the wood, using the herbal medicine of Nv Zhen Zi (Fructus Ligustri Lucidi), which enters the meridians liver and kidney and nourishes them. The second herbal medicine is Gou Qi Zi (*Fructus lycii*) which also enters the meridians of the liver and kidney and nourishes them. Both medicines also have the benefits of promoting eye sight, which is closely related to the health of the liver. Because of the mother-child relationship between the kidney and the liver, the Yang of the liver is astringed by enhancing the kidney.

4.3. Results from the Experimental Studies. A series of experimental studies has been carried out in the last six years to systematically test the therapeutic treatment based on the cybernetic model of Wuxing and the engineering concept of

disturbance rejection. These experiments are designed to test if the results agree with the theory, using the herbal medicine discussed above, including curcumin, ursolic acid, baicalin, paeonol, tetramethylpyrazine, as well as the combination of curcumin and *Rhizoma dioscoreae*, and the combination of ursolic acid and *Fructus lycii*. The treatments were applied to the precancerous lesion of mice liver, induced by using the diethylnitrosamine (DEN) [21–24], a standard method.

The effects of the curcumin and *Rhizoma dioscoreae* combination were first examined by the liver histological analysis and activities of serum marker enzymes. The experimental results show that DEN initiation led to a remarkable increase of serum marker enzymes, and abnormality such as bile canaliculi hyperplasia and presence of tumor cells were observed in liver histopathological examination in the model mice, while the control ones revealed the normal architecture.

Oral treatment of curcumin and the treatment of the curcumin and *Rhizoma dioscoreae* combination resulted in a marked reduction in serum marker enzymes and improvement in liver histopathology compared with the model ones. The conclusion was that curcumin can protect the hepatic precancerous lesions in the mice attacked by diethylnitrosamine, and combination use of curcumin and *Rhizoma dioscoreae* can be more effective [16, 25]. This shows the effectiveness of the method of inhibiting the wood and replenishing the earth discussed above. Moreover, it demonstrates that treating the liver and, at the same time, nourishing the spleen greatly enhanced the treatment, just as the cybernetic model of Wuxing and the active disturbance rejection principle suggested.

Similarly, the method of nourishing both liver and kidney is also put to test by using the ursolic acid from *Fructus ligustri lucidi* and by combining ursolic acid with *Fructus lycii*. Results show that both treatments lead to improvements but the latter is better [26]. This confirms the method of replenishing the water to nourish the wood, based on the Wuxing and the disturbance rejection principles.

Later experiments show that tetramethylpyrazine, paeonol, and baicalin were partially effective to protect liver from the carcinogenesis initiated by DEN; tetramethylpyrazine and paeonol both activate the blood and play the role of relieving the child in the cybernetic model of Wuxing. The readers are referred to [27–29] for more detailed results and other related studies.

In summary, the pathogenesis of the liver cancer is complex because of the mutually dependent relations among all five Zang organs. The treatment of the liver must be accompanied by the treatment of other organs according to the cybernetic model of Wuxing and the principle of disturbance rejection.

5. Conclusions

In this paper we attempt to establish a framework for the study of the TCM where a new language of engineering cybernetics is introduced to delineate, with clarity, the principles and practice of the TCM. In engineering cybernetic we find a holistic view, a science of interrelations, with strong resemblance to what the Wuxing attempts to do: describing the inner workings of nature and human body alike as mutually connected components, that is, closed-loop dynamic systems. EC and the TCM also share a commitment and goal to attain and maintain a quality, that is, described as balance, equilibrium, or homeostasis; correspondingly there is a great similarity in how such quality is maintained, by what is called "disturbance rejection" in EC. The engineering invention of active disturbance rejection shares the common goal with the TCM in anticipating and rejecting the disturbances before the balance is disrupted. Combining the vocabulary of EC and the TCM we found the cybernetic model of Wuxing as the common language and a promising new path towards the quantitative study of the TCM. As an initial study and illustration, the pathogenesis and treatment of the liver cancer based on the TCM are explained using the language of the cybernetic model of Wuxing and the principles of disturbance rejection. The results from a six-year study seem to support this line of investigation and to provide a clear exposition of both the principles of the TCM and how they are practical with herbal medicine.

Human body is a complex, large, and open system that defies our complete grasp. It has always been a great challenge to organically combine the best offerings from the Western medicine and the Eastern medicine, largely due to the lack of common language. In EC we find the language of a holistic science for the West, that is, inherently congenial to the Eastern thinking in Yin-Yang and Wuxing. It is our hope that what started here could help future researchers to build the bridge from the general system qualities and behaviors to the properties of the system components from the qualitative to the quantitative and from the abstract concepts to the concrete practice.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgment

This study was supported by Grant no. 2011-CXTD-04 from Beijing University of Chinese Medicine innovation team project.

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Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2014, Article ID 472638, 11 pages
http://dx.doi.org/10.1155/2014/472638

Research Article

Supplementation with the Extract of Schisandrae Fructus Pulp, Seed, or Their Combination Influences the Metabolism of Lipids and Glucose in Mice Fed with Normal and Hypercholesterolemic Diet

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Received 16 February 2014; Revised 17 March 2014; Accepted 17 March 2014; Published 30 April 2014

Academic Editor: Gerhard Litscher

Schisandrae Fructus (SF), which possesses five tastes: sweet (fruit skin), sour (pulp), bitter/pungent (seed core), and saltiness (all parts), can produce a wide spectrum of biological activities in the body. Here, we investigated the effects of the ethanolic extract of SF pulp, seed, or their combination (namely, EtSF-P, EtSF-S, or EtSF-P/S, resp.; collectively called EtSF) on the metabolism of lipids and glucose in normal diet- (ND-) and hypercholesterolemic diet- (HCLD-) fed mice. Supplementation with EtSF significantly reduced hepatic triglyceride and cholesterol levels by 18–47% in both ND- and HCLD-fed mice. EtSF supplementation reduced serum triglyceride levels (approximately 29%), whereas EtSF-P and EtSF-S/P elevated serum cholesterol (up to 26 and 44%, resp.) in HCLD-fed mice. Treatment with EtSF decreased hepatic glucose levels (by 9–44%) in both ND- and HCLD-fed mice. Supplementation with EtSF-S or EtSF-S/P (at 1 and 3%) increased biliary or fecal TC contents in HCLD-fed mice. However, supplementation with EtSF-S/P at 9% reduced biliary TC levels in HCLD-fed mice. EtSF-P or EtSF-S/P supplementation reduced serum alanine aminotransferase activity in HCLD-fed mice. The findings suggested that supplementation with EtSF lowered lipid and glucose accumulation in the liver and increased fecal cholesterol contents in mice. Dietary supplementation with EtSF-P or EtSF-S/P attenuated liver damage in HCLD-fed mice.

1. Introduction

Hyperlipidemia (HLD) refers to increased levels of lipids in the blood, including cholesterol and triglyceride. It is well known that HLD, the leading cause of death and disability over the world, significantly increases the risk of cardiovascular diseases, nonalcoholic fatty liver disease (NAFLD), metabolic syndrome, stroke, or cerebrovascular accident [1–3]. NAFLD, which is the most common liver disease in western countries and with a clinical manifestation of steatosis and nonalcoholic steatohepatitis, is also recognized as a cause of cryptogenic cirrhosis and hepatocellular carcinoma [4]. A recent study has shown that the incidence of highly differentiated colorectal adenocarcinoma in NAFLD group is significantly higher than that in the control group [5]. In addition, NAFLD is associated with increased risk of hypertension [6], higher incidence of type 2 diabetes [7], and high levels of serum uric acid [8].

Since the incidences of HLD and NAFLD are increasing rapidly, it is of therapeutic interest to search for effective agents that can lower lipid contents in the blood and liver [9]. Although much effort has been put to develop drugs used for the prevention and treatment of HLD-related diseases, effective drugs for treating HLD, especially NAFLD, are

yet to be discovered. In addition, synthetic lipid-lowering drugs have many potential adverse/side effects, such as muscle tenderness [10, 11], renal failure [12], and others, including headache, bowel upset, nausea, sleep disturbances, and hepatomegaly [13, 14]. In recent years, people prefer to use natural remedy such as dietary supplement/functional food for the prevention and treatment of NAFLD and/or lipid disorders [15, 16].

Schisandrae Fructus (SF, Wu-Wei-Zi in Chinese) has been used for thousands of years as a "superior" drug in the practice of Chinese medicine in China. Previous studies have shown that SF and its related chemical components possess a wide spectrum of biological activities such as antioxidation, antitumor [17, 18], hepatoprotection against chemically and virally induced hepatic injury [19], antifatigue, immunostimulation, and antiaging [20]. A recent study has demonstrated that SF extract can prevent ethanol-induced fatty liver, possibly through activation of AMPK (AMP kinase) and peroxisome proliferator-activated receptor α (PPARα) signaling pathway [21]. Gomisin N, a diastereomer of schisandrin B (Sch B, a major active ingredient of SF), inhibited DNA damage checkpoint signaling by stereospecifically interacting with ataxia telangiectasia and Rad-3-related (ATR) protein kinase [22]. Our previous studies have shown that SF extracts [23, 24], Sch B [25], bicycol [26], and bifendate [27] can reduce hepatic triglyceride (TG) and total cholesterol (TC) levels in hypercholesterolemic (HCL) mice. In the present study, we endeavored to compare the effects of ethanol extracts of SF pulp, seed, or a mixture of pulp/seed on serum and hepatic lipid/glucose (GLU) levels, as well as liver function in mice under the normal and HCL conditions. Fenofibrate (FF) was used as a positive control for comparison.

2. Materials and Methods

2.1. Herbal Material and Extraction Procedure. SF, which is the fruit of *Schisandra chinensis* (Turcz.) Baillon (Bei-Wu-Wei-Zi in Chinese), was purchased from the Anguo Chinese herbs market in Hebei province, China, and authenticated by Professor Chun-Sheng Liu at the Beijing University of Chinese Medicine. The fruit pulp and seed were manually separated and then dried at room temperature. The weight of pulp and seed was 63 and 37% of total weight, respectively. For the preparation of SF extracts, SF pulp, seed, or both were crushed into small pieces using an industrial grinder and extracted twice (first, 1.5 h; second, 2 h) with 5 volumes of 80% (v/v, in H_2O) ethanol under reflux after soaking for half an hour. The pooled extract was filtered by filter paper and concentrated by rotary evaporation to obtain the SF seed ethanolic extract (EtSF-S), SF pulp ethanolic extract (EtSF-P), and their combination extracts (EtSF-S/P). The extracts were stored at 4°C until use.

2.2. Chemicals and Regents. Cholesterol (certificate number 20120614) and bile salt (certificate number 20121210) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). FF (certificate number 18246) was obtained from Beijing Yongkang Medical Ltd. (Beijing, China). Assay kits for TC, TG, and GLU were bought from Zhongsheng Beikong Biotechnology and Science Inc. (Beijing, China). Assay kits for high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), and alanine aminotransferase (ALT) were purchased from Beijing Leadman Biochemistry Co., Ltd. (Beijing, China).

2.3. Animal and Treatment. All experimental procedures were approved by the University Committee on Research Practice at Beijing University of Chinese Medicine. Male ICR mice (grade II, certificate number SCXK (jing) 2012-0001), weighing 18–20 g, were supplied by Vital River Lab Animal Co. Ltd. (Beijing, China). All animals were maintained on a 12 h (light on 700–1900 h) light-dark cycle at 20–21°C, with a relative humidity of 50–55%. They were allowed for free access to water and food.

2.4. Experimental Design

2.4.1. Design 1. In this study, the effects of dietary supplementation with SF extracts or FF on lipids, GLU, and liver were investigated in normal mice. Animals were divided into four groups of 10 animals in each: (1) mice fed with normal diet (ND); (2) and (3) mice fed with diet supplemented with 1% and 9% SF extracts (w/w), respectively; (4) mice fed with diet supplemented with 0.1% FF. After 10 days, mice were sacrificed under light ether anesthesia. Blood, collected from the orbital vein, and liver tissue samples were obtained and subjected to biochemical analysis.

2.4.2. Design 2. This study was designed to investigate the effects of SF extracts on serum and hepatic parameters in mice fed with HCL diet (HCLD) containing cholesterol/bill salt (1/0.3%, w/w). Mice were randomly divided into six groups (10 in each group): (1) mice fed with ND; (2) mice fed with HCLD; (3), (4), and (5) mice fed with HCLD supplemented with 1, 3, and 9% EtSF-S, EtSF-P, or EtSF-S/P, respectively; (6) mice fed with HCLD supplemented with 0.1% FF. Ten days later, animals were sacrificed and blood/liver tissue samples were collected for biochemical analysis. Figure 1 shows the design of the present study.

2.5. Serum and Hepatic Biochemical Analysis. Serum samples were prepared by centrifuging the clotted blood for 8 min at 2000 ×g and stored at −20°C until used for biochemical analysis. Liver tissue samples were homogenized in 9 volumes of 0.9% (w/v) NaCl solution by two 10 s bursts of a tissue disintegrator at 13,500 rpm and then centrifuged at 2000 ×g for 15 min to obtain the supernatants. Ten μL of serum and 40 μL of the hepatic supernatant were used to determine the TG and TC levels with GPO-PAP and COD-PAP methods, respectively. Ten μL serum and 5 μL hepatic supernatant were used to determine the GLU levels with GOD-POD method. Serum HDL and LDL levels, as well as ALT activity, were determined using automatic biochemistry analyzer (Beckman Coulter Synchron CX4 PRO.Brea, CA, USA).

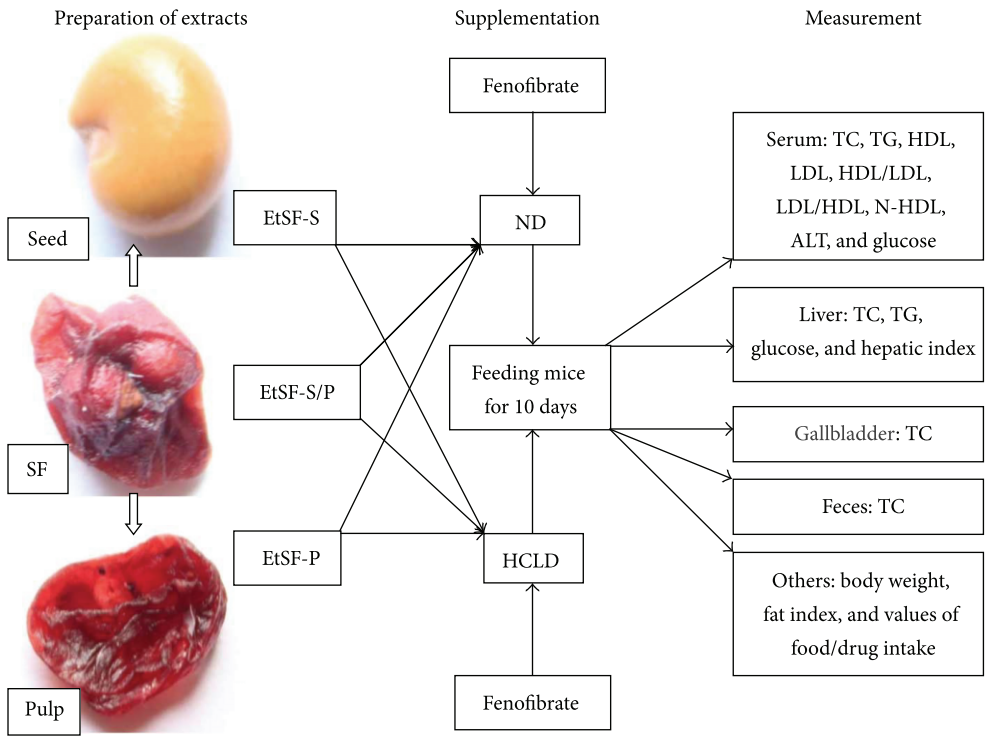

Figure 1: The design of the current study. SF: Schisandra Fructus; EtSF-S: ethanolic extract of SF seed; EtSF-P: ethanolic extract of SF pulp; EtSF-S/P: ethanolic extract of SF seed/pulp; TC: total cholesterol; TG: triglyceride; LDL: low-density lipoprotein; HDL: high-density lipoprotein; N-HDL: non-HDL; ALT: alanine aminotransferase; HCLD: hypercholesterolemic diet; ND: normal diet.

2.6. Measurement of Biliary and Fecal TC Contents. Mouse gallbladder was removed from the liver and soaked in 1 mL TC reagent for 16 h. Then TC concentrations (μmol/gallbladder) were measured using the method described above. For the determination of TC contents in feces, mouse feces were collected and dried at room temperature. Dried feces (approximately 30 mg) were extracted with 0.5 mL chloroform-methanol (1 : 1, v/v) mixture for 12 h and then centrifuged at 2000 ×g for 5 min to obtain the supernatants. Thirty μL fecal supernatants were used to measure the TC levels (μmol/g feces) using assay kit.

2.7. Measurement of Hepatic Index. Body and liver weights were measured. Hepatic index was estimated from the ratio of total liver weight to body weight (liver weight/body weight × 100).

2.8. Statistical Analysis. Values given are means ± SEM. Data were analyzed by one-way ANOVA using SPSS statistical analysis program and then differences among means were analyzed by Dunnett's multiple comparisons test or post hoc analysis. $P < 0.05$ was considered significant.

3. Results

3.1. Effects of EtSF Supplementation on Serum Lipid Profiles. As shown in Table 1, daily supplementation with EtSF (i.e., EtSF-S, EtSF-P, and EtSF-S/P) did not affect serum TC, TG, and HDL levels in mice fed with ND. However, both EtSF-P and EtSF-S/P supplementation markedly increased serum HDL and LDL levels (up to 15–47% and 14–73%, resp.) in ND- and HCLD-fed mice. All 3 tested EtSF extracts decreased serum TG levels (up to 25%) in HCLD-fed mice, but EtSF-P and EtSF-S/P markedly increased serum TC levels (approximately 26 and 44%, resp.) in mice fed with HCLD. Feeding mice with HCLD markedly increased serum TC, LDL, and N-HDL levels, as well as LDL/HDL ratio. HCLD decreased serum TG level (up to 60%) and HDL/LDL ratio, when compared with ND-fed mice. EtSF-S supplementation did not affect serum HDL/LDL and LDL/HDL ratios, but decreased N-HDL level in ND-fed mice. EtSF-P and EtSF-S/P supplementation decreased serum HDL/LDL ratio and increased LDL/HDL ratio and N-HDL levels in both ND-fed and HCBD-fed mice. FF supplementation reduced serum TC (32%), TG (52%), HDL (34%), or HDL/LDL ratio but increased LDL/HDL ratio, in normal mice. Serum TC, TG, LDL, and N-HDL levels were reduced by 29, 38, 66, and 69%, respectively, in mice fed with FF-supplemented HCLD diet, when compared with those fed with HCLD only. Moreover, FF elevated serum HDL/LDL ratio but decreased LDL/HDL ratio in HCLD-fed mice.

3.2. Effects of EtSF Supplementation on Hepatic Lipid/Glucose Levels. Supplementation with EtSF-S, EtSF-P, or EtSF-S/P decreased hepatic TC and TG contents (up to 47%) in ND-fed mice. Feeding mice with HCLD markedly increased hepatic TC and TG contents (up to 447 and 402%, resp.), when

Table 1: Effects of EtSF supplementation on serum lipid profiles in normal and HCL mice.

Groups	Drug (%)	TC (mmol/L)	TG (mmol/L)	HDL (mmol/L)	LDL (mmol/L)	HDL/LDL	LDL/HDL	N-HDL (mmol/L)
ND-fed mice								
ND	—	3.39 ± 0.10	1.24 ± 0.06	3.57 ± 0.12	0.44 ± 0.02	8.18 ± 0.35	0.13 ± 0.01	0.65 ± 0.02
ND/EtSF-S	1	3.37 ± 0.14	1.29 ± 0.08	3.66 ± 0.19	0.45 ± 0.02	8.27 ± 0.57	0.13 ± 0.01	0.53 ± 0.05*
	9	3.25 ± 0.12	1.05 ± 0.15	3.58 ± 0.14	0.45 ± 0.03	8.07 ± 0.43	0.13 ± 0.01	0.46 ± 0.05**
ND/FF	0.1	2.32 ± 0.10**	0.60 ± 0.03**	2.36 ± 0.14**	0.40 ± 0.03	6.12 ± 0.38**	0.17 ± 0.01**	0.68 ± 0.03
ND	—	4.23 ± 0.25	1.81 ± 0.05	3.64 ± 0.18	0.47 ± 0.03	7.90 ± 0.38	0.13 ± 0.01	0.71 ± 0.12
ND/EtSF-P	1	4.28 ± 0.16	1.81 ± 0.06	3.66 ± 0.17	0.49 ± 0.03	7.62 ± 0.42	0.14 ± 0.01	0.71 ± 0.09
	9	4.44 ± 0.19	1.78 ± 0.17	3.72 ± 0.14	0.62 ± 0.05*	6.25 ± 0.57*	0.17 ± 0.02*	0.83 ± 0.09
ND	—	4.28 ± 0.14	1.82 ± 0.18	3.67 ± 0.16	0.59 ± 0.04	6.44 ± 0.42	0.16 ± 0.01	0.61 ± 0.04
ND/EtSF-S/P	1	4.31 ± 0.17	1.93 ± 0.19	3.52 ± 0.14	0.78 ± 0.04**	4.62 ± 0.20**	0.22 ± 0.01**	0.78 ± 0.06*
	9	4.67 ± 0.15	2.09 ± 0.11	4.09 ± 0.18	0.74 ± 0.03**	5.52 ± 0.12*	0.18 ± 0.004	0.58 ± 0.05
HCLD-fed mice								
ND	—	4.15 ± 0.16	1.79 ± 0.10	3.70 ± 0.19	0.56 ± 0.04	6.71 ± 0.33	0.15 ± 0.01	0.45 ± 0.05
HCLD	—	5.59 ± 0.27**	1.24 ± 0.06**	3.60 ± 0.17	2.21 ± 0.12**	1.64 ± 0.06**	0.62 ± 0.02**	1.99 ± 0.15**
HCLD/EtSF-S	1	5.74 ± 0.23	0.97 ± 0.05††	3.57 ± 0.09	2.38 ± 0.13	1.52 ± 0.06	0.67 ± 0.03	2.18 ± 0.17
	3	5.68 ± 0.14	1.08 ± 0.05	3.70 ± 0.08	2.23 ± 0.08	1.67 ± 0.05	0.60 ± 0.02	1.98 ± 0.10
	9	5.68 ± 0.16	0.96 ± 0.07††	3.68 ± 0.09	2.31 ± 0.13	1.63 ± 0.08	0.63 ± 0.03	2.00 ± 0.12
HCLD/FF	0.1	3.95 ± 0.22††	0.77 ± 0.04††	3.34 ± 0.23	0.76 ± 0.07††	4.62 ± 0.31††	0.23 ± 0.02††	0.61 ± 0.06††
ND	—	4.23 ± 0.25	1.81 ± 0.05	3.64 ± 0.18	0.47 ± 0.03	7.90 ± 0.38	0.13 ± 0.01	0.71 ± 0.12
HCLD	—	5.06 ± 0.23*	1.01 ± 0.06**	3.22 ± 0.11	1.55 ± 0.05**	2.05 ± 0.11**	0.50 ± 0.02**	1.95 ± 0.15**
HCLD/EtSF-P	1	4.98 ± 0.15	1.04 ± 0.08	3.23 ± 0.09	1.61 ± 0.07	2.03 ± 0.08	0.50 ± 0.02	1.75 ± 0.13
	3	5.61 ± 0.20	0.92 ± 0.08	3.60 ± 0.14	1.74 ± 0.09	2.03 ± 0.09	0.50 ± 0.02	2.01 ± 0.10
	9	6.38 ± 0.20††	0.76 ± 0.06††	3.70 ± 0.14†	2.28 ± 0.09††	1.59 ± 0.07††	0.64 ± 0.03††	2.69 ± 0.13††
ND	—	4.28 ± 0.14	1.82 ± 0.18	3.67 ± 0.16	0.59 ± 0.04	6.44 ± 0.42	0.16 ± 0.01	0.61 ± 0.04
HCLD	—	5.10 ± 0.18**	0.73 ± 0.06**	3.74 ± 0.13	1.97 ± 0.11**	1.95 ± 0.12**	0.53 ± 0.04**	1.35 ± 0.13**
HCLD/EtSF-S/P	1	6.40 ± 0.16††	0.69 ± 0.07	4.26 ± 0.09††	2.88 ± 0.11††	1.50 ± 0.07††	0.68 ± 0.03††	2.14 ± 0.12††
	3	6.34 ± 0.31††	0.57 ± 0.04†	4.01 ± 0.19	2.80 ± 0.16††	1.45 ± 0.07††	0.70 ± 0.03††	2.33 ± 0.18††
	9	7.32 ± 0.23††	0.60 ± 0.05	4.38 ± 0.12††	3.41 ± 0.16††	1.30 ± 0.06††	0.78 ± 0.04††	2.94 ± 0.20††

Mice were fed with normal diet (ND) or hypercholesterolemic diet (HCLD) without and with the ethanolic extract of Schisandrae Fructus (SF) pulp, seed, or their combination (namely, EtSF-P, EtSF-S, and EtSF-P/S, resp.) and fenofibrate (FF) at the indicated doses (%, w/w), which was estimated on the basis of crude herbal material, for 10 days. Then serum total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and non-HDL (N-HDL) levels, as well as HDL/LDL and LDL/HDL ratios, were measured. HCLD was constituted of 1% cholesterol and 0.3% bile salt (w/w). Values given are the means ± SEM, with $n = 10$. $^{*}P < 0.05$, $^{**}P < 0.01$ versus ND; $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$ versus HCLD. Statistical significant differences were determined using a one-way ANOVA followed by Dunnett's multiple comparisons test or post hoc analysis.

compared with those of mice fed with ND. Supplementation with EtSF-S, EtSF-P, or EtSF-S/P reduced the hepatic TC and TG contents by 18–37% and 23–30%, respectively, in HCL mice. FF supplementation lowered hepatic TC/TG contents by 64/49 and 81/55% in both normal and HCL mice, respectively (Figures 2(a) and 2(b)). Dietary supplementation with 3 tested SF extracts and FF reduced hepatic GLU contents by 10/44% and 58/44% in ND-/HCLD-fed mice, respectively (Figure 2(c)).

3.3. Effects of EtSF Supplementation on Biliary and Fecal Cholesterol Contents. EtSF-S and 1% EtSF-S/P, but not EtSF-P, supplementation increased biliary TC concentrations in HCLD-fed mice (up to 136 and 60%, resp.). However, supplementation with 9% EtSF-S/P reduced biliary TC by 30% in HCL mice (Figure 3(a)). Feeding mice with 9% EtSF-P and EtSF-S/P elevated the fecal cholesterol excretion (by 21 and 62%, resp.) (Figure 3(b)).

3.4. Effects of EtSF Supplementation on Hepatic Index and Function. Feeding mice with EtSF-S/P or HCLD increased hepatic index by 10 or 18%, respectively, when compared with control ND group. FF increased hepatic index by 95 and 79%, respectively, in normal and HCL mice, respectively (Figure 4(a)). EtSF did not alter the serum ALT activity in normal mice, but EtSF-P and EtSF-S/P lowered the ALT activity (33 and 24% decrease, resp.) in HCL mice. FF supplementation significantly elevated serum ALT activity by 209 and 650%, respectively, in ND- and HCLD-fed mice (Figure 4(b)).

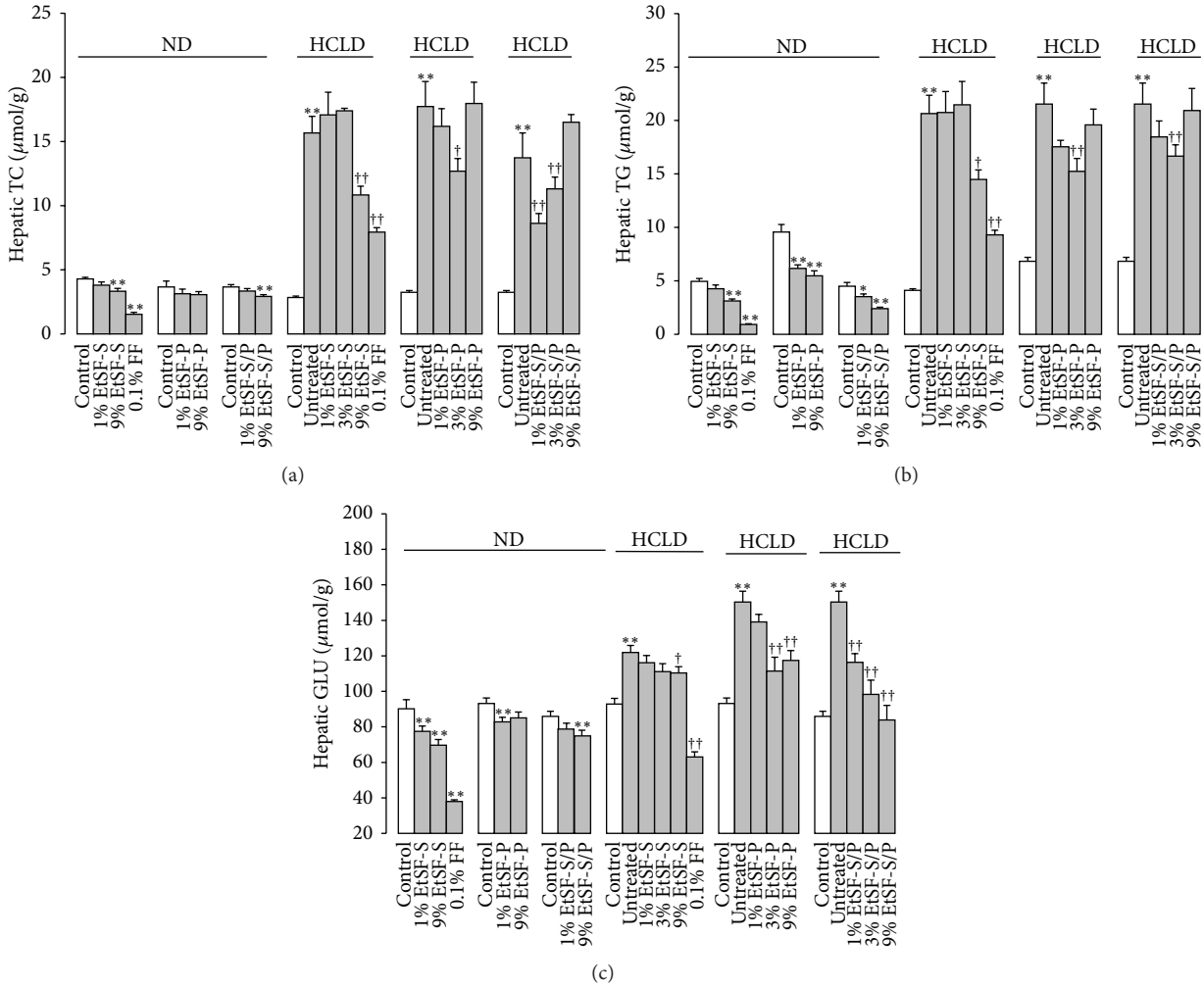

Figure 2: Effects of EtSF supplementation on hepatic lipid/glucose contents in normal and HCL mice. Experimental details were described in Table 1. Mice were fed with ND and HCLD without or with EtSF or FF supplementation, as indicated in the figure. Ten days later, hepatic TC (a), TG (b), and glucose (c) contents were measured. Values given are the means ± SEM, with $n = 10$. $^{*}P < 0.05$, $^{**}P < 0.01$ versus mice fed with ND and $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$ versus mice fed with HCLD alone. Statistically significant differences were determined using a one-way ANOVA followed by Dunnett's multiple comparisons test or post hoc analysis.

3.5. Effects of EtSF Supplementation on Body Weight and Food/Drug Intake. EtSF-P supplementation decreased the body weight (up to 6%; $P < 0.05$) in ND-, but not HCLD-, fed mice. However, no detectable changes in body weight between EtSF-S/EtSF-S/P supplemented and unsupplemented mice fed with ND and HCLD were observed. In addition, weight loss was observed in FF-supplemented mice with ND (by 8%) and HCLD (by 16%). Daily intake of EtSF-S, EtSF-P, or EtSF-S/P was estimated to be 1.47–1.68 g/kg (based on crude herb equivalent) at 1% supplementation, 4.23–5.47 g/kg at 3% supplementation, and 12.72–15.43 g/kg, at 9% supplementation. The human equivalent dose of 1% EtSF is estimated to be 0.15–0.17 g crude herb/kg. The daily intake of FF was estimated to be 0.15 and 0.13 g/kg in normal and HCL mice, respectively (Table 2).

4. Discussion

While genetic inheritance may contribute to the development of HLD and its related NAFLD in some patients, the main pathological causes are related to the lack of exercise and diet with high levels of saturated fats and carbohydrates [3]. In the present study, mice fed with a diet containing cholesterol and bile salt for 10 days exhibited elevations in serum TC, LDL levels, and ALT activity, as well as hepatic TC, TG, and GLU levels, which were associated with hepatomegaly and liver injury. Biliary and fecal TC concentrations were also increased in mice fed with HCLD. It was observed that feeding mice with HCLD for ten days was able to increase serum LDL level but causes no detectable change in serum HDL. The findings indicated that HLD and/or NAFLD in humans were successfully mimicked by a mouse model of feeding

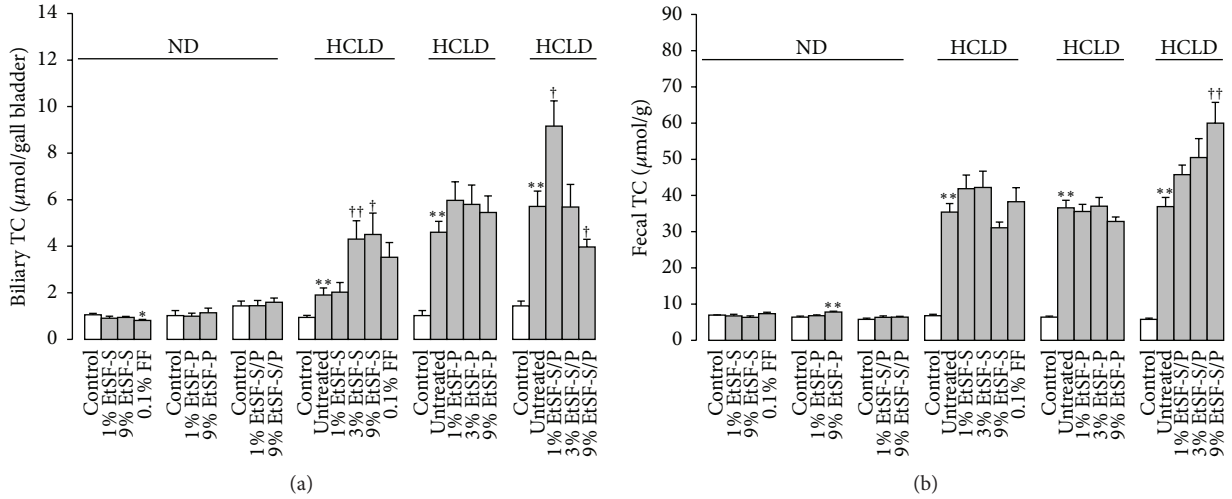

Figure 3: Effects of EtSF supplementation on biliary and fecal cholesterol contents in normal and HCL mice. Experimental details were described in Table 1. Mice were fed with ND and HCLD without or with EtSF or FF supplementation, as indicated in the figure. Ten days later, biliary (a) and fecal (b) TC contents were measured. Values given are the means ± SEM, with $n = 10$. $^{*}P < 0.05$, $^{**}P < 0.01$ versus mice fed with ND and $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$ versus mice fed with HCLD alone. Statistically significant differences were determined using a one-way ANOVA followed by Dunnett's multiple comparisons test or post hoc analysis.

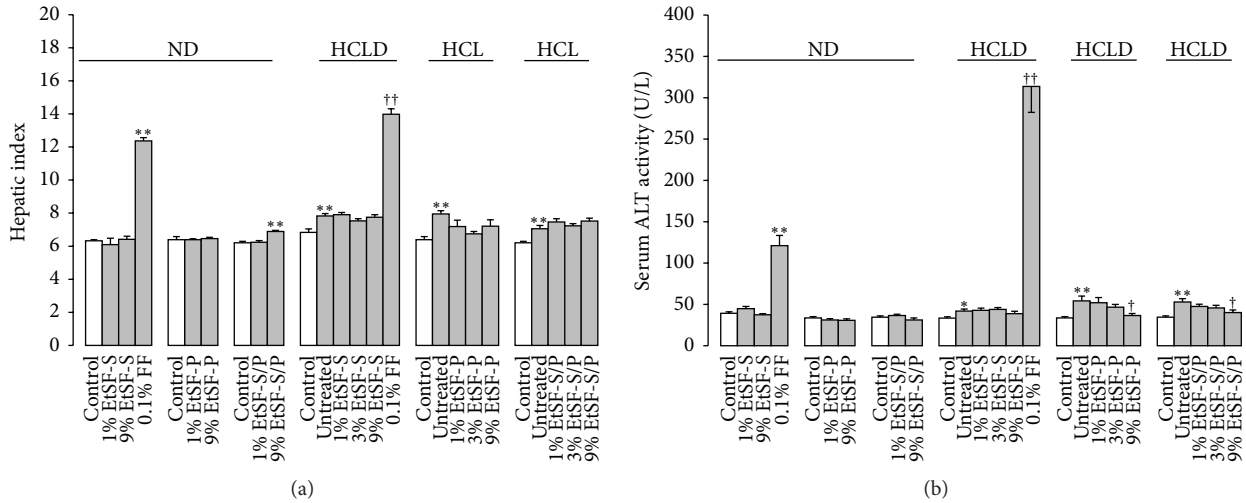

Figure 4: Effects of EtSF supplementation on hepatic index and function in normal and HCL mice. Experimental details were described in Table 1. Mice were fed with ND and HCLD without or with EtSF or FF supplementation, as indicated in the figure. Ten days later, hepatic index (a) and serum alanine aminotransferase (ALT) activity (b) were measured. Values given are the means ± SEM, with $n = 10$. $^{*}P < 0.05$, $^{**}P < 0.01$ versus mice fed with ND and $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$ versus mice fed with HCLD alone. Statistically significant differences were determined using a one-way ANOVA followed by Dunnett's multiple comparisons test or post hoc analysis.

HCLD. Nevertheless, the low levels of serum TG, as observed in the mouse model, may be related to the short period (10 days) of modeling, HCLD composition, and/or interspecies differences [28, 29]. In clinical situation, however, serum TG level is often not a concomitant parameter in HCL; it may increase or remain unchanged but never be lower than the normal range.

Given that N-HDL, HDL/LDL ratio, and LDL/HDL ratio are clinical parameters for assessing the risk of cardiovascular diseases, NAFLD, and metabolic syndrome in humans [30–32], these parameters were also adopted for evaluating the effectiveness of the tested EtSF extracts in mice fed with HCLD. Feeding mice with HCLD increased serum LDL/HDL ratio and N-HDL levels but decreased the HDL/LDL ratio, which are consistent with the clinical manifestation of HLD [33–35]. FF supplementation increased HDL/LDL ratio and decreased N-HDL levels and LDL/HDL ratio in HCL mice. On the other hand, the supplementation with EtSF-P and EtSF-S/P decreased HDL/LDL ratio and elevated N-HDL levels and LDL/HDL ratio. While EtSF-P and EtSF-S/P lowered serum TG and enhanced serum HDL, both EtSF extracts increased serum TC and N-HDL levels and LDL/HDL ratio,

Table 2: Effects of EtSF supplementation on body weight and food/drug intake in normal and HCL mice.

Groups	Drug concentration (%, w/w)	Body weight (g) in D 1	Body weight (g) in D 10	Food intake (g/kg/day)	Drug intake (g/kg/day)
ND-fed mice					
ND	—	18.55 ± 0.09	28.70 ± 0.53	148.54	—
ND/EtSF-S	1	18.58 ± 0.09	28.10 ± 0.45	147.80	1.48
	9	18.56 ± 0.09	28.24 ± 0.53	151.48	13.63
ND/FF	0.1	18.52 ± 0.11	26.36 ± 0.65*	145.27	0.15
ND	—	18.14 ± 0.18	28.02 ± 0.45	158.60	—
ND/EtSF-P	1	18.08 ± 0.18	26.55 ± 0.52*	147.39	1.47
	9	18.03 ± 0.19	26.38 ± 0.41*	165.29	14.88
ND		18.52 ± 0.10	28.02 ± 0.51	154.29	—
ND/EtSF-S/P	1	18.48 ± 0.11	27.87 ± 0.50	146.88	1.47
	9	18.55 ± 0.11	28.42 ± 0.45	145.30	13.08
HCLD-fed mice					
ND	—	18.60 ± 0.16	28.48 ± 0.50	156.21	—
HCLD	—	18.68 ± 0.18	28.48 ± 0.49	143.35	—
HCLD/EtSF-S	1	18.55 ± 0.17	29.49 ± 0.53	145.75	1.46
	3	18.65 ± 0.16	28.48 ± 0.72	141.09	4.23
	9	18.62 ± 0.17	28.07 ± 0.47	141.31	12.72
HCLD/FF	0.1	18.67 ± 0.19	23.81 ± 0.62††	132.00	0.13
ND	—	18.14 ± 0.18	28.02 ± 0.45	158.60	—
HCLD	—	18.63 ± 0.22	27.74 ± 0.59	159.60	—
HCLD/EtSF-P	1	18.92 ± 0.34	28.17 ± 0.62	164.80	1.65
	3	18.45 ± 0.25	28.55 ± 0.79	182.16	5.47
	9	18.41 ± 0.29	27.06 ± 0.45	171.46	15.43
ND	—	18.52 ± 0.10	28.02 ± 0.51	154.29	—
HCLD	—	19.00 ± 0.26	27.37 ± 0.48	157.86	—
HCLD/EtSF-S/P	1	18.88 ± 0.24	29.04 ± 0.92	167.58	1.68
	3	19.14 ± 0.30	28.17 ± 0.47	172.16	5.17
	9	18.90 ± 0.30	27.47 ± 0.42	166.41	14.98

Experimental details were described in Table 1. The dosages (g/kg/day) based on crude herbal material were determined with the amount of ingested diet (g/kg/day) and drug concentrations in the diet. Values given are the means ± SEM, with $n = 10$. $^{*}P < 0.05$ versus mice fed with ND; $^{††}P < 0.01$ versus mice fed with HCLD. Statistical significant differences were determined using a one-way ANOVA followed by Dunnett's multiple comparisons test or post hoc analysis.

as well as decreased HDL/LDL ratio. These findings suggest that EtSF-P and EtSF-S/P (but not EtSF-S) supplementation may lead to further worsening of lipid parameters in mice under HCL condition. However, it has been reported that the baseline levels of plasma TC, HDL, LDL, and TG in mice were marginally higher than the reference ranges prior to the experiment and 2 weeks of EtSF supplementation did not cause any significant changes in lipid parameters [36].

It is well established that lipid metabolism is closely related to GLU metabolism in the body. The relevant metabolic disorders constitute the pathological basis of hyperlipidemia, metabolic syndrome, type 2 diabetes, fatty liver disease, and obesity [37]. In the present study, supplementation with EtSF-S, EtSF-P, or EtSF-S/P did not change serum TC and TG levels but altered hepatic lipid contents and GLU levels in HCL mice. While EtSF-P and EtSF-S/P supplementation increased hepatic TC contents in HCLD-fed mice, EtSF-S lowered the hepatic TC content. Serum levels of HDL and LDL (often referred to as "good" cholesterol and "bad" cholesterol, resp.) [38] were increased in mice fed with HCLD or ND supplemented with EtSF-P or EtSF-S/P. The elevation of serum HDL and LDL levels by EtSF extracts, in particular the EtSF-S/P, might result from a metabolic response to hypercholesterolemia, wherein the increased cholesterol content necessitates higher levels of LDL and HDL for transportation in the blood. Supplementation with EtSF was found to markedly decrease hepatic TC, TG, and GLU contents in both ND- and HCLD-fed mice. EtSF-S/P supplementation at 1% increased biliary TC level; however, the supplementation at 9% reduced biliary TC level and

increased fecal TC excretion. While EtSF-S attenuated serum TG levels, both EtSF-P and EtSF-S/P did not cause any changes in normal and HCL mice. Taken together, the results suggest that SF can influence the lipid and GLU metabolism in mice in a complex manner, especially under the HCL condition.

A previous study in our laboratory has shown that Sch B lowered fat accumulation in L-02 cells incubated with free fat acid via inhibition of adipose differentiation-related protein (ADRP) and sterol regulatory element-binding protein (SREBP-1) expression [39]. It is known that ADRP is closely associated with intracellular lipid droplets and upregulated in hepatic steatosis [40]. SREBP-1 is the most important transcription factor regulating *de novo* lipogenesis in the liver and induces insulin resistance [41]. Kwon et al. [42] reported that SF lignans could improve insulin sensitivity via the PPAR-γ pathways. Therefore, it is possible that the EtSF extracts tested in the present study may protect against NAFLD and decrease hepatic GLU contents through a similar action mechanism. Hyperlipidemia is commonly associated with insulin resistance, which may result in hyperinsulinemia and hyperglycemia. However, in present study, feeding mice with HCLD did not increase serum GLU levels (data not shown). Instead, HCLD elevated hepatic GLU, which may be related to the stimulation of hepatic gluconeogenesis, an indicative of insulin resistance in extrahepatic tissues. In addition, based on the reduction of hepatic TC content and elevation of serum TC/LDL levels by EtSF extracts in HCLD-fed mice, it is possible that EtSF extracts, particularly the EtSF-S/P, can stimulate the release of TC from the liver and thereby ameliorate hepatic steatosis.

The observations of increased serum ALT activity, enlarged liver size, and increased lipid accumulation in the HCLD-fed mice suggest the presence of liver damage, which may result from the accumulation of lipids in hepatic tissue [43] and/or activation of signaling pathways in hepatocytes that stimulate the production of proinflammatory mediators [44]. Supplementation with EtSF-P and EtSF-S/P protected against liver damage in HCL mice, as evidenced by the decrease in serum ALT activity. It is believed that the dibenzocyclooctadiene-type lignans such as schisandrin A and Sch B are the active components of SF in protecting against liver injury [45]. As to why the lignan-enriched EtSF-S was unable to protect against liver damage in HCLD-fed mice remains to be investigated,

FF, the fibrates class of lipid-lowering drugs, is commonly used in the treatment of HLD as a PPARα agonist for reducing cardiovascular risks and treating NAFLD/nonalcoholic steatohepatitis [46, 47], as well as improving the GLU tolerance and lowering adiposity [48]. Significant lowering of serum and hepatic lipid/GLU levels, as well as body weight and fat mass, was observed following FF supplementation (data not shown). As about one-fourth to one-third of blood cholesterol is carried by HDL, hence, low serum HDL levels caused by FF might result from the drug-induced hypocholesterolemia in normal mice. Although fibrate treatment improved liver function in patients with metabolic syndrome in clinic situation [49] and ameliorated concanavalin A-induced hepatitis in rats [50], FF can cause acute cholestatic hepatitis in patients [51–53]. In the present study, the daily supplementation with FF (130–150 mg/kg; about 30-fold higher than the human dose) induced hepatomegaly and increased ALT levels in normal and HCL mice. FF-induced elevation in serum ALT activity might be partly due to the increased expression of hepatic transaminase gene [54].

Herbal drugs, which contain a mixture of chemical components, can produce a wide spectrum of biological actions. According to the theory of Chinese medicine, SF possesses five tastes (Wu Wei in Chinese)—sweet (fruit skin), sour (pulp), bitter/pungent (seed core), and saltiness (all parts). SF pulp, which mainly contains polysaccharides/sugars and organic acids [55, 56] as well as dibenzocyclooctadiene lignans such as schisandrin A, B, and C and gomisin A and N, is responsible for producing most of the pharmacological activities. Although lignans are most abundantly found in SF seeds [57], EtSF-S is not the most biologically active among the 3 tested SF fractions, as observed in the present study. In addition, the pharmacological actions produced by EtSF do not always display a dose-response relationship. For instance, EtSF-S/P reduced hepatic GLU level in a dose-dependent manner, but it did not lower hepatic TC at the highest tested dose (i.e., 9%). While three doses (1, 3, and 9%) were tested in HCL mice, two doses were adopted in normal mice to examine the possible toxicity of EtSF in mice. Although the daily doses of 9% EtSF could reach about 15 g/kg/day for 10 days, they did not affect the behaviors in mice (data not shown).

In conclusion, results obtained from the present study showed that the supplementation with EtSF produced a significant influence on lipid/GLU metabolism in ND- and HCLD-fed mice, especially in HCL mice. Dietary supplementation with EtSF-P or EtSF-S/P elevated serum lipid levels, except for that of serum TG levels which was lowered, in HCL mice. Dietary supplementation with EtSF-S, EtSF-P, or EtSF-S/P reduced hepatic lipid and GLU concentrations in both normal and HCL mice. EtSF-S/P, but not EtSF-S and EtSF-P, supplementation increased fecal cholesterol excretion in HCLD-fed mice. EtSF-P and EtSF-S/P attenuated the HCLD-induced hepatotoxicity. Supplementation with FF decreased serum and hepatic lipid and GLU levels, as well as increased serum ALT activity and liver weight in mice fed with ND and/or HCLD (see the summary of results in Table 3). The ensemble of results indicates a differential effect between SF seed and pulp on lipid and GLU metabolism, particularly in HCL mice. Supplementation with EtSF might ameliorate the lipid accumulation in liver cells and thus protect against liver injury in HCL mice.

Conflict of Interests

The authors declare that there is no conflict of interests with respect to the authorship and/or publication of this paper.

Authors' Contribution

Xiao-Yan Wang and Zhi-Ling Yu contributed equally to the work.

Table 3: A summary of results from the study.

	EtSF-S dietary supplement	EtSF-P dietary supplement	EtSF-S/P dietary supplement	FF dietary supplement
ND-fed mice				
Serum TC	—	—	—	↓
TG	—	—	—	↓
HDL	—	—	—	↓
LDL	—	↑	↑	—
ALT activity	—	—	—	↑
Hepatic TC	↓	—	↓	↓
TG	↓	↓	↓	↓
Glucose	↓	↓	↓	↓
Index	—	—	↑	↑
Biliary TC	—	—	—	↓
Fecal TC	—	↑	—	—
Body weight gain	—	↓	—	↓
HCLD-fed mice (*change versus ND-fed mice*)				
Serum TC (↑)	—	↑	↑	↓
TG (↓)	↓	↓	↓	↓
HDL (—)	—	↑	↑	—
LDL (↑)	—	↑	↑	↓
ALT activity (↑)	—	↓	↓	↑
Hepatic TC (↑)	↓	↓	↓	↓
TG (↑)	↓	↓	↓	↓
Glucose (↑)	↓	↓	↓	↓
Index (↑)	—	—	—	↑
Biliary TC (↑)	↑	—	↑(1%) ↓(9%)	↑
Fecal TC (↑)	—	—	↑	—
Body weight gain (—)	—	—	—	↓

↑: increased or elevated; ↓: decreased or inhibited; —: unaltered.
TC: total cholesterol; TG: triglyceride; HDL: high-density lipoprotein; LDL: low-density lipoprotein; ALT: alanine aminotransferase; FF: fenofibrate.

Acknowledgments

This paper was supported by the National Natural Science Foundation of China (Grant no. 31071989) and the Outstanding Teaching Team of Chinese Herbal Pharmacology in Beijing City (Grant no. 201006).

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Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2014, Article ID 525340, 20 pages
http://dx.doi.org/10.1155/2014/525340

Review Article

Historical Perspective of Traditional Indigenous Medical Practices: The Current Renaissance and Conservation of Herbal Resources

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Received 30 November 2013; Revised 1 February 2014; Accepted 23 March 2014; Published 27 April 2014

Academic Editor: Kelvin Chan

In recent years, increasing numbers of people have been choosing herbal medicines or products to improve their health conditions, either alone or in combination with others. Herbs are staging a comeback and herbal "renaissance" occurs all over the world. According to the World Health Organization, 75% of the world's populations are using herbs for basic healthcare needs. Since the dawn of mankind, in fact, the use of herbs/plants has offered an effective medicine for the treatment of illnesses. Moreover, many conventional/pharmaceutical drugs are derived directly from both nature and traditional remedies distributed around the world. Up to now, the practice of herbal medicine entails the use of more than 53,000 species, and a number of these are facing the threat of extinction due to overexploitation. This paper aims to provide a review of the history and *status quo* of Chinese, Indian, and Arabic herbal medicines in terms of their significant contribution to the health promotion in present-day over-populated and aging societies. Attention will be focused on the depletion of plant resources on earth in meeting the increasing demand for herbs.

1. Introduction

Herbalism is a traditional medicinal or folk medicine practice based on the use of plants and plant extracts. Herbs/plants, the major component of traditional materia medica in the world, are of the main forms of life on earth. It is estimated that there are about 350,000 species of existing plants (including seed plants, bryophytes, and ferns), among which 287,655 species have been identified as of 2004 [1]. Herbal medicine (HM), also called botanical medicine, phytomedicine, or phytotherapy, refers to herbs, herbal materials, herbal preparations, and finished herbal products that contain parts of plants or other materials as active ingredients [2]. The plant parts used in herbal therapy include seeds, berries, roots, leaves, fruits, bark, flowers, or even the whole plants. Man was mainly dependent on crude botanical material for medical needs to retain vitality and cure diseases [3] prior to the introduction of aspirin derived from *Spiraea ulmaria* which was already prescribed for fever and swelling in Egyptian papyri and recommended by the Greek Hippocrates for pain and fever.

Although written records about medicinal plants dated back at least 5,000 years to the Sumerians, who described well-established medicinal uses for such plants as laurel, caraway, and thyme [4], archeological studies have shown that the practice of herbal medicine dates as far back as

60,000 years ago in Iraq and 8,000 years ago in China [5, 6]. With the advent of western medicine (or "conventional" medicine) over the past century, herbal medicine has been challenged by practitioners of mainstream medicine because of the lack of scientific evidence in the context of contemporary medicine, despite its long history of effective use. Interestingly, things change with time. In recent years, there has been a resurgence of the use of herbs due to the side effects of chemical drugs, lack of curative modern therapies for several chronic diseases, and microbial resistance, as well as the unprecedented investment in pharmaceutical research and development (R&D) [7]. For example, only about 1,200 new drugs have been approved by the US Food and Drug Administration (FDA) since 1950 [8]. As a result, the use of herbs and herbal products for health purposes has increased in popularity worldwide over the past 40 years, in both the developing and the industrialized countries [9]. Moreover, global pharmaceutical companies armed with modern science/technology and ideas have begun to rediscover herbs as a potential source of new drug candidates and renewed their strategies in favor of natural product drug development and discovery [10–13].

Nowadays, many practitioners of "conventional" medicine do not hesitate to recommend herbs, herbal products, or complementary and alternative medicine (CAM) therapy to their patients for the effective treatment of certain diseases [14, 15]. A survey in 2007 indicated that about 40% of adults and 11% of children used CAM therapy (CAMT), and among the adult users, white and black adults constituted 43.1% and 25.5%, respectively [16]. In addition, CAM and herbal medicines are more commonly used by people with higher levels of education and income [17, 18]. In this context, a 2012 study indicated that the use of CAM significantly correlated with higher education level, with a trend towards greater use in younger patients with breast cancer [19]. Although at present, we do not fully understand the exact facts and mechanisms underlying most traditional remedies and/or how they prevent disease that does not affect the enthusiasm of the public to accept CAM/CAMT [20]. Although there is a wide variety of CAM and CAMT around the world, they can all be divided into two main categories, namely, drug-based CAM/CAMT and non-drug-based CAM/CAMT [21].

Our earlier endeavors, which focused on discussing the current research and development of Chinese herbal medicine (CHM), and the trend in drug discovery, as well as a variety of CAM, aimed to promote the utilization of natural and traditional resources for contemporary health care, including food/diet therapy [7, 21–24]. As a continuing effort, the current paper will give an overview on herbal medicine from China, India, and Arabia, which are the three most influential traditional medicine systems to improve public health problems.

2. Chinese Herbal Medicine (CHM)

In ancient Chinese times "medicine" (traditional Chinese medicine, TCM, e.g., *Zhong-Yi* in Chinese) and "pharmacy" (CHM, e.g., *Zhong-Yao* in Chinese) were already described as distinct disciplines. More than 85% of Chinese materia medica (CMM) originates from plants, but animal parts/insects, minerals, and crude synthetic compounds are also prescribed by TCM practitioners. In addition, the term "CHM" also encompasses a number of ethnic herbal medicines and folk medicines in China.

2.1. Literature Overview of CHM. CHM is traditionally one of the most important modalities utilized in TCM. It has an extremely valuable, rich, lengthy, and extensive treatment history. CHM was firstly described by a legendary figure called *Shen-Nong*, who is said to have lived from 2737 BCE to 2697 BCE, nearly 5,000 years ago [25, 26]. It is said that *Shen-Nong, by* tasting hundreds of herbs on one day, found more than 70 herbs that had medicinal value, selected those that were suitable as remedies, and described their properties [27]. As a result of his efforts, numerous herbs ("herbal" medicine) became routinely used for health care in ancient China [28]. *Shen-Nong-Ben-Cao-Jing*, the first known user guide to CHM, was written by authors who lived during the period immediately following the fall of the *Han* dynasty (202 BCE–220 CE). The compendium documented 365 Chinese herbal preparations, including 252 kinds of plant parts, 67 kinds of animal parts, and 46 kinds of minerals for medication, and it also described their therapeutic effects.

Prior to the time of *Shen-Nong-Ben-Cao-Jing*, some ancient Chinese scripts, such as *Shang-Shu*, *Shi-Jing* (The book of songs), *Shan-Hai-Jing*, *Zhou-Li*, *Li-Ji* (The book of rites), and *Zuo-Zhuan*, recorded the use of herbal remedies. *Shi-Jing*, which first recorded the use of herbal remedies, illustrated not only the therapeutic effects of the herbs, but also the places where the herbs were grown and their harvesting season. It recorded 170 kinds of CHMs, including 80 plant species and 90 insect species [29]. *Shan-Hai-Jing*, the oldest Chinese book dealing with geography, recorded 9 species of plants with food value, 45 species of plants with medicinal value, 6 plants with some kind of efficacy, 6 plants poisonous to animals and pests, 6 species of plants with mood-elevating effects, 6 plants with health-promoting properties, 19 species of plants for the treatment of diseases, and 2 plants that are poisonous for humans [30].

More than 240 herbal drugs and 52 prescriptions were described in the book named *52 Bing-Fang* (Recipes for 52 Ailments), which was unearthed in an ancient tomb (*Ma-Wang-Dui*) in China [31]. *Xin-Xiu-Ben-Cao* (Newly revised materia medica), which was promulgated in 659 CE and recorded 850 kinds of herbal drugs, was the first pharmacopoeia in China, even in the world [32]. Oracle bone, a form of divination in ancient China, recorded more than 60 kinds of plants and animals, but they were not described as medication.

The *epic* book of materia medica in TCM history, *Ben-Cao-Gang-Mu* (*Compendium of Materia Medica*) written by *Li Shi-Zhen* (1528–1593), was published in 1596 in China. This book recorded 1,892 kinds of herbal medicines and 11,096 herbal formulae. After Charles Darwin (1809–1882) had read the book, he stated that *The Compendium of Materia*

Table 1: Some important texts in the historical developmental process of Chinese materia medica [33, 34].

Lectures	Issued date	Total	Plant	Animal	Mineral	Processing products	Formulae	Other
52 Bing-Fang	200 BCE	247	115	48	21			63
Shen-Nong-Ben-Cao-Jing	202 BCE–220	365	252	67	46			
Xin-Xiou-Ben-Cao	659	850	635	128	87			
Zheng-Lei-Ben-Cao	1082	1,746	1,151	342	253		>3,000	
Ben-Cao-Gang-Mu	1596	1,892	1,094	443	161		11,096	194
Znong-Yao-Da-Ci-Dian	1977	5,767	4,773	740	82	172		
Zhong-Hua-Ben-Cao	1999	8,980	7,815	1,051	114			
Chinese Pharmacopoeia	2010	2,165	680	36	18	1,384		47

Medica was the encyclopedia of 16th century in China. This book was later translated into different languages, including Japanese, Korean, English, French, Russian, and Latin, and it has become a major historical reference on CMM.

The founding of China has brought about a hitherto unprecedented development of CHM in Chinese history. The holistic and systematic development of CHM has resulted in an increase in the number of approved CHMs. *Zhong-Hua-Ben-Cao*, the most authoritative Chinese book with a complete record of CMM issued in 1999, lists 8,980 kinds of CHMs that are divided into 34 volumes and summarizes the contemporary research of Chinese medicine with modern science and technology. *Zhong-Yao-Da-Ci-Dian* (a dictionary of traditional Chinese medicine), published in 1997, recorded 5,767 CHMs; when it was reprinted in 2006, the number of CHMs had increased to 6,008. The Chinese Pharmacopoeia (2010 version) listed 2,165 CHMs and their products. About 300 of them are commonly used in clinical practice, and many others are used locally as folk medicines. In terms of the literature on CHM, the theoretical aspects and practical experiences of several thousand years of usage are documented in more than 8,000 books; the total number of ancient literature about both CHM and TCM reached 13,000. Therefore, the documentation of knowledge in CHM is unique in the world (Table 1).

2.2. The Contribution of CHM to the World's Pharmacy. CHM has been influencing the world since ancient times. The famous Italian traveler Marco Polo (1254–1324) described a scene of merchants shipping Chinese herbs in Aden and Alexandria in his *Travel Book*. During the sea voyage of *Zheng He* between 1405 and 1433, China exported a large number of herbs including rhubarb, angelica, velvet, poria, taurine, ginseng, and cinnamon to other Southeast Asian countries. In return, over the past 2,000 years, more than 40 kinds of foreign herbs were imported into China and eventually adopted by TCM; they include kelp from Korea, turmeric and styrax from Southeast Asia, and others such as borneol, clove, frankincense, myrrh, benzoin, senna, and saffron [35].

In the 18th century, with reference to Chinese ginseng, *Panax quinquefolium* (also called American ginseng) was first discovered. It is indigenous to the southern regions of Ontario and Quebec in Canada and the midwestern, southern, and eastern parts of the United States [36]. In recent decades, studies have shown that American ginseng, like the Chinese one, also possesses neuroprotective, cardioprotective, antidiabetic, antioxidant, and anticancer properties, as well as the ability to alleviate symptoms of the common cold [37, 38]. The proven similarities between American ginseng and Chinese ginseng have been instrumental in boosting the market of the American product. From 1960 to 1992, both the demand and the price for American ginseng increased, with the export value being over US $104 million in 1992 in the USA alone. During the period from 1997 to 2007, the average export price of cultivated ginseng from the USA was US $19.30/lb and that of wild ginseng was US $84.50/lb [39]. The Panax family consists of at least nine species, including *ginseng*, *panax quinquefolium*, p*anax notoginseng* (*Sanqi*), and *Panax japonicus* (Japanese ginseng) [40]. If Chinese ginseng had not served as a reference herb, the Panax family would certainly not have become so popular and might still be treated as ordinary grass. Similarly, if the Chinese had not recognized the medicinal value of bezoars (gallstones from cattle), they would only have been treated as waste. Currently, China imports more than 100,000 kilograms of gallstones each year, about 60% of which comes from Africa, and the total value amounts to US $100 million. A good quality gallstone sells for between US $15 and 20 per gram [41].

In the winter of 753, *Jian Zhen* (688–763), a famous Chinese master of Buddhism in the *Tang* Dynasty, arrived in Nara, Japan, after several unsuccessful attempts, and he brought with him a number of CHMs. To date, more than 60 kinds of CHMs are still kept in the Nara Shosoin. According to the official body of Japanese kampo medicine (the practice of CHM in Japan), 36 kinds of CHMs were brought by *Jian Zhen* for use in Japan; they include ephedra, asarum, peony, monkshood, polygalaceae, astragalus, licorice root, angelica, bupleurum, Chuanxiong, scrophulariaceae, scutellaria, platycodon, anemarrhena, pinellia, schisandra, and eucommia [42]. To recognize the contribution of *Jian Zhen*, he was renowned as the father of kampo medicine by the Japanese. The 14th edition of the *Japanese Pharmacopoeia* (JP), issued in 1993, listed 165 herbal ingredients, the majority of Chinese origin, that are approved to be used in kampo remedies [43]; the 16th *Japanese Pharmacopoeia*, published in 2012, listed 276 kinds of crude drugs (e.g., herbal medicines and/or their extractions) [44].

All in all, the development of CHM has emerged from thousands of years of Chinese civilization. It is therefore no surprise that CHM is of great worth for mankind.

2.3. Species in China and CHM. Nature has endowed China with a vast landscape with varied geographical features and a resultant wealth of medicinal plants. Geographically, China (from south to north) covers equatorial, tropical, subtropical, warm-temperate, temperate, and cold-temperate zones. Therefore, Chinese climatic conditions are suitable for the growth and reproduction of various animals and plants. In China, there are 499 kinds of mammals, 1,186 kinds of birds, 376 kinds of reptiles, 279 kinds of amphibians, and 2,084 kinds of fish, which account for 12.5, 13.1, 6.0, 7.0, and 12.1% of their respective species in the world [45–50]. China has more than 31,000 higher plants, 256 endemic genera, and 15,000–18,000 endemic species (50–60% of the total on earth), many of which are living fossils, such as dawn redwood (*Metasequoia glyptostroboides* Hu and Cheng), ginkgo (*Ginkgo biloba* L.), silver fir (*Cathaya argyrophylla* Chun and kuang), and tulip tree (*Liriodendron chinense* (Hemsl.) Sarg.) [51]. The increasing demand for herbal products in the global market is likely to challenge herbal resources in the world. In the *China Plant Red Data Book* published in 1992, 388 species of plants are listed as threatened, which include 121 as endangered (i.e., first grade national protection), 110 as rare (second grade national protection), and 157 as vulnerable (third grade national protection). Among these plant species, 77 are typical CHMs that account for 19.86% of the total threatened species [52]. Besides, 257 kinds of animal medicine appear in the national key protection name list of wild animals (Figure 1).

In CHM, there are 11,146 different kinds of plants, 1,581 kinds of animals/animal parts and insects, 80 kinds of mineral drugs, and more than 50 kinds of crude chemical preparations, as well as 5,000 (total one million) clinically validated herbal formulations. Unlike other herbal medicines and western medicines, CHMs are often prescribed as formulas under the guidance of TCM's theories and practice. Each herbal medicine prescription (formula, *Fang-Ji* in Chinese) is a cocktail of many herbs tailored to the individual patient. It allows us to blend herbs to enhance their positive effects and reduce or eliminate any negative side effects they may have, when they are used each alone (Figure 2).

Because of the differences in geographical and climatic conditions, residents in various geographical regions in China have distinctive lifestyles, customs, and cultures, as well as disease spectra. These variations have brought about the development of a wide variety of traditional medicine practices. China has 56 ethnic groups, meaning that there are 56 kinds of culture, language, and herbal medicine. CHM (also called *Han* medicine) was developed by the *Han* ethnic group. Table 2 shows the number of plant-derived herbal medicines in various ethnic medicines as recorded in the database of China plant species. In fact, the number of herbal medicines is far bigger than that recorded in the database. For example, Tibetan herbal drugs have 2,172 rather than 1,085 varieties, not including 214 kinds of animal drugs and 50 kinds of mineral drugs [53]. There is no doubt that CHM, together with other ethnic herbal medicines in China, comprises a gold mine of potential modern medicines and health products.

Table 2: Ethnic materia medica (EMM) in China.

Ethnic group	EMMs
Han	11,146
Tibetan	1,085
Miao	718
Dai	707
Yi	564
Li-Su	494
Zhuang	473
Mongolian	397
Wa	332
Tu-Jia	330
Ha-Ni	302
De-Ang	272
A-Chang	263
Ji-Nuo	250
Du-Long	165
She	161
Mu-Lao	152
La-Hu	151
Uighur	143
Shui	129
Korean	121
Na-Xi	103
Bai	90
Mao-Nan	75
Pu-Mi	49
Bu-Lang	44
Bu-Yi	32
Beng-Long	28
Jing	20
Ge-Lao	18
Daur	14
Kazak	14
O-Lun-Chun	12
Hui	11
Manchu	9
Li	9
Yu-Gu	5
Gao-Shan	4
Tajik	1
Russian	1
Nu-Jiang	1
Wei-Xi	1
Total	18,891

Data from "scientific database of China plant species. http://apps.searo.who.int/PDS_DOC".

2.4. Pharmaceutics of CHM. Dosage form, also known as routes of administration, is a mixture of components with medicinal properties and nondrug components (excipient or vehicle). It describes the physical form in which medication will be delivered into the body. Currently, there are

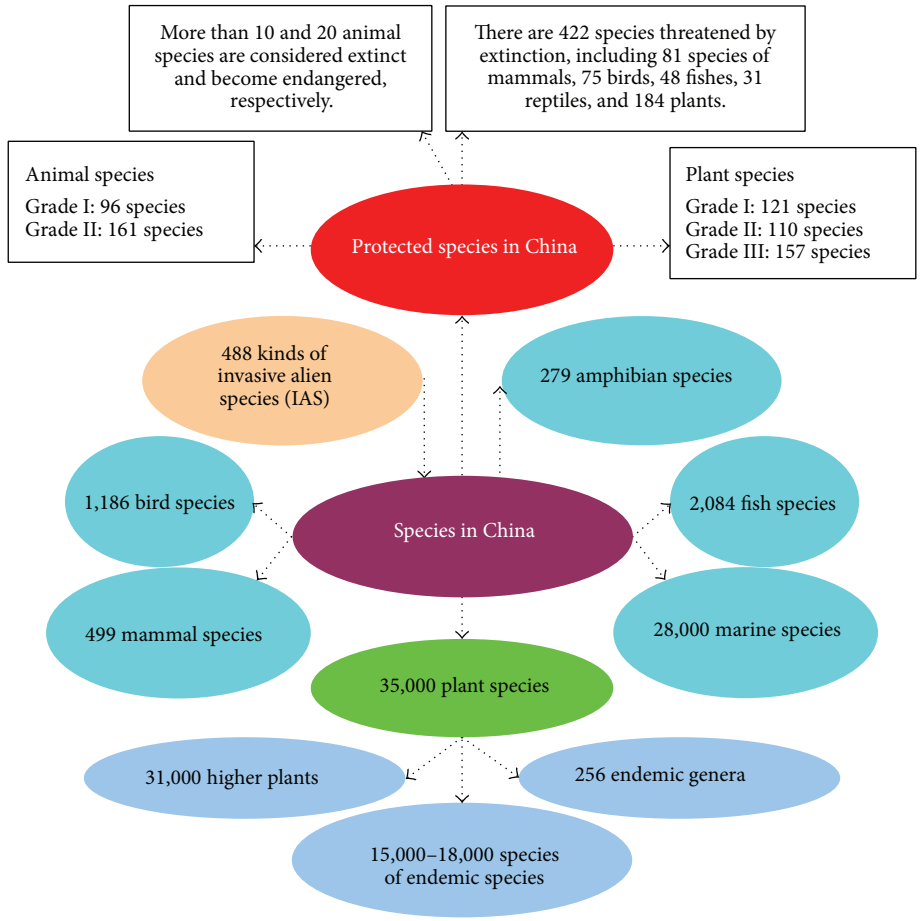

Figure 1: Species and protected species in China.

more than 40 available dosage forms of CHM products in the market. They include decoction (*Tang-Ji*, hot water extract), tincture (*Ting-Ji*, ethanol extract), powders (*San-Ji*, powder form), bolus/pills (*Wan-Ji*, boluses or small pills containing herbal ingredients), pastes (*Jin-Gao*, extracts from organic solvents), granules, tablets, oral liquids, and injection liquids. Liniment, poultices, plasters, and ointments are adopted for external use of CHM. Recently "nanomized" and "aerosol" herbs have emerged as new dosage forms of CHM [54–56].

Unlike synthetic drugs, CHMs are usually subjected to specific treatments (processing, the process of preparing CHM), also known as *Pao-Zhi* in Chinese, prior to use. *Pao-Zhi* is a very ancient part of the practice of Chinese medicine, dating back at least 2,000 years. There are more than 30 kinds of procedures involving stir-frying (*Chao*), calcining (*Daun*), steaming (*Zheng*), boiling (*Zhu*), and so forth. The herbal effects and compositions/ingredient structures are changed after *Pao-Zhi*, as compared to the unprocessed version. Experience has shown that the effectiveness and security of some CHMs are dependent upon their correct *Pao-Zhi* before being used in clinic. This is one of the reasons why CHM is different from the plant drug and/or natural drug. Regardless of the primitive processing technology that was used in ancient times, the rationale underlying the traditional processing of CHM has been supported by scientific evidence in modern research. Figure 3 shows the traditional processing methods of CHM, together with their pharmaceutical processing procedures, which are still employed in the pharmaceutical industry of CMM in China [57].

2.5. The Status Quo of CHM. The CHM industry has always been one of China's traditional competitive industries. In 2010, CHM manufacturing assets in China exceeded 300 billion RMB, an increase of about 18%, nearly 5 percentage points higher than the year before; the number of CMM pharmaceutical enterprises amounts to more than 2,300, total investment in fixed assets totaled nearly 500 billion RMB, an increase of about 16% compared to the previous year [58]. As of today, there are 8,000 products related to CHM in the China market. In 2010, China manufactured 2.384 million tons of Chinese herbal products, with sales amounting to 417.875 billion RMB [59].

More than 8,000 varieties of CHMs or related herbal products are now exported from China to more than 130 countries and regions worldwide; each year, more than 50 kind of CMMs are exported to the United States, including

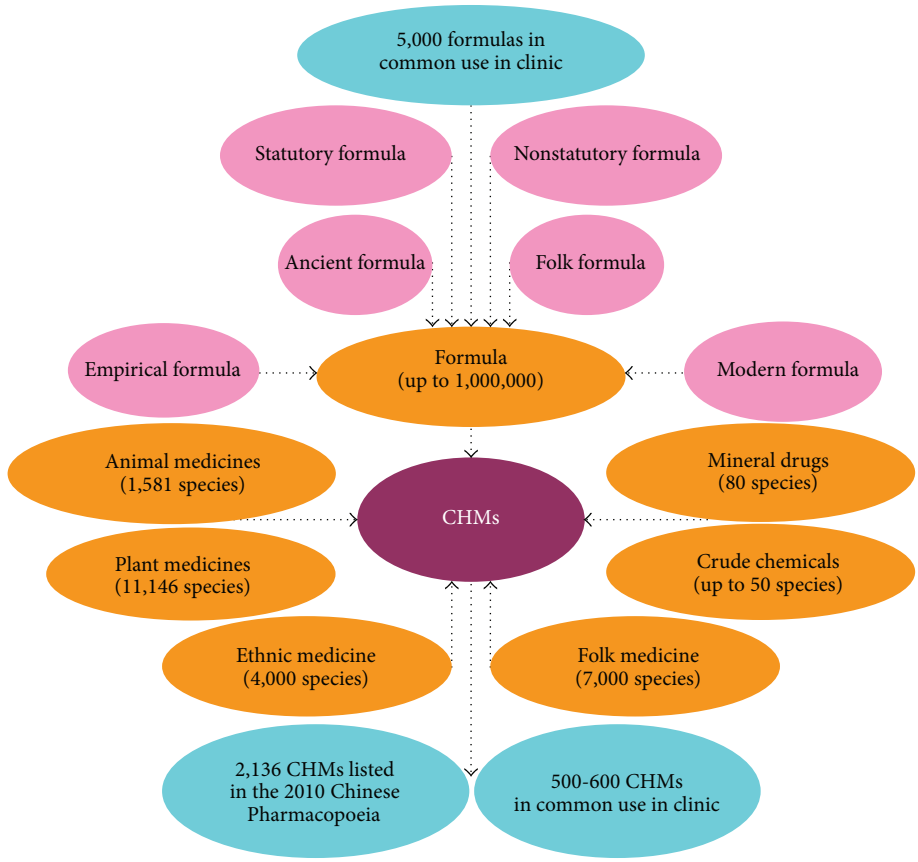

Figure 2: Chinese herbal medicine (CHM) in China.

berberine, angelica, licorice, Fritillaria, turmeric, frankincense, Tianma, rhubarb, Eucommia, cloves, wolfberry, Panax, fresh ginseng, and pinellia [60]. Over the past few years, herbal exports have steadily increased from US $1.09 billion in 2006 to US $1.46 billion in 2009 [61].

More importantly, in recent decades, China has put a great deal of human efforts and financial resources to promote research and development in the area of CHM in a systematic manner, and this enormous effort is unmatched by other traditional medicines around the world. In this context, we have published reviews on the status of CHM research and development as well as drug discovery in China [24]. In China, 3,563 extracts, 64,715 compositions, 5,000 single compounds, and 130 kinds of CHM-related chemical drugs have been developed [22]. From the marketing perspective, currently, four models of application and five types of Chinese herbal products can be adopted in the international arena. The same approach may also be applied to other herbal medicines (Figure 4).

3. Indian Herbal Medicine (IHM)

Indian medicine/materia medica/herbal medicine (IM/IMM/IHM), also called Ayurvedic medicine/materia medica (AYM/AYMM), belongs to the traditional health care and longevity systems. Because the belief that "everything can be a drug" is deeply rooted in Indian culture, Ayurvedic physicians made use of an extensive collection of medications, herbs/plants, even the urine of animals, and described their effects meticulously. Currently, 70 percent of Indians still rely on IM for their primary health care [66].

3.1. Literature Overview of IHM. In India, the history of using plant resources for treating diseases can be dated back to 6,000 to 4,000 BCE, the Buddhist period. AYM has a vast literature in Sanskrit and various Indian languages, covering various aspects of diseases, therapeutics, and pharmacy. The earliest references to such plants, minerals, and animal products with their usage for medical purposes are found in the *Rig veda*, an ancient Indian sacred collection of Vedic Sanskrit hymns, and the *Atharvaveda*, the fourth and last Veda of Hindu literature [67]. *Bhava Prakasha*, written by Bhava-Mishra, is the most important text on herbs/plants and is held in high esteem by modern Ayurvedic practitioners [68–70].

The oldest text of AMM, the *Rasa Vaisesika* of Nagarjuna, who is considered the most important Buddhist philosopher after Buddha's death [71], was composed during the 5th century CE. In this text the various concepts of drug composition and action were described [72]. The *Charaka Samhita* is the first recorded treatise fully devoted to the concepts and practice of Ayurveda, with a primary focus on therapeutics

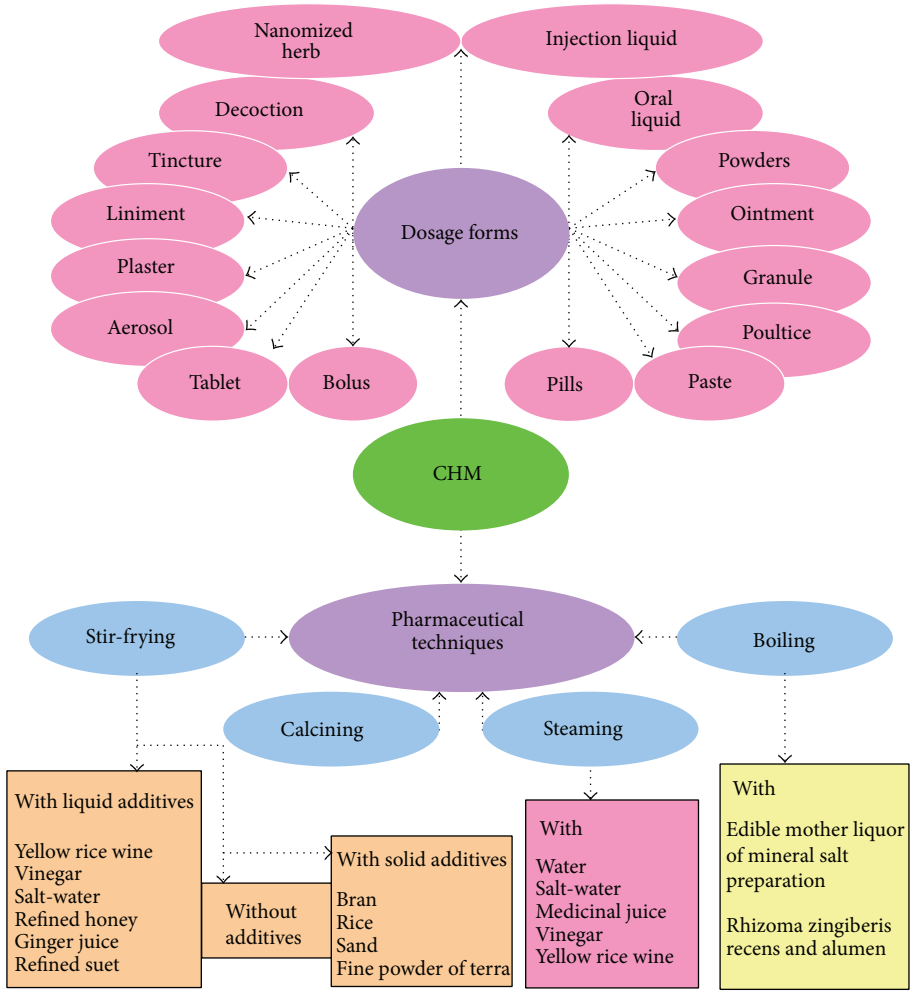

Figure 3: Dosage forms and pharmaceutical techniques in Chinese herbal medicine (CHM).

[73]. In the *Charaka Samhita*, plant-derived drugs are divided into 50 groups according to their pharmacologic/therapeutic actions. The next landmark in Ayurvedic literature was the *Sushruta Samhita*. Although the text places special emphasis on surgery, it also describes 395 medicinal plants, 57 drugs of animal origin, and 64 minerals or metals as therapeutic agents [74] (Figure 5). In ancient times, Ayurvedic texts were very respected in the neighboring countries, and they were also translated into Greek (300 BCE), Tibetan and Chinese (300 CE), Persian and Arabic (700 CE), and so forth [75].

3.2. Plant Species in India and IHM. India possesses almost 8% of the estimated biodiversity of the world with around 126,000 species; there are about 400 families of flowering plants in the world, at least 315 of these can be found in India [79]. Currently, about 45,000 species (nearly 20% of the global species) are found in the Indian subcontinent: ~3,500 species of plants are of medicinal value; 500 medicinal plant species are used by the contemporary Ayurvedic industry; ~80% of the medicinal plant species are procured from wild areas; and 10% of medicinal plants involved in active trade are obtained from cultivation in farms [80]. The western Himalayan region provides about 80% of herbal drugs in Ayurveda, 46% of Unani, and 33% of allopathic systems [81]; 50% of drugs recorded in the British Pharmacopoeia are related to medicinal plants growing in this region [82].

In India, approximately 25,000 plant-based formulations are used in traditional and folk medicines [83]. The number of plant species used in various IM is as follows: Ayurveda, 2,000; Siddha (a type of ancient traditional Indian medicine), 1,300; Unani (a system of alternative medicine first developed by the Islamic physician Avicenna in about 1025 CE), 1,000; homeopathy, 800; Tibetan, 500; modern, 200, and folk, 4,500 [84]. More than 7,500 plant species are currently used in IM, including tonics, antimalarials, antipyretics, aphrodisiacs, expectorants, hepatoprotectants, antirheumatics, and diuretics [85, 86], as well as for the therapy of certain central nervous system disorders [87, 88].

The IHMs are derived either from the whole plant or from different organs, like leaves, stem, bark, root, flower, seed, and so forth, but also include animals and minerals. Some drugs are prepared from excretory plant products such as gum, resins, and latex. Commonly used spices, herbs, and herbal

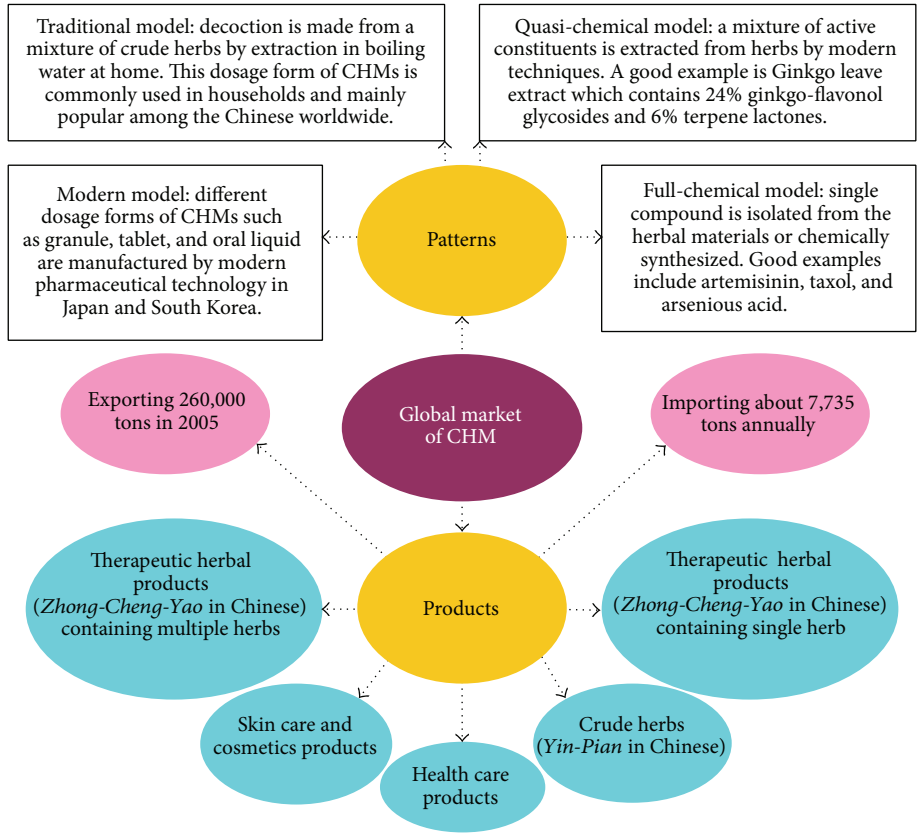

Figure 4: Styles of Chinese herbal medicine (CHM) in the global market (see also [62] of quasi-chemical model and [63–65] of full-chemical model).

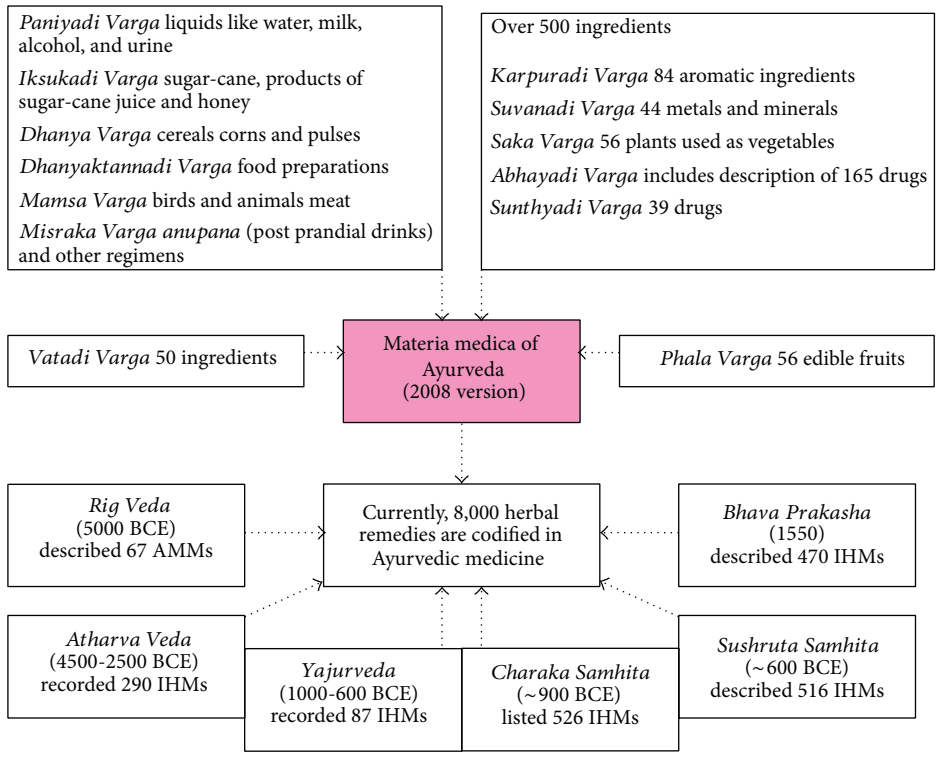

Figure 5: Some important texts of Indian herbal medicine [76–78].

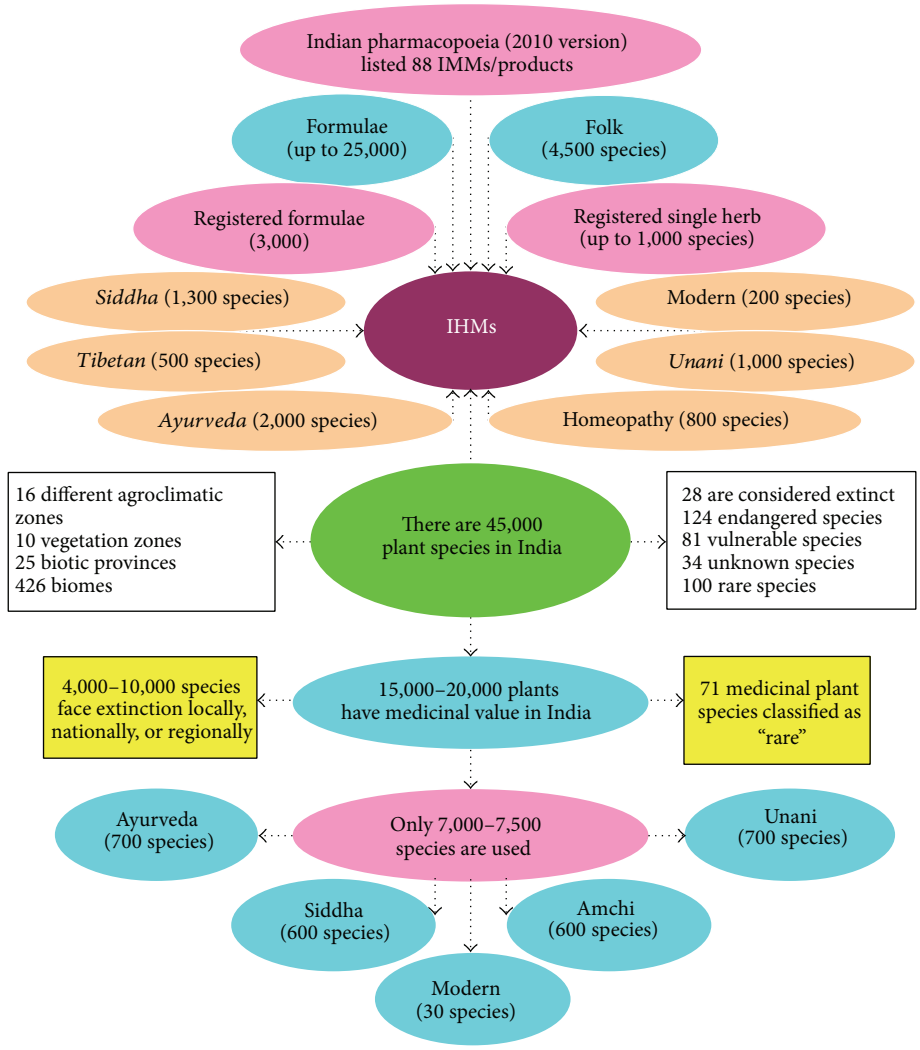

Figure 6: Plant species in India and Indian herbal medicine (IHM).

formulae are utilized for therapeutic interventions in about 28 kinds of chronic diseases in humans [89]. Special herbal preparations, known as *Rasayans*, are used for rejuvenation and retarding the aging process, thereby promoting longevity [90].

Of the 700 plant species commonly used in the Indian herbal industry, 90% of them are collected from the wild. About 50% of the tropical forests, the treasure house of plant, and animal diversity have already been destroyed. Many valuable medicinal plants are on the verge of extinction. The *Red Data Book of India* in 1997 has 427 entries of endangered species of which 28 are considered extinct, 124 endangered, 81 vulnerable, 100 rare, and 34 insufficiently known species [91]. The *Red Data Book of India* released in 2012 described 3,947 species as "critically endangered", 5,766 as "endangered", and more than 10,000 species as "vulnerable" [92] (Figure 6).

3.3. Pharmaceutics of IHM. Compared to those of CMM, AYM possesses very complex formulae consisting of 30 or more ingredients. In the formula, a number of ingredients, which are properly processed for pharmaceutical application, are chosen to balance the three humoral doctrines ("*Vata*", "*Pitta*," and "*Kapha*"). Herbs used in AYM include essential oils extracted from plants, fruits, vegetables, and common spices. The crude herbal material may be ground into powders and put into capsules, cooked into teas, used topically, taken raw, and so forth. IMM preparations on the market and/or Ayurvedic medical practice are complex mixtures including plant- and animal-derived products, minerals, and metals, as well as involving several specific preparatory steps or manufacturing processes.

Kasthoushadhies (herbal preparations) and *Rasaoushadhies* (herbo-bio-mineral-metallic preparations) are the two major groups of IMM preparations [93]. The latter has a metallic base but ordinarily does not contain active metal, since the metal is converted into an ash or oxide and forms an organometallic compound with a number of organic materials used for trituration as *Bhavana Dravya* [94, 95].

Medicinal principles are present in different parts of the plant such as root, stem, bark, heartwood, leaf, flower, fruit, or plant exudates. Generally, the herbal remedies can be

in various crude dosage forms like pills, powders, essential oil, infusions, or poultices. AYM believes that *Sandhana kalpana* (biomedical fermented formulations), a unique and complex dosage form containing acidic and alcoholic fermented components, is one of the most effective dosage forms of Ayurveda in practice for thousands of years [96]. During the fermentation process of liquid basic drugs, such as juices or decoctions, alcohol is produced by in-source material used in pharmaceutical procedure. Thus, extraction of active principles of the herbal drugs is done through self-generated alcohol. This formulation has longer shelf life, quick absorption and action, and excellent therapeutic efficacy as compared to other preparations [97].

The specific media are usually used in the manufacturing process of IMM products according to the different preparation. This plays a very important role in either breaking down the chemical compound(s) that is not required or forming the novel active ingredient(s) that is of value to the people, for example, *Shodhana* (purification/potentiation) of particular poisonous herbs, like *Gomutra* (cow's urine) for *Shodhana* of *Vatsanabha* (*Aconitum ferox* Wall.) and *Godugdha* (cow's milk) for *kupeelu* (*Strychnos nux-vomica Linn*.) [98]. On the other hand, Ayurvedic drugs are usually administered orally along with vehicle materials (*Anupana*) such as honey, sugar, jaggery, ghee, milk, warm water, and juice of some medicinal herbs. These Ayurvedic *Anupana* (i.e., drug vehicles serving as a medium of administration) can improve acceptability and palatability and help in absorption of the main herbal remedy; moreover, they may also act as an early antidote (Figure 7).

3.4. The Status Quo of IHM. The treatment of disease by Ayurveda is highly individualized and depends on the psychophysiologic status of the patient, particularly in relation to the season of the year [101]. Currently, more than 600 herbal formulas and 250 single plant drugs are included in the "Pharmacy" of Ayurvedic treatments [102]; about 1,000 single herbal remedies and 3,000 compound herbal formulations are registered in India. More than 600 herbal formulae and 250 single plants are included in the "Pharmacy" of Ayurvedic treatment [103]. The 6th *Indian Pharmacopeia* released in 2010 recognized 55 crude herbal drugs, 26 extracts, 3 finished formulations, and 2 pharmaceutical aids that are marketed [104].

According to a study commissioned by the Associated Chamber of Commerce and Industry, the Indian herbal industry is projected to double to 150 billion Rs. by 2015, from the current 75 billion business [105]. In the 1990s, the annual sales of the Indian herbal industry were about 23 billion Rs. (as compared to 145 billion Rs. in the pharmaceutical industry), with a growth rate of 15% [106]. By the end of 2012, the domestic market is expected to reach 145 billion Rs. and the export market 90 billion Rs., with compound annual growth rates of 20 and 25%, respectively [105]. The export market for medicinal plants appears to be growing faster than the Indian domestic market.

India not only has a great role to play as a supplier of herbal products for the domestic market, but it can also benefit from the tremendous potential afforded by overseas markets. Currently, the Indian herbal market is valued at 70 billion Rs., and over 36 billion Rs. worth of raw herbal materials and herbal products is exported [106]. The export of crude herbal extracts amounted to US $80 million, and the total sales of herbal products added up to US $1 billion [107]. Among the exported herbal products, 60% are processed plant materials that are unique to India, 30% are plant extracts, and 10% are Ayurvedic preparations [108]. The plant-derived pharmaceuticals exported from India include isabgol, opium alkaloids, senna derivatives, vinca extract, cinchona alkaloids, ipecac root alkaloids, solasodine, diosgenin/16DPA, menthol, gudmar herb, mehdi leaves, papian, rauwolfia guar gum, jasmine oil, agar wood oil, and sandal wood oil [109]. However, the export of 29 medicinal plants, including plant parts and their derivatives/extracts, obtained from wild sources, is prohibited by the Indian government [109]. Indian herbal medicine has now become a rich source of innovative drug discovery [110].

In India, the turnover of IHM industry is estimated to be more than 88 billion Rs; the domestic market is of the order of 40 billion Rs with a total consumption of all IMMs to a figure of 177,000 metric tons (MT). India has 9,493 HM manufacturing units, but 8,000 of them are small scale, one having an annual turnover of less than 10 million Rs. Some of the well-known units (with an annual turnover of more than 500 million Rs.) include Dabur, Zandu, Himalaya, Shree Baidyanath, and Arya Vaidya. They consume about 35% of the total raw IHMs [111].

4. Arabic Herbal Medicine (AHM)

It is well known that ancient Hippocratic-Greek medical knowhow was adapted and improved by Arabian herbalists, pharmacologists, chemists, and physicians in the Middle Ages. Furthermore, the majority of Arabs are Muslims, and Arabic culture and Islamic ideology are closely related. As such, Arabic medicine/materia medica/herbal medicine (AM/AMM/AHM) may also be called Greco-Arab or Islamic medicine.

4.1. Achievements of AM. The Arabic world used to be the center of scientific and medical knowledge for many centuries (from 632 to 1258 CE) after the fall of the Roman Empire. *The Legacy of Islam* (published in 1931; edited by the late Sir Thomas Arnold and Alfred Guillaume) states, "Looking back we may say that Islamic medicine and science reflected the light of the Hellenic sun, when its day had fled, and that they shone like a moon, illuminating the darkest night of the European Middle Ages; that some bright stars lent their own light, and that moon and stars alike faded at the dawn of a new day: the Renaissance. Since they had their share in the direction and introduction of that great movement, it may reasonably be claimed that they are with us yet." [112].

During the middle ages, AM contributed greatly to the development of modern medicine and pharmacy in Europe.

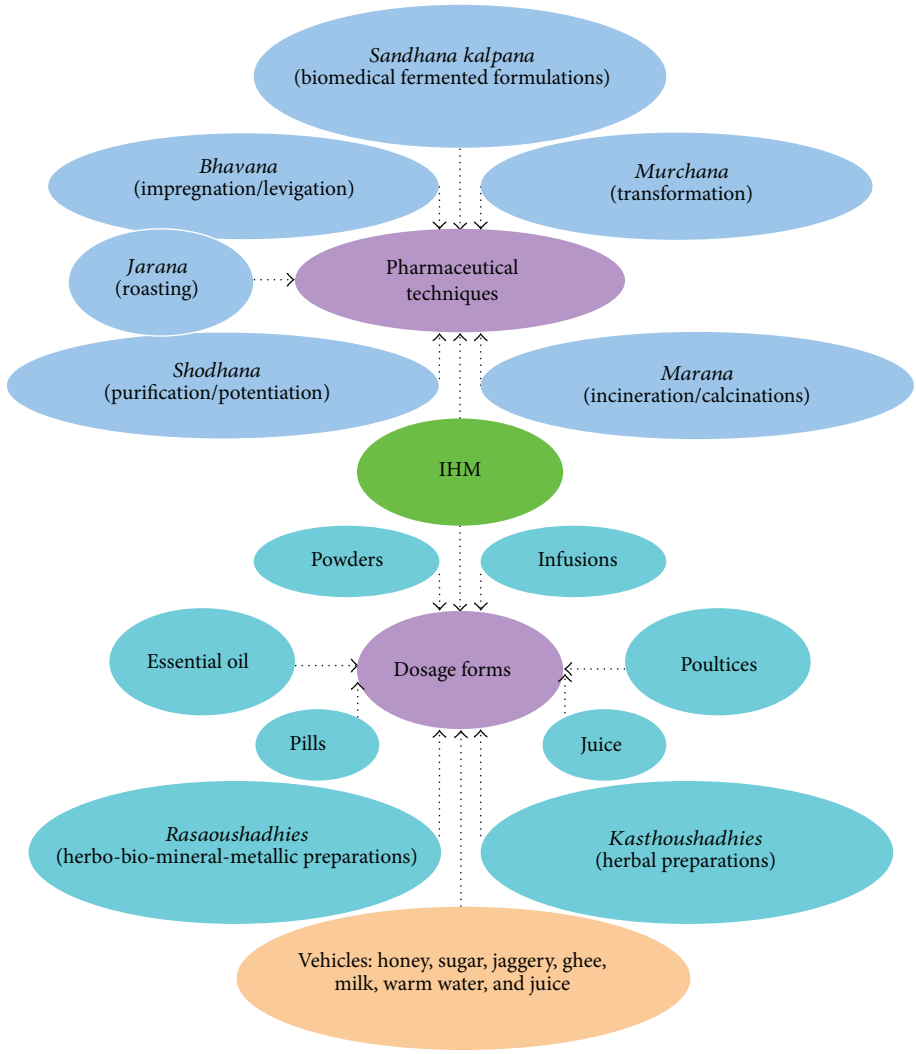

Figure 7: Dosage forms and pharmaceutical techniques in Indian herbal medicine (IHM) [99, 100].

For instance, the European pharmacopoeia relied on Muslim writings and information therein until the late 19th century. Despite the scarcity of medical knowledge in the Koran, Arabs adopted the ancient medical practices that originated from Mesopotamia, Greece, Rome, Persia, and India (or even China) [113, 114]. In the early 11th century, Avicenna (980–1037), a great philosopher and physician, incorporated a number of Chinese herbal preparations in his book *Pharmacopoeia*. Ancient Arabs established their "Pharmacy" on the basis of physicochemical techniques such as evaporation, filtration, distillation, sublimation, and crystallization used in "alchemy" which was invented by the Chinese [115].

Alchemy is the predecessor of chemical discipline that led to the development of natural science in modern times. Therefore, China is regarded as one of the key players in advancing modern civilization, particularly in the area of scientific methodology. It has been stated that if Greece was the theoretical founder of modern civilization, the *Qin/Han* dynasty in ancient China was the technical founder of modern natural sciences. Alchemy was invented during the period of Warring States in ancient China, but it vanished for no apparent reason in the middle of the *Tang* Dynasty. Nevertheless, the spirit of exploration of the ancient Chinese is praise-worthy. Several inventions by Taoist alchemists, such as cinnabar (*Zhu-Sha*), orpiment (*Ci-Huang*), and realgar (*Xiong-Huang*) in CHM, particularly gunpowder, have had a far-reaching impact on modern medicine and on the world in general [115].

Although AM was at the forefront of medical knowledge in Renaissance Europe of the 15th century, unlike CM and IM, its herbal medicine was not well developed from the start. The theory of AM is based on the "humours" of Hippocrates and Galen. There were more "modern" than "traditional" elements in AM; therefore, it played a pivotal role in the early formation and development of modern medicine. AM mainly integrated various herbal medicines and related technologies that originated from other countries and regions and established the foundation for the development of medicine

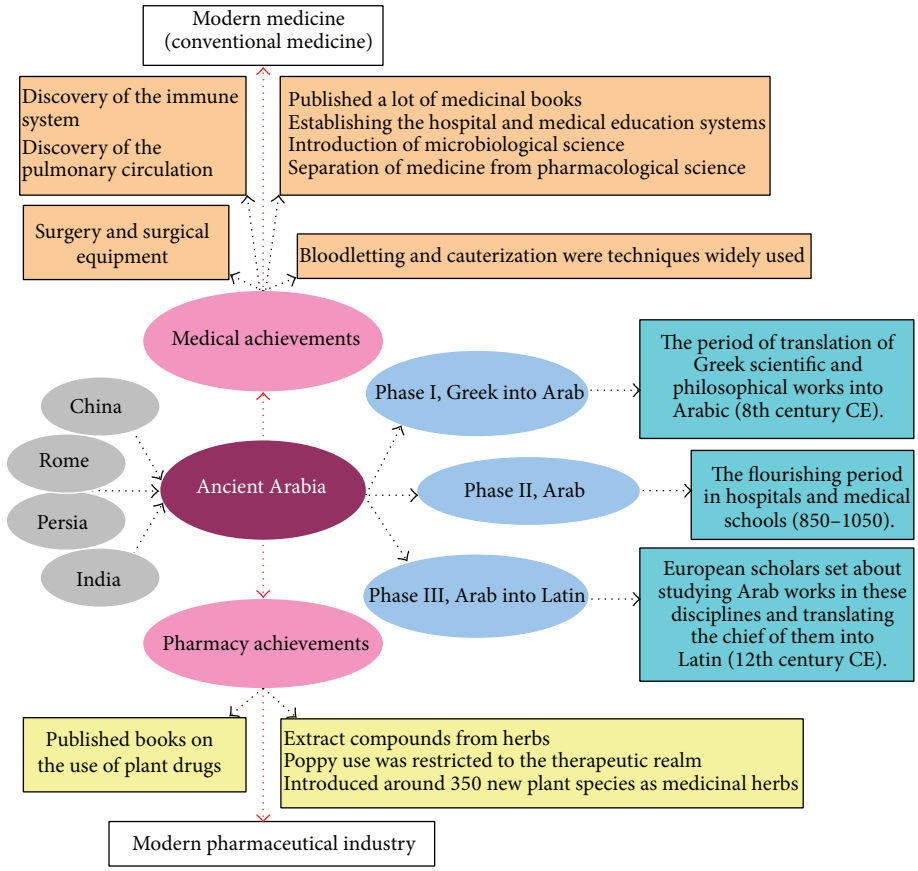

Figure 8: Achievements of Arabic medicine and pharmacy [118, 119].

and pharmacy in modern medicine [116, 117]. Therefore, AM carried on the past heritage and opened up the future in the history of the development of human medicine (Figure 8).

4.2. Past and Present of AHM. During the 8th century, Arabs in the Baghdad region were the first in history to separate medicine from pharmacological science. The world's first drug stores were established in the Arab world (Baghdad, 754 CE). The forms employed in that period are still used in therapy, and some formulations of drugs can be found in pharmacopoeias even today [120]. The earliest records of herbs, which were written on clay tablets in cuneiform, were from Mesopotamia (dating back to 2600 BCE). The best known Egyptian pharmaceutical record is the *Ebers* Papyrus (dating back to 1500 BCE), which documented some 700 herbal medicines (mostly from plants), with dosage forms including gargles, snuffs, poultices, infusions, pills, and ointments and vehicles using beer, milk, wine, and honey [121].

Since the 8th century, the practice of AHM has been using natural remedies, both organic (such as camel urine) and inorganic types, for the prevention and treatment of diseases [122]. Interestingly, pharmacological studies have revealed that camel urine treatment caused a significant cytotoxic effect on bone marrow cells in mice [123]. The Middle East region is inhabited by more than 2,600 plant species, of which more than 700 species are noted for their use as medicinal herbs or botanical pesticides; however, only 200–250 plant species are still in use in traditional Arab medicine for the treatment of various diseases [124]. Plant species from the western Mediterranean coastal region (from Alexandria to Sallum, Egypt) comprise 230 species belonging to 48 families; 89% of the species had medicinal value, 62% of the species were common, approximately 24.9% were occasional, and 13% were rare [118].

Until now, 236 plant species, 30 animal species, 29 organic substances, and 9 materials of other or mixed origins are still being used in treating human diseases and are sold or traded in the Mediterranean region and/or in the global market [124]. A survey of the plant species in the Mediterranean region by ethnopharmacologists indicated that 250–290 plant species are still in use [125, 126]. In Israel, 129 plant species are used in AM for the treatment of various diseases. Among these plants, there are 40 species used for treating skin diseases, 27 species for treating digestive disorders, 22 species for treating liver diseases, 16 species for treating respiratory diseases and coughing, 22 species for treating various forms of cancer, and 9 species for weight loss and lowering cholesterol [127]. However, more than 1,400 kinds of herbal medicines were used by Islamic physicians during the period of the Arab Empire (632–1258).

Figure 9: Past and present of Arabic herbal medicine [130].

The dosage forms utilized in AHM include decoction, infusion, oil, juice, syrup, roasted materials, fresh salads or fruits, macerated plant parts, milky sap, poultice, and paste, of which some formulations of herbal drugs are still used today [128]. Although AHM is the first choice for many people in dealing with ailments in the Middle East, most of the herbalists (such as those in Jordan), who acquire the expertise from their predecessors, are not properly trained in herbal medicine [129] (Figure 9).

In contrast to CHM or IHM, the physical characteristics of the herbal size, shape, color, texture, and taste traditionally served as important criteria in their selection for therapeutic purposes. For example, seeds with kidney shape are used for treating kidney stones; roots shaped similar to the human body or fruits that resemble human testicles are traditionally used to stimulate sexual desire or treat sexual weakness; a yellow decoction or juice obtained from herbal leaves is used for treating jaundice and liver diseases [131, 132].

5. Discussion

In this section, two important issues related to herbal medicine are discussed.

5.1. The Theoretical Advantages of Herbal Medicine. Due to shortage of scientific evidence on the molecular mechanism of herbs, it is often considered as only an alternative choice to conventional drugs. Here, we attempt to describe the feasibility and superiority of herbal medicine containing complex and multicompounds as medication using logical concepts in philosophy.

Currently, multidrug therapy or polypharmacy, also known as multiple drug intake or cocktail treatment, which involves therapeutic interventions using combinations of drugs (herbal versus chemical, herbal versus herbal, and chemical versus chemical) through pharmacokinetic and pharmacodynamic pathways or both [133–136], is commonly practiced in clinical situations. It is believed that multidrug therapy produces beneficial effects that do not occur when using each drug alone. Due to the additive and/or synergistic interactions among the drugs, or the suppression of adverse effects, multidrug therapy appears to be effective in treating diseases such as cancer, AIDS, malaria, diabetes, hypertension, MRSA, and chronic diseases associated with old age. Nevertheless adverse drug reaction (ADR), another important public health problem, may be enhanced after multidrug combination treatment through not only drug-drug interaction, but also herb-herb or drug-herb interaction [137, 138]. For example, as a monotherapy, St John's wort extract has an encouraging safety profile. However, in some cases, life-threatening interactions were reported when used together with other drugs [139]. Therefore, the possibility of drug-drug interaction (DDI), including both beneficial effects and ADR, has caused the FDA and European Medicines Agency (EMA)

to encourage the industry to perform drug interaction studies [140]. In the new postgenomic era DDI can be predicted with the data from pharmacogenetic information which may have an important implication for the development of personalized medicine and drug R&D for clinic and pharmaceutical industry, respectively [141].

More often than not, the pathogenesis of diseases is related to multiple targets rather than a single target. Asai et al. found that nonsteroidal anti-inflammatory drugs, cholesterol-lowering statins, and β- or γ-secretase inhibitors can produce additive effects on the reduction of Aβ-amyloid levels in cultured neuronal cells [142]. Combination therapy of PA-824-moxifloxacin and pyrazinamide can kill over 99% of drug-sensitive and multidrug-resistant *Mycobacterium tuberculosis* in patients with tuberculosis (TB) within 2 weeks. However, at present, the treatment of patients with TB or multidrug-resistant TB using conventional drug therapy requires 6 or 18–24 months, respectively [143]. A polypill containing amlodipine, losartan, hydrochlorothiazide, and simvastatin produces a significant effect in preventing heart attacks and strokes [144]. *Liu Wei Di Huang Wan* (Rehmannia Six Formula), which is a well-known Chinese herbal formula used for the treatment of 137 kinds of diseases in China, consists of six Chinese herbs: *Radix Rehmanniae* nourishes kidney *Yin* and *essence* (minute substances for supporting life); *Fructus Corni* nourishes the liver/kidney and restrains the leakage of the *essence*; *Rhizoma Dioscoreae* tonifies spleen *Yin* and consolidates the essence; *Rhizoma Alismatis* promotes urination to prevent buildup of significant fluids; *Poria* drains dampness from the spleen; *Cortex Paeoniae* clears liver fire [145–147]. Therefore, the multitarget herbal formula can produce a wide range of therapeutic effects.

Herbal formulations evolved from thousands of years of experience in practicing herbal medicine. While therapeutic interventions using multiple drugs in modern medicine are based on an understanding of disease processes and drug mechanisms, the use of multicomponent herbal formulae (*Fu-Fang* in Chinese herbal medicine) is based on CM theory and practical experience. Unlike using a single drug in orthodox medicine, raw plant or plant extracts contain an array of bioactive ingredients (a single plant contains 100–1,000 compounds of 20–50 different structure types) that can produce additive and/or synergistic actions [148]. The multi-ingredient herbal drug/formula allows for a multitarget interaction in treating diseases. For instance, the common cold is a viral infectious disease of the upper respiratory system, which primarily affects the nasal cavity. However, cold symptoms typically include coughing, sore throat, runny nose, headache, fever, and discomfort in the entire body. So far, no single chemical entity can simultaneously alleviate all clinical manifestations of common cold. Therefore a typical over-the-counter cold remedy is composed of multiple drugs, such as aspirin (A), phenacetin (P), and caffeine (C), in an APC tablet.

One and one can add up to more than two. Therefore, herbal treatment resembles a cocktail treatment or "magic shrapnel" (multidrugs act on multiple targets) [149]. The chemical compounds residing in an herbal drug or formulation work together within the body to maintain health and/or fight against diseases. The concept of synergism in modern pharmacology encompasses two aspects: (1) pharmacodynamic synergy results from the enhancement of action when multiple biologically active substances are directed at related targets in a physiological system, which are often linked to the pathogenesis of a disease and (2) pharmacokinetic synergy can result from alterations in drug absorption, distribution, metabolism, and/or elimination (Figure 10).

5.2. Resource Conservation in Herbal Medicine. Excessive medical treatment and medication, including the consumption of herbal medications, is a global trend, especially in developed countries. Countless facts have indicated that herbal preparations or formulations can be used for the treatment of many common as well as complex diseases for all ages, with a minimum of adverse side effects compared to conventional drugs. Together with the long history of their use, plant-derived herbs and herbal products are gaining popularity in the global market as registered drugs, dietary supplements, health care products, cosmetics, and so forth. Medicinal plants are highly esteemed as a rich source of new therapeutic agents for the prevention and treatment of diseases. Nowadays, the public acceptance of herbal medicine increases not only in Asian countries (49% in Japan, 45% in Singapore, 70% in China, and 80% in India), but also in western countries [150]. The sales of herbal drugs or related products are expected to increase at an annual rate of 6.4%. In the USA, the use of herbal products by consumers was less than 5% in 1991, but it increased to 50% in 2004, and the amount of botanical remedies constitutes as much as 25% of total medications. According to a WHO report, the global market value of herbal products to date is US $61 billion, but it is predicted to grow to US $5 trillion by 2050 [151]. The market shares in Europe and the USA are 41 and 20%, respectively [152].

Of the 250,000 higher plant species on earth, more than 80,000 are of medicinal value even in the genome era. In Brazil, it is estimated that there are almost 55,000 native species, at least 1,200 documented medicinal plants, and probably many more undocumented species used by various indigenous groups [153]. It can be expected that natural medicines, particularly herbal medicine, will make a growing or even a decisive contribution to human health care again. By 2001, researchers had identified 122 compounds used in modern medicine which were derived from plant/herb sources. Of these, 80% have an ethnic medical use which is identical or related to the current use of the active component(s) of the plant [154]. Some of these compounds include tubocurarine, morphine, codeine, aspirin, atropine, pilocarpine, ephedrine, vinblastine, vincristine, taxol, podophyllotoxin, camptothecin, digitoxigenin, gitoxigenin, digoxigenin, capsaicin, allicin, curcumin, and artemisinin. Unfortunately, many plant species on earth have become endangered as the consumption of herbs and herbal products continues to increase world-wide.

Traditional herbal medicine uses remedies derived from plants, animals, metals, and minerals. If herbal resources are inappropriately exploited, the extinction of many plant

Figure 10: Pharmacodynamic synergism and pharmacokinetic synergism of herbal medicines.

species will inevitably occur, with a resulting adverse alteration of the ecological environment. For example, for more than 30 years wild *Panax notoginseng* has no longer been found in Yunnan province (the origin of the plant) in China [155]; the acquisition of one kilogram of wild licorice will destroy 8–10 acres of grasslands [156]; digging of one Cordyceps can cause direct damage to about 30 cm^2 of grassland [157]. To treasure and maintain the gifts from mother nature (Hindu philosophy regards the Earth as a living being, i.e., mother nature), governments should install measures to ensure the ethical exploitation of herbal resources in their countries or societies. Therefore, it is high time to formulate strategies to avoid the overexploitation of herbal resources.

6. Concluding Remarks

Since ancient times, disease has been a leading cause of morbidity/mortality, and it is associated with a heavy economic burden among people with diseases. Despite current advances in science and medicine, disease remains a serious threat to public health in both developed and developing countries, urban and rural areas, and all ethnic groups. Ancient and modern people take medicines to fight illness or to feel better when they are sick. Most medicines (conventional drugs) at present are chemically synthesized and some are isolated from naturally occurring plants on the basis of their use in traditional medicine. However, our ancestors took only certain kinds of specific natural remedies to fight or prevent a specific illness. Because modern drug development is a high-risk (and therefore high-failure) commercial endeavor and synthetic drugs have a high rate of adverse events; there is a universal trend of using herbal medications or related products.

Based on cultures and geographical regions, various kinds of herbal remedies have evolved. Herbal medicines are therefore an integral part of culture and geographical environment, and various kinds of herbal medicines have their own unique way of understanding and treating a disease. However, the globalization of trade and market has brought about an integration of different kinds of herbal medicines over the world. At present, herbal medications or related products in the global market are derived from Chinese herbs, Indian herbs, Arabic herbs, and Western herbs. Herbal remedies may also be classified into three categories, namely, modern herbs, theoretical herbs, and empirical herbs, in accordance with their nature/characteristics and the nature of current usage [158]. As for the medications derived from herbs, they no longer belong to any herbal series or category and have essentially become equivalent to conventional drugs. In general, most herbal remedies/formulae are considered to be safe and are well tolerated because they have been

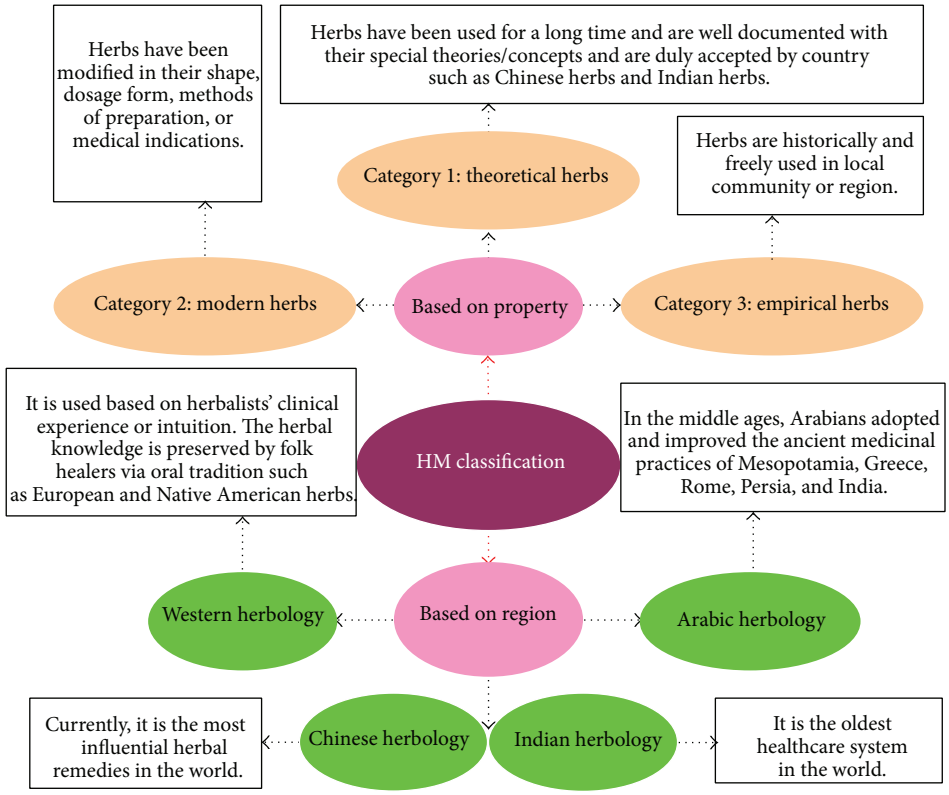

Figure 11: Classification of herbal medicines (HMs) in the international market.

successfully used for thousands of years as foods to promote health and as medicines to treat diseases. To date, herbal products are widely available to consumers and have become increasingly popular throughout the world. There is no doubt that herbal products will continue to play a crucial role in the health care system of human societies, not to mention that secondary metabolites of plants are economically important as drugs, fragrances, pigments, food additives, and pesticides (Figure 11) [159].

Conflict of Interests

The authors declared no conflict of interests with respect to the authorship and/or publication of this paper.

Authors' Contribution

Si-Yuan Pan, Gerhard Litscher, Si-Hua Gao, Shu-Feng Zhou, and Zhi-Ling Yu equally contributed to this work.

Acknowledgments

This paper was supported by the National Natural Science Foundation of China (Grant no. 31071989). The work has also been supported by Sino-Austrian Projects (Austrian Federal Ministries of Economy and Science and of Health) and the European Academy of TCM.

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Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2014, Article ID 735423, 11 pages
http://dx.doi.org/10.1155/2014/735423

Research Article

Phytotherapeutic Information on Plants Used for the Treatment of Tuberculosis in Eastern Cape Province, South Africa

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Received 2 February 2014; Revised 18 March 2014; Accepted 26 March 2014; Published 22 April 2014

Academic Editor: Gerhard Litscher

The current rate of deforestation in Africa constitutes a serious danger to the future of medicinal plants on this continent. Conservation of these medicinal plants in the field and the scientific documentation of our knowledge about them are therefore crucial. An ethnobotanical survey of plants used for the treatment of tuberculosis (TB) was carried out in selected areas of the Eastern Cape, South Africa. These areas were Hala, Ncera, Sheshegu, and Gquamashe, all within the Nkonkobe Municipality. One hundred informants were interviewed. The survey included the identification of scientific and vernacular names of the plants used for treatment of TB as well as the methods of preparation and administration, the part used, dosage, and duration of treatment. The survey revealed 30 plants belonging to 21 families which are commonly used by traditional healers for the treatment of TB and associated diseases. Of these plants *Clausena anisata, Haemanthus albiflos,* and *Artemisia afra* were the most cited. The leaves were the most common part used in the medicinal preparations. Our findings are discussed in relation to the importance of the documentation of medicinal plants.

1. Introduction

Tuberculosis (TB) is a fearful disease in developing nations, especially in the Asian and African continents, probably due to their inadequate means for the management and treatment of the disease. It is caused by a bacterium called *Mycobacterium tuberculosis*. TB infects nine million people every year most of them being children, and it leads to approximately two million deaths annually [1, 2]. These statistics are likely to increase in future because the human immunodeficiency virus (HIV) is entwined with TB and also due to the surfacing of multidrug-resistant strains of TB organisms [3–5].

TB is a very common disease in South Africa. According to reports, it is the fifth largest cause of death in this country [6, 7]. For example, approximately 285,000 cases of TB were reported in South Africa in 2005. In fact, South Africa has the seventh highest number of people suffering from TB in the world and the second highest in Africa [7]. In addition, the country has the fifth highest burden of drug-resistant tuberculosis cases in the world [8].

Africa is endowed with an enormous wealth of plant resources [9]. Herbal remedies from these plants have contributed to the reduction of excessive mortality, morbidity, and disability brought about by diseases such as HIV/AIDS, malaria, tuberculosis, sickle cell anaemia, diabetes, mental, disorder and microbial infections [10]. In addition to treating infectious diseases, phytomedicines have been reported to limit the side effects associated with synthetic antimicrobial drugs [11].

The Eastern Cape Province of South Africa is known for its richness in plant species [13]. The inhabitants of this province have a long history of traditional plant usage for the treatment of various diseases including TB [14]. Herbal medicine, being a significant element in the cultural patrimony, still remains the main recourse for a large majority of people for addressing health problems.

The aim of this study was to document the plant species used exclusively for the treatment of TB by the traditional healers in selected areas of Nkonkobe Municipality, Eastern Cape Province of South Africa (Figure 1).

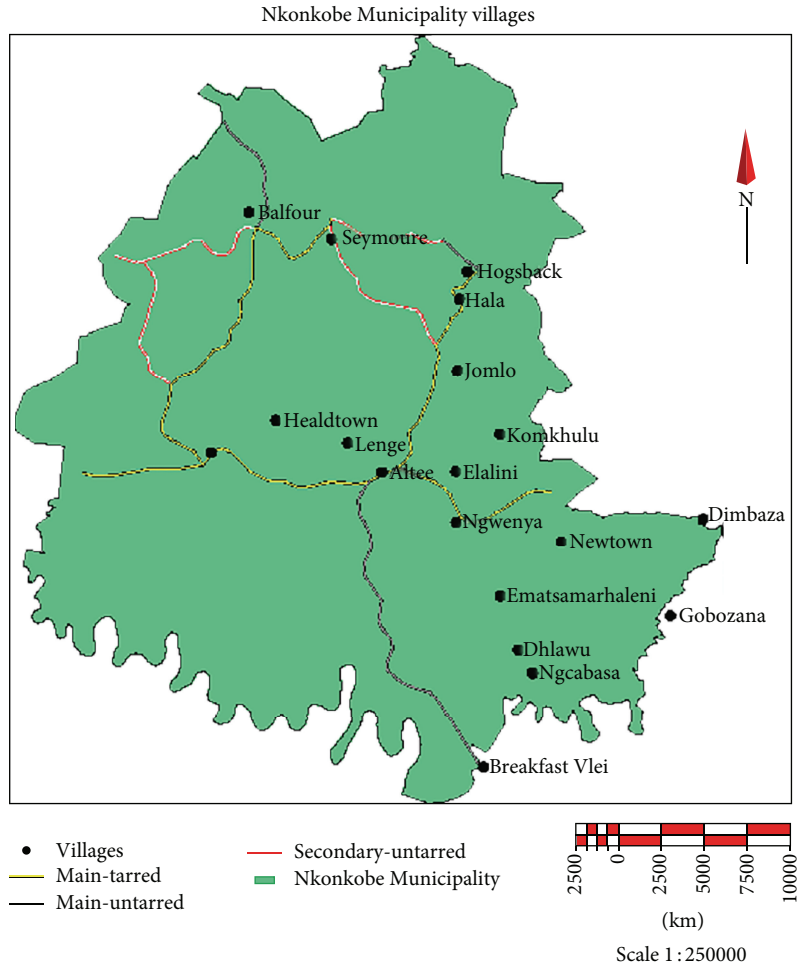

Figure 1: Nkonkobe Municipality, Eastern Cape Province, South Africa. Source: Omoruyi et al. [12].

2. Methodology

2.1. Study Area. The study area falls within Amathole district area latitudes from 30°00 to 34°15′S and longitudes from 22°C 45′ to 30°15′E. This area consists of many villages which are generally described as rural and poor with high prevalence of TB.

An ethnobotanical survey on medicinal plants was carried out in order to obtain phytotherapeutic information on plants useful for the treatment of tuberculosis in Eastern Cape Province, South Africa. This survey was conducted in the following villages within the Nkonkobe Municipality: Hala, Ncera, Sheshegu, and Gquamashe, using a well-structured questionnaire. One hundred informants were interviewed using semistructured questionnaire in order to obtain information from the rural dwellers including traditional healers known as *Sangomas* in xhosa speaking communities. Knowledgeable village elders who use medicinal plants were also consulted to provide information on the medicinal plants and their importance in the treatment of tuberculosis. This method proved to be a very viable and an effective option of data collection. The choice to employ this particular method was heavily influenced by the literacy levels, remote locations visited, and willingness of the respondents that supplied the information needed for the study. The questionnaire was designed to elicit information on the demographic structures of the respondents, names of plants commonly used in the treatment of tuberculosis, plant parts used, the methods of preparation, and therapeutic application including details on administration and dosages. Interviews were conducted in the local language of the respondents and, with the help of an interpreter, it was translated to English. Samples of the plant material used for the management of tuberculosis were collected from the wild. Scientific identification of samples collected was done in the Department of Botany, University of Fort Hare, Giffen Herbarium, where herbarium specimens are kept; and thereby reference to standard botanical classification and nomenclature [15, 16]. Identification was further confirmed by Professor D. S. Grierson, Department of Botany, University of Fort Hare. Plant species were grouped into their respective families along with local and common names.

2.2. Data Analysis. For quantitative analysis, an ethnobotanical index was used to evaluate the local importance of those

species in the study area using the relative frequency of citations (RFC).

The ethnobotanical data on plant species collected for this study were elaborated and analysed. The different plant species were grouped into their respective families, with information on most cited species, plant parts used method of preparation, therapeutic application, dosage, mode of use and mode of treatment was also provided. Quantitative analysis of the data was done to know the diversity of species of plants in the study area, to verify the potential of local knowledge (importance) of the communities studied. Therefore, from the citations, the number of species, the number of respondents who gave some information, and information on the plant species were supplied. The ethnobotanical indices are found on the basic structure of the ethnobotanical information. Data analyses were followed by ethnobotanical indexes using relative frequency of citation.

Relative frequency of citation (RFC) is used to find out probability between number of people who give citation to each species and number of all respondents. The result described local importance of each species. RFC was calculated thus

$$\mathrm{RFC} = \frac{\mathrm{FC}}{N}, \quad (1)$$

where RFC is the relative frequency of citation, FC is the number of respondents who gave citations at each species, and N is the number of respondents.

2.3. Intellectual Property Agreement Statement. The traditional healers who participated and also shared their wealth of knowledge on the information of plant usage during the ethnobotanical survey were adequately informed that this research shall not be for commercial purposes but will serve as a way of conserving indigenous knowledge as regards the traditional management of tuberculosis in Nkonkobe Municipality, Eastern Cape, South Africa. Ethical approval for the study was granted by the University of Fort Hare's Ethics Committee (UREC).

3. Results

In this study, 80% of the respondents were males and 20% were females (Table 1). Generally, older people (34%) were mostly engaged in the practice of using herbs for healing. This culture is still prominent in Africa. 80% of the respondents claimed to have inherited the healing knowledge from their parents and has become a norm in the family. Out of the 100 respondents, a total of 80 males (80%) and 20 females (20%) were interviewed. Most of the respondents were between the ages of 21 and 80. Only 8 respondents were between the ages of 21 and 30, 9 between 31 and 40, 12 respondents between 41 and 50, 19 were between 51 and 60, 20 respondents were between 61 and 70, and 32 respondents between 71 and 80. The majority of the traditional medicine practitioners had primary school education with 78% out of the total respondents, while 10% had secondary school education, 2% out of the total respondents had vocational education/training, and 10% had no education. This is an indication that in the study area (Nkonkobe Municipality) education within the tradomedical practitioner is still at infancy. Furthermore, the result revealed that all the respondents have experience in the treatment of TB in the villages. We are also informed that the patients are diagnosed by observing the rate of coughing or cough sputum with blood stain.

A total of 30 plant species belonging to 21 families were indicated as being used traditionally for the treatment of TB (Table 2). Rutaceae and Alliaceae have the highest proportion of species used for the treatment of TB and associated diseases. At least one of these plants was mentioned by two or more respondents to be contained in their recipes for the treatment of TB; of these plants, the most frequently mentioned were *Clausena anisata* Hook, *Haemanthus albiflos* L,

Table 1: Demographic characteristics of the respondents in the study area.

Characteristics	Frequency	Percentage (%)
Age (years)		
Less than 20	0	0
21–30	8	8
31–40	9	9
41–50	12	12
51–60	19	19
61–70	20	20
71–80	32	32
Total	100	100
Gender		
Male	80	80
Female	20	20
Total	100	100
Sources of information		
Ancestral	80	80
Others	20	20
Total	100	100
Years of experience		
Less than 5	3	3
6–10	4	4
11–15	7	7
16–20	20	20
Above 21	66	66
Total	100	100
Experience in treating TB		
Yes	100	100
No	0	0
Total	100	100
Level of education		
Primary education	78	78
Secondary education	10	10
Adult/vocational education	2	2
No education	10	10
Total	100	100

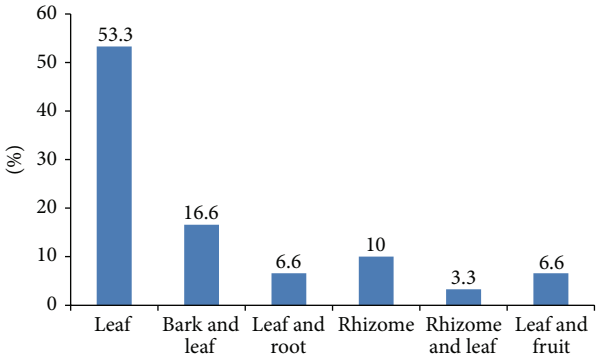

Figure 2: Variation in the parts of the plants used in the treatment of ailments.

Table 2: Distribution of plants family showing their percentage occurrence.

Family	Distribution	% occurrence
Alliaceae	3	10
Rutaceae	3	10
Apiaceae	2	6.6
Asteraceae	2	6.6
Lamiaceae	2	6.6
Myrtaceae	2	3.3
Rosaceae	2	6.6
Solanaceae	2	6.6
Aizoaceae	1	3.3
Amaryllidaceae	1	3.3
Apocynaceae	1	3.3
Asparagaceae	1	3.3
Cannabaceae	1	3.3
Caryophyllaceae	1	3.3
Dispaceae	1	3.3
Fabaceae	1	3.3
Hypoxidaceae	1	3.3
Moraceae	1	3.3
Rhamnaceae	1	3.3
Rubiaceae	1	3.3
Sapindaceae	1	3.3

Artemisia afra Jacq. ex Willd, *Carpobrotus edulis* (L.) Bolus, *Ptaeroxylon obliquum* Thunb, and *Tulbaghia violaceae* Harv. (Table 3).

The plant parts used in most of the herbal preparations include the leaves, leaves combined with bark, leaves combined with root, rhizome combined with leaves, and fruit combined with leaves. The leaves were the most frequently used (53.3%) (Figure 2) followed by the bark combined with leaves (16.6%) and rhizome (10%). The herbal medical practitioners, however, indicated that leaves are more effective than the other parts of the plants. They also claimed that it will take about 1–4 months for a TB patient to be healed during treatment. According to the healers, the healing of a patient begins with the reduction in chronic coughing and bloody sputum. An important finding from this study is that the inhabitants of the villages consult herbal practitioners because of their belief in holistic nature of treatment and the cost of treatment which is relatively cheaper than the orthodox medicines. Another finding was that the herbal medical practitioners believe that certain ailments are cured with the aid of herbal medicine and consultation with the ancestors for bewitched persons who are believed to be difficult to manage with orthodox medicine.

Table 3: Distribution of plant species according to the ethnobotanical survey.

Species	Number of occurrences
Clausena anisata	10
Haemanthus albiflos	7
Artemisia afra	6
Cannabis sativa	5
Carpobrotus edulis	5
Mentha longifolia	5
Ptaeroxylon obliquum	5
Tulbaghia violacea	5
Agathosma betulina	4
Hippobromus pauciflorus	4
Hypoxis argentea	4
Allium spp.	3
Capsicum frutescens	3
Corymbia citriodora	3
Daucus carota	3
Ficus spp.	3
Rosmarinus officinalis	3
Scabiosa albanensis	3
Tulbaghia acutiloba	3
Withania somnifera	3
Acacia karroo	2
Araujia sericifera	2
Bidens pilosa	2
Asparagus africanus	1
Centella coriacea	1
Prunus africana	1
Rubia petiolaris	1
Silene undulata	1
Syzygium cordatum	1
Ziziphus mucronata	1

3.1. Local Importance of the Plant Species Sampled. Based on the local importance analysis of each of the plant species used for the treatment of TB in the four villages in Nkonkobe Municipality (Table 4), *Clausena anisata* was the most useful plant species (RFC = 0.1) with 10 citations (10% of the informants). It was followed by *Haemanthus albiflos* (RFC = 0.07) with 7 citations, and also *Artemisia afra* (RFC = 0.006) *Clausena anisata* was the most cited plant in this study. The recipes for the treatment of tuberculosis within the study area were made up of plant parts such as leaves, bark, fruit, and root only. These recipes were prepared as concoctions,

Table 4: Result of analysis used by RFC for the medicinal plant species. $n = 100$.

No.	Species	Basic value		Ethnobotanical index	Ranking of RFC
		FC	*N* (%)	RFC	
1	*Clausena anisata*	10	10	0.1	1
2	*Haemanthus albiflos*	7	7	0.07	2
3	*Artemisia afra*	6	6	0.06	3
4	*Cannabis sativa*	5	5	0.05	4
5	*Carpobrotus edulis*	5	5	0.05	4
6	*Mentha longifolia*	5	5	0.05	4
7	*Ptaeroxylon obliquum*	5	5	0.05	4
8	*Tulbaghia violacea*	5	5	0.05	4
9	*Agathosma betulina*	4	4	0.04	5
10	*Hippobromus pauciflorus*	4	4	0.04	5
11	*Hypoxis argentea*	4	4	0.04	5
12	*Allium spp.*	3	3	0.03	6
13	*Capsicum frutescens*	3	3	0.03	6
14	*Corymbia citriodora*	3	3	0.03	6
15	*Daucus carota*	3	3	0.03	6
16	*Ficus spp.*	3	3	0.03	6
17	*Rosmarinus officinalis*	3	3	0.03	6
18	*Scabiosa albanensis*	3	3	0.03	6
19	*Tulbaghia acutiloba*	3	3	0.03	6
20	*Withania somnifera*	3	3	0.03	6
21	*Acacia karroo*	2	2	0.02	7
22	*Araujia sericifera*	2	2	0.02	7
23	*Bidens pilosa*	2	2	0.02	7
24	*Asparagus africanus*	1	1	0.01	8
25	*Centella coriacea*	1	1	0.01	8
26	*Prunus africana*	1	1	0.01	8
27	*Rubia petiolaris*	1	1	0.01	8
28	*Silene undulata*	1	1	0.01	8
29	*Syzygium cordatum*	1	1	0.01	8
30	*Ziziphus mucronata*	1	1	0.01	8

FC: number of informant who gave citation at each species, *N* (%): the number of participants mentioning the use of the plant species as medicine for the treatment of tuberculosis in percentage of the total participants, RFC: the relative frequency of citation.

macerations, and infusions while the mode of administration is oral (Table 5).

4. Discussion

4.1. Plant Use Based on Indigenous Knowledge. Indigenous knowledge (IK) is one of unique experiences applied to traditional knowledge that is transferred to younger generation and is still developed by rural indigenous communities in specific geographical areas. The characteristics of IK come into view and are developed in specific society; they are unique and exclusive [17].

The observation that a greater proportion of the informants were males could be attributed to the fact that male respondents were bold and courageous to talk and have rapport with the interviewer unlike their female counterparts who prefer to shy away.

Generally, older people were mostly engaged in the practice of using herbs for healing. This information on the age of the respondents implies that youths in the study areas are not fully engaged in the traditional medicine practice and this suggests a breakdown in dissemination of knowledge between the old and younger generation. This simply implies that with time this knowledge may be lost (due to the death of the old people with vast knowledge of herbal medicine) unless efforts are made to reverse the situation.

A great proportion of the plant species documented have been validated through phytochemical and pharmaceutical research; some, although, not evaluated for their efficacy are used to treat TB and opportunistic diseases associated with tuberculosis in South Africa and other parts of the world. For instance, *Artemisia afra* is used by the traditional healers in Amathole District to treat Flu and TB, and it is also used by the Zulu people [18–20]. The leaf extract of *Haemanthus albiflos* was reported to have shown significant difference against DNA viruses and all RNA viruses [21] likewise the leaf of *Carpobrotus edulis* was reported to have showed significant difference for the treatment of TB and as

Table 5: Plant species used for the treatment of TB.

Botanical name	Family	Local name	Plant part used	Method of preparation	Therapeutical application	Dosage, mode of use, and duration of the treatment	Development of plant for use	Plant status
Agathosma betulina (Berg)	Rutaceae	Ibuchu	Leaf	3000 mL of boiled poured on 500 g tender leaves collected freshly; it was allowed to steep for 30 minutes before taken	Chronic cough	150 mL of the extract taken orally 2 × 2 for 3 weeks	Tender leaf	Fresh
Artemisia afra (Jacq. ex Willd)	Asteraceae	Umhlonyane	Leaf	2000 mL of boiled water was poured on 400 g tender leaves collected freshly; it was allowed to steep for 30 minutes before taken	Flu, excessive cough, tuberculosis	100 mL of the extract taken orally 2 × 1 for 2 weeks	Tender leaf	Fresh
Acacia karroo (Hayne)	Fabaceae	Umnga	Bark and leaf	2000 mL of hot water poured on 400 g air dried leaves, for 25 minutes, while the grounded bark can be also infused alternatively	Tuberculosis	75 mL of the infused plant substance taken orally. 1 × 1, for 1 month	Tender and mature	Air dried
Ptaeroxylon obliquum (Thumb)	Rutaceae	Umpafa	Leaf	The air dried leaves of the plant left in contact with the menstruum (Alcohol) for five days which was later sieve to get the extract for the treatment	Tuberculosis and Chest complaints	100 mL of the extract taken orally 3 × 1, for 3 weeks	Tender leaf	Air dried
Tulbaghia violacea (Harv.)	Alliaceae	Clausena anisata	Rhizome	This was extemporaneously prepared; cold water was added to 1000 g of air dried herbal substance and boiled under reduced temperature for 45 minutes	Ulceration of the lung	150 mL taken orally 3 × 1, for 3 weeks	Mature	Air dried
Prunus africana Hook.	Rosaceae	Umkakase	Bark and leaf	This was extemporaneously prepared; cold water was added to 1000 g of air dried bark and leaves of herbal substance, boiled under reduced temperature for 45 minutes	Whooping cough and Tuberculosis	150 mL taken orally for 3 × 1, for 3 weeks	Tender and mature	Air dried
Clausena africana (Hook)	Rutaceae	Ipereres	Leaf and Bark	2000 mL of hot water will be poured on 400 g air dried leaves, for 25 minutes. The grounded bark can also be infused alternatively	Tuberculosis and chest complaints	150 mL taken orally for 3 × 1, for 2 weeks	Tender leaf	Air dried

Table 5: Continued.

Botanical name	Family	Local name	Plant part used	Method of preparation	Therapeutical application	Dosage, mode of use, and duration of the treatment	Development of plant for use	Plant status
Haemanthus albiflos (L)	Amaryllidaceae	Istitibala	Leaf and root	The air dried leaf and bark of the plant will be soaked in alcohol for six days and will be shaken occasionally	Tuberculosis	75 mL taken orally 3 × 1, for 3 weeks	Mature and tender	Air dried
Mentha longifolia (L)	Lamiaceae	Inxina	Leaf	2000 mL of boiled water was poured on 400 g tender leaves collected freshly, allowed to steep for 30 minutes before taken	Ulceration of the lung	100 mL of herb taken orally 3 × 1 for 2 month	Tender leaf	Fresh
Hypoxis argentea (Fiscand)	Hypoxidaceae	Inongwe	Leaf	2000 mL of hot water will be poured on 400 g of fresh leaves and allowed to steep for 30 minutes	Tuberculosis	100 mL of the prepared herbs taken 3 × 1 for 3 weeks	Tender leaf	Fresh
Ficus sur (Forrssk)	Moraceae	Mngxam	Leaf	2000 mL of hot water poured on 400 g of fresh leaves and allowed to steep for 30 minutes	Ulceration of the lung and tuberculosis	100 mL of the prepared herb will be taken orally 3 × 1 for 1 month	Tender leaf	Fresh
Hippobromus pauciflorus (Radlk)	Sapindaceae	Mfazionegxolo	Leaf	2000 mL of hot water poured on 400 g of fresh leaves and allowed to steep for 30 minutes	Tuberculosis	100 mL of prepared herbs taken 3 × 1, for 2 months	Mature	Fresh
Araujia sericifera (Brot)	Apocynaceae	Impinda	Rhizome and leaf	2000 mL of hot water poured on 400 g of fresh leaves and matured rhizome of the plant and allowed to steep for 30 minutes	Tuberculosis	75 mL of prepared herbs taken orally 2 × 1, for 2 months	Mature	Fresh
Allium sativum (L)	Alliaceae	Ikoronofile	Rhizome	2000 mL of hot water poured on 400 g of fresh rhizomes and allowed to steep for 30 minutes	Bloody cough and ulceration of the lung	50 mL of the prepared herbs taken 2 × 1, for 3 weeks	Mature	Fresh
Rosmarinus officinalis (L)	Lamiaceae		Leaf	2000 mL of hot water will be poured on 400 g of matured leaves and allowed to steep for 30 minutes.	Tuberculosis	150 mL of the prepared herbs taken 3 × 1 for 3 weeks	Mature	Fresh
Cannabis sativa (L)	Cannabaceae	Umya	Leaf	2000 mL of hot water poured on 400 g of matured leaves and allowed to steep for 30 minutes	Tuberculosis	150 mL of prepared herbs will be taken orally 3 × 1 for 3 weeks	Mature	Fresh

Table 5: Continued.

Botanical name	Family	Local name	Plant part used	Method of preparation	Therapeutical application	Dosage, mode of use, and duration of the treatment	Development of plant for use	Plant status
Daucus carota (L)	Apiaceae		Leaf and fruit	2000 mL of hot water will be poured on 400 g of matured leaves and allowed to steep for 30 minutes. Or the leaves and the fruit can be soaked with alcohol for seven days	Tuberculosis	75 mL of the prepared herbs taken orally 3 × 1, for 1 month	Mature and tender	Air dried
Bidens pilosa (L)	Asteraceae	Imbikicane	Leaf and bark	This was extemporaneously prepared; cold water was added to 1000 g of air dried bark and leaves of herbal substance and boiled under reduced temperature for 45 minutes	Tuberculosis	75 mL of prepared herbs taken orally 3 × 1, for 3 weeks	Mature and tender	Air dried
Corymbia citriodora (L)	Myrtaceae		Leaf	2000 mL of hot water will be poured on 400 g of tender leaves and allowed to steep for 30 minutes	Tuberculosis	75 mL of prepared herbs taken orally 3 × 1, for 3 weeks	Tender leaf	Fresh
Ziziphus mucronata (Willd subsp.)	Rhamnaceae		Leaf	2000 mL of hot water poured on 400 g of tender leaves and allowed to steep for 30 minutes	Tuberculosis	75 mL of the prepared herbs taken 3 × 1, for 1 month	Tender leaf	Fresh
Capsicum frutescens (L)	Solanaceae	Ikhanakhana	Fruit and leaf	This was extemporaneously prepared; cold water was added to 1000 g of air dried fruit and leaves of herbal substance and boiled under reduced temperature for 35 minutes	Tuberculosis	50 mL of the prepared herbs taken orally 2 × 1, for 3 weeks	Mature	Air dried
Withania somnifera (L) Dunal	Solanaceae	Ubuvimba	Leaf	2000 mL of hot water poured on 400 g of tender leaves and allowed to steep for 30 minutes	Tuberculosis	150 mL of prepared herbs taken orally 3 × 1 for 3 weeks	Tender leaf	Fresh
Silene undulata (L)	Caryophyllaceae	Isilawu	Leaf	2000 mL of hot water poured on 400 g of tender leaves and allowed to steep for 30 minutes	Tuberculosis	75 mL of the prepared herbs taken orally 3 × 1, for 1 month	Tender leaf	Fresh

Table 5: Continued.

Botanical name	Family	Local name	Plant part used	Method of preparation	Therapeutical application	Dosage, mode of use, and duration of the treatment	Development of plant for use	Plant status
Scabiosa albanensis (L)	Dipsacaceae	Umsilawu	Leaf and root	The root and leaves of the plant left in contact with the menstruum (alcohol) for five days which was later sieved to get the extract for the treatment	Tuberculosis	50 mL of the extract taken orally 3 × 1, for 1 month	Mature and Tender	Fresh
Rubia petiolaris (DC)	Rubiaceae	Impendulo	Leaf	2000 mL of hot water poured on 400 g of tender leaves and allowed to steep for 30 minutes	Tuberculosis	150 mL of the prepared herbs taken 3 × 1 for 3 weeks	Tender leaf	Fresh
Tulbaghia acutiloba (Harv.)	Alliaceae	Isivumbampunzi	Rhizome	2000 mL of hot water poured on 400 g of air dried rhizomes and allowed to steep for 30 minutes	Tuberculosis	150 mL of the prepared herbs taken 3 × 1 for 3 weeks	Mature	Air dried
Asparagus africanus (Lam)	Asparagaceae	Ikhubalo	Leaf	Infusion 2000 mL of hot water poured on 400 g of tender leaves and allowed to steep for 30 minutes	Tuberculosis	150 mL of the prepared herbs taken orally 3 × 1 for 3 weeks	Tender leaf	Fresh
Centella coriacea (L)	Apiaceae	Unongotyozana	Leaf	2000 mL of hot water poured on 400 g of fresh leaves and allowed to steep for 30 minutes	Tuberculosis	100 mL of the prepared herbs taken orally 3 × 1 for 3 weeks	Tender leaf	Fresh
Carpobrotus edulis (L) Bolus	Aizoaceae	Unomatyumtyum	Leaf	2000 mL of hot water will be poured on 400 g of fresh leaves and allowed to steep for 30 minutes	Tuberculosis	100 mL of the herbs taken orally 3 × 1 for 3 weeks	Tender leaf	Fresh
Eucalyptus camadulensis	Myrtaceae	Gumtriya	Leaf and bark	2000 mL of hot water poured on 400 g of fresh leaves and barks and allowed to steep for 45 minutes	Tuberculosis/constant coughing	100 mL taken orally 3 × 1 for 3 weeks	Mature and tender	Fresh

Key: 3 × 1: thrice daily; 2 × 1: twice daily; 1 × 1: once daily.

immune booster for HIV patients in Nkonkobe Municipality [12, 20]. Furthermore, Lall and Meyer [22] reported that individuals infected with HIV/AIDS are also susceptible to TB and often develop this disease before other manifestations become apparent.

Cannabis sativa was also reported, through infusion and inhalation, to be used for the treatment of TB among the Zulu people, Hutchings et al. [23]. Leaf extract of *Eucalyptus camaldulensis* inhibited the growth of *Bacillus cereus*, *Escherichia coli*, *Klebsiella pneumonia*, and *Staphylococcus aureus* [24]. Madikizela et al. [25] documented *Asparagus africanus* and *Ficus sur* which showed positive result against *Mycobacterium tuberculosis*; similarly, Ghosal et al. 1985 [26] reported that species in the *Haemanthus* genus produce several alkaloids which have therapeutic effect against coughs and dropsy asthma and as topical antiseptics. Also extracts from several species of Amaryllidaceae plants have been found to possess pronounced antibacterial and antifungal activities [27].

To the best of our knowledge *Clausena anisata, Haemanthus albiflos, Asparagus africanus, Araujia sericifera, Scabiosa albanensis*, and *Silene undulate* were recorded for the first time for the management of TB in Nkonkobe Municipality, Eastern Cape Province. This investigation plays a significant role in the medicinal plant research in the context of management of TB and its opportunistic diseases in Eastern Cape Province. Hence, the above listed plants are widely used for treating different ailment; for instance *C. anisata* was reported to treat measles and bronchial problem in Nigeria [28]. In Tanzania, traditional healers use *Clausena anisata* against oral candidiasis and fungal infections of the skin [29].

Hutching et al. [23] reported that *C. anisata* leaf have been used for the treatment of respiratory ailments. This finding is in line with the present study which justifies the widespread use of *C. anisata* for the treatment of TB in Nkonkobe Municipality. York et al. [30] reported that the leaf extract of the plant was tested against *Cryptococcus neoformans, Klebsiella pneumonia, Moraxella catarrhalis, Mycobacterium smegmatis,* and *Staphylococcus aureus* in KwaZulu-Natal. The aqueous and methanol leaf extract of *C. anisata* was also reported in Ethiopia to have exhibited anti-antimycobacterium properties against *Mycobacterium tuberculosis* and *Mycobacterium bovis*.

5. Conclusion

The current study was undertaken to investigate local communities in Nkonkobe Municipality, Eastern Cape Province, as regard the treatment and management of TB. The documented medicinal plants used by the Xhosa herbalist reflect a rich ethnomedicinal knowledge in the municipality. These results strengthen the firm belief that traditional medicines are readily accessible and still play an important role in meeting the basic health care of many people in African communities.

Phytomedicinal information on the treatment of TB in this region is well established. Thirty plants belonging to 21 families were mentioned to be used for the treatment of TB and associated diseases. Other diseases treated using these plants were respiratory infections. The commonly mentioned species are *Clausena anisata*, *Haemanthus albiflos, Artemisia afra, Carpobrotus edulis, Ptaeroxylon obliquum,* and *Tulbaghia violacea*. The most frequently mentioned species was *Clausena anisata* known locally as *Iperepes*. Many studies have revealed some bioactive chemical compositions in these plants which probably justify their pharmacological properties. The following five species were recorded for the first time for the management of TB in Eastern Cape Province, South Africa: *Clausena anisata, Haemanthus albiflos, Araujia sericifera, Scabiosa albanensis, and Silene undulate*. This study has contributed to the scientific documentation of medicinal plants used for the treatment of TB. This is necessary in the rural communities to avert the erosion of traditional medicine knowledge. The larger percentage of the traditional healers is old people; therefore, this legacy needs to be conserved. Further studies are in progress on the antituberculosis assay to validate ethnopharmacology relevance of the most mentioned plants in the study area.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

This work was financially supported by Govan-Mbeki Research and Development Centre (GMRDC) of the University of Fort Hare, South Africa, and the Medicinal Plant and Economic Development Research Centre (MPED).

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Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2014, Article ID 393680, 6 pages
http://dx.doi.org/10.1155/2014/393680

Review Article

New Perspectives on Specific Immune-Depletion Technique Using Monoclonal Antibodies against Small Active Molecules in Herbs

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Received 6 January 2014; Accepted 16 February 2014; Published 17 March 2014

Academic Editor: Si-Yuan Pan

One of the main focuses in Chinese Medicine research is the identification of efficacious components in Chinese herbal medicine (CHM). Studies in such area are difficult due to the complexity and the synergistic characteristics of CHM. Current methods to track and separate active components are not adequate to meet the needs of revealing effects and identify substances and pharmacological mechanisms, which directly restrict the modernization and globalization of CHM. In this paper, a new methodology to deplete a single active component via immunoassay was introduced. The specific active component in a CHM mixture can then be identified and studied through comparative analyses of the pharmacological effects before and after immune depletion. With this new methodology, degree of contribution of a particular component to the whole complex herbal mixture can be elucidated, and its synergistic property with other components can be determined. The new method can reflect not only the overall combined pharmacological effects of CHM but also the effect of individual component. It is an effective way to explain the degree of contribution of one specific component to the overall activity of a CHM prescription.

1. Introduction

Complexities are the basic character of Chinese Herbal Medicine (CHM, Zhong Yao in Chinese). The composition of CHM is complex in terms of its chemical structure and therapeutic effects. The pharmacodynamics of CHM is generated from the combined effects of different herbal components in a complex CHM mixture. And such combination is a result of a linear or nonlinear accumulation of synergistic or antagonistic medicinal interactions in vivo. In order to properly and comprehensively study and understand the pharmacological mechanism and synergistic effects in CHM and to establish a standard CHM quality control (QC), it is vital to identify the most active component in any given CHM prescription, the "principal component" (PC). And how to accurately explain the relationship between the therapeutic effects of a CHM preparation and the variation in concentration of principal compound is one of the key questions that needed answers in CHM research. This will be a key to develop CHM with factual bases.

When it comes to establishing connections between a principal component and a primary medicinal property of a herb, simply focusing on one specific chemical compound is insufficient. The pharmacological action of a herb is far more sophisticated and every active component should be taken into consideration and analysed as a whole. Even though the dominant component may be the major contributor to the herb's pharmacological function, but its synergistic relationship with other components is just as important. Current existing methods are unsubstantial to satisfy the needs of material-based CHM research. New ideas and perspectives are needed to explore new techniques and applications.

The primary objective in the CHM research is to identify the principal component and determine its main contributions to the pharmacodynamics of component herbs. In recent years, with the advancements in detection method development and the application of "fingerprint" technology, researchers have successfully isolated and analysed the primary active components in herbs and herbal mixtures, but study on CHM trace elements has just begun. It was necessary to study herbal medicine in vivo and define the absorption, metabolism, and distribution in different organs, tissues and cells, but to reveal the whole medicinal component system was not easy. Moreover, after the absorption and metabolism, it was exceedingly difficult to identify the pharmacological mechanism in vivo because of the content descend and conformational change of active components. Due to the complexity of CHM, it has been difficult to examine its pharmacodynamics and pharmacokinetics in vivo. However, scholars in the field of CHM research continuously come up with better strategies and have made some breakthrough in the area [1, 2]. One practical and effective research strategy currently adopted in the field is using monomeric component as a starting point for quality control, pharmacokinetic analysis, and drug innovation [3].

2. Current Status on Material-Based CHM Research

Progress has been made in principal component identification [4–7], which leads to making great strides in both of pharmacodynamics and pharmacokinetics determinations in CHM research. Advancements have been made in correlating the pharmacological properties of primary active components in certain CHM prescriptions and their consistency of therapeutic effects [8]. Some scholars have introduced the best ratio of principal components in some CHM decoction and documented the influences of different decocting methods. These new research updates provide foundations for new experiments and assist in the establishment of standard CHM QC systems [9]. The degree of contribution of principal components in herbal medicine is still a high-profile and also a difficult topic in the CHM research field. Many questions have been raised. Can an identified principal component truly represent the therapeutic effect of a CHM prescription? What are the real-world applications of such principal component? In a complex CHM mixture, which component can serve as the standard QC? And how can altering the level of this component influence the efficacy of CHM? Much work is still needed to be done in the future.

The unique nature of CHM is created from the combined actions of different components. In order to achieve standardized QC, establish material-based pharmacodynamics, and analyse the metabolism of any of the CHM prescriptions, it is necessary to correlate the medicinal property of the principal component identified and the prescription's known therapeutic effects. And it is also necessary to correlate the amount of principal component present and the overall CHM therapeutic performance. A "quantity-effect" (QE) relationship of the principal component can then be generated and studied. This QE relationship can then be further applied into other different research aspects, such as the metabolism, the in vivo distribution, and the medicinal behaviors of CHM. Then, the accurate functional consistency of herbs and the main active component will be described. How to accurately and precisely establish such relationship is a critical issue being faced in basic CHM research. Such relationship can also affect the planting, harvesting, processing, and quality control processes of CHM. It is also a significant foundation for future CHM researches in areas such as herbal compatibility, CHM pharmacokinetics, and CHM pharmacodynamics, which can ultimately lead to a more effective, more stable, and safer application of CHM principal component.

3. Limitations of Current Research Methods

Defining the corresponding mechanisms of CHMs is enormously challenging due to the complexity and unique nature of each herb. A single herb possesses a complex system that consists of a broad spectrum of compounds. When a herb is introduced into an animal or a human, each of the compound's pharmacodynamic characteristics will be altered after they are metabolized or interacted with other compounds, producing a different therapeutic effect. The interactions between various components in herbal medicine can be synergistic and antagonistic. Due to technical limitations and sample acquisition difficulties, the pharmacokinetic study on a single active component has become a great challenge. On top of that, synergistic nature of CHM compounds makes the study even more complicated.

There has long been an interest in CHM innovation and pressing demand for drug development in the field of material basis of CHM research. Researchers who hold "Reductionist" opinions worldwide have launched a research campaign focusing on herbal medicinal chemical compositions and their role in CHM efficacy. Some studies have tried to isolate the primary compound that is directly associated with the pharmacodynamics of a CHM prescription through direct separation analysis of herbal chemical compositions [10, 11]. This approach helps isolate the principal components, partly identify their role played in the overall efficacy, and also describe the consistency of them. But there are difficulties in tracing processes, combined effects of multiple components introduce complexities and affect accuracy of the relevancy description in medicinal efficacy. Thus, it is almost impossible to study the synergistic medicinal value of multiple components. The analysis cannot reflect the influence of synergy by principal component and other components and also the composite effect of entire herbal compound. Other researchers premise the objective to find and identify the common components of serum samples and herbal materials. They set studies to focus on the common components presence in serum sample and herbs, evaluate the pharmacological and pharmacodynamic activities, and finally interpret the correlations of the common components with the overall CHM pharmacodynamics [12]. Although this approach demonstrates the synergistic effects of different

CHM components, it is incapable of interpreting the extent contribution of a single component to whole efficacy.

4. Advantages of Targeted Depletion of Principal Components

In the interpretation of the material basis of CHM, one approach for multicomponent analysis in herbal medicine is to remove one component at a time and eventually identify the target component of interest according to the changes inefficacy. This methodology is derived from the philosophy of reductionism. It highlights the individual component and elicits its pharmacological effect. However, such approach may not be the best option in analysing CHM on the material basis due to the following reasons. (1) Reductionistic thinking is contradictory to the basic philosophy of Chinese medicine, which emphasizes the "on a whole" approach to every matter. (2) The research object is centred on the effect and mechanism of one single component, which can result in a loss of focus of the overall performance of a CHM mixture. (3) The reduction process of ridding one compound at a time eliminates any synergistic or antagonistic activities between compounds and is not suitable for studies that desire multicomponent interactions. Therefore, the steps of "track" then "separation" are not fully applicable to the material basis of CHM.

The goal of pharmacodynamic analysis of whole compound system of CHM material-based research is difficult to realize in the near future. And the approach to track and analyse single active component still possesses a certain degree of technical difficulties and the results obtained are still questionable. New strategies and methodologies are needed. Protein specific immunodepletion assay may be a valuable solution to the technical obstacles mentioned above.

The basic principle of immunodepletion assay is to utilize antigen-antibody reaction to remove specific proteins in a complex CHM mixture. The objective is to establish and study the correlation between one specific protein and the overall efficacy of a CHM prescription. Comparing the pharmacological properties of a CHM mixture before and after protein deletion can then reveal the specific function of that particular protein. The advantage of adopting this method is the specific nature of antibody, which can selectively target the protein of interest and eliminate all other undesirable factors. The method not only discloses the pharmacodynamics and pharmacokinetics of a single active component but also reveals properties of other untargeted components and their synergistic behaviors. This method allows us to analyse the target component within the herbs and can accurately describe the degree of contribution of the target component to the overall efficacy without destroying the relations of other components.

After the removal of a target protein in a herbal extract, the protein can then be reintroduced into the solution at variable concentration. And a quantity-efficacy (QE) correlation of that protein can then be generated, which demonstrates that the variation in concentration of a principal component can affect the overall therapeutic performance of a CHM preparation. Obtaining the QE correlation of the principal component can provide valuable support for new drug development. Mixing different monoclonal antibodies to create a cocktail can remove several different target proteins at one time. And through orthogonal design and titration method, the synergistic relationships between different active components can then be revealed. Illustrating such synergistic interactions between CHM compounds can provide better scientific information for future CHM formulation and compatibility studies. The immunodepletion of specific CHM proteins allows the analysis of a single component independent of others. It can also be used to study the combined effects of certain different components or all the components altogether and other various related issues. It is a more reasonable way to carry out future CHM researches.

Monoclonal antibody is currently one of the most important research tools in life science research. And monoclonal antibody against CHM compound, if can be widely accepted, will be a very well-prospected research application. CHM researchers are now learning antibody technologies to study the complexities of herbal medicine. In 2005, Zhang et al. [13] reported a work on SiNi-powder. They utilised antiglycyrrhizin polyclonal antibodies to selectively deplete glycyrrhizin and determine its role in contact hypersensitivity in mice. They proposed the idea that a better way to identify the compound of interest on a molecular basis is to selectively remove certain components. In 2006, Chen et al. [14] also used immunoaffinity chromatography to efficiently remove Naringin for research, proving that the method of high specificity removal of Naringin did not influence other components and is worth promoting. A research article in 2011 described the changes in nitric oxide (NO) and nitric oxide synthase (iNOS protein) levels from a liquorice-specific deletion and glycyrrhizin addition experiment. The studies provided a clear efficacy correlation between glycyrrhizin and liquorice and exhibited the unique technical advantage of utilizing protein specific knockout technique [15]. These exploratory efforts have provided new research ideas on CHM components. The adaptation of protein specific deletion technology using monoclonal antibodies can minimize the effect of other components and focus solely on the target component's identification and pharmacological property determination. This new method will have good prospects in the material-based CHM research and in CHM pharmacological evaluation.

5. Monoclonal Antibody in Components Depletion

There is no essential difference between the path of making monoclonal antibodies of TCM components and other small molecules. Scholars around the globe have established several hapten synthesizing methods, such as the carbodiimide method [16] and the active-ester method [17]. These methodologies are mature and can provide valuable technical support for CHM hapten synthesis. With improvements in the synthesis of monoclonal antibody, researches in the small molecule immunoassay depending on the use of monoclonal

or polyclonal antibodies have gotten a boost as well. Preliminary applications of these immunoassays include pesticide detection in food, rapid drug testing, and environmental monitoring [18–21]. Some research has even demonstrated a very low hapten detection level reaching femtomolar [22].

In recent years, a variety of monoclonal antibodies against active components in herbal medicine have been successfully prepared against liquorice acid [23], paeoniflorin [24], aconitine [25], ginsenosides Rg1 [26], aristolochic acid [27], berberine [28], saikosaponin [29], and so forth. The emergence of all these different antibodies has promoted development in the related research field, for example, CHM quality assessments, serum CHM concentration testings [30, 31], integrating western blot and immunoassays in the analysis of CHM chemical composition, identification of tissue localization [26]. In 2011, Current Drug Discovery Technologies, an internationally renowned journal, published a series of reviews and research articles focusing on monoclonal antibodies [32–34]. This can be a good indication that monoclonal antibody-based small molecule drug discovery technology has gotten more and more attention and become the new drug discovery platform in the field of metabolic mechanism research.

In contrast, limited by outdated research strategies, the usage of specific protein immunodepletion in the field of CHM basic research has not truly begun. It is also due to the lack of high titer monoclonal antibodies and lack of suitable instruments. Another reason for the setback is that more than 90% of the small molecules (MW < 2500) isolated from CHM are haptens, which are incapable of direct stimulation of antibody production in vivo. Another reason is that there are compounds that have similar structures with the active components in a CHM mixture (e.g., isomers) and may cause undesired cross-reaction with the monoclonal antibody, which further complicates the process of polyclonal antibody preparation and antigen synthesis and clone selection. Furthermore, the structures of the active components in CHM are diverse and can create obstacles during the coupling process with macromolecules. Therefore, preparation of high potent monoclonal antibodies for small CHM molecules and establishing a CHM small molecule monoclonal antibody library are the foundations for promoting protein specific immunodepletion technology.

6. Research Example

Pueraria root (*Radix puerariae*) is a sweet, acrid, cool, and nontoxic herbal remedy. It benefits the spleen and stomach. It is commonly used in the treatment for diarrhoea, fever, thirst, exogenous fever, headache, neck and shoulder pain, chest pain, measles, and so forth. Clinical application of *R. puerariae* is widespread in both classic and modern Chinese medicine prescriptions, such as GuiZhi-JiaGeGen decoction, GeGenJiaBanXia decoction, GeGen-HuanQinHuangLian decoction, Puerarin injection, GanMao QingRe granules, XinKeShu tablets, YuFengNingXin Tablets, TianBaoNingYinXing preparations, and XiaoKe capsules.

Radix puerariae contains more than 30 types of flavonoid and isoflavonoid compounds, including daidzein and puerarin. It also contains triterpenoids, steroids, coumarin, GE phenolic glycosides, amino acids, and starch [35]. Puerarin is a type of isoflavone found in *Pueraria*. A typical *Pueraria* usually contains from 3% to 5% of puerarin, which is the primary therapeutic component of *Pueraria* [36]. Testing for the presence of puerarin is a commonly accepted practice to determine the quality of a *Pueraria* root and also as a QC standard for any medications that use *Pueraria.*

Puerarin's pharmacological activity has been a primary research focus in the field of medicine. Literatures suggest that the pharmacological action of *Pueraria* is associated with its antisympathetic, calcium antagonistic, and extensive β receptor blocking effects which result in vasodilation, lowering blood sugar and lipoproteins, and lowering blood pressure and subsequently protects the heart, liver, and kidneys [37–40]. The medicinal value of purarin is well established, but questions such as "how is the variation of puerarin concentration in different kinds of *Pueraria* root affecting *Pueraria's* efficacy?" and "should puerarin be taken as the QC of any *Pueraria's* preparations?" need to be explicitly delineated in order to guide the real-world usage to *Pueraria*.

Series of research in the production of monoclonal antibody against the active CHM component were launched since 2008. The study involves the synthesis of artificial antigens by conjugating CHM micromolecules with macromolecules, such as bovine serum albumin. The conjugate obtained was then used to immunize lab animals and generate corresponding cell fusions, which were then screened for positive clones. Researchers have successfully generated monoclonal antibodies against the hormone geniposide, Radix puerariae-baicalin, berberine, and many other CHM components. Mastering and widely adopting monoclonal antibody technology have made significant advancements in the CHM industry. Geniposide and puerarin immunoassay kits were introduced, allowing the completion of study in allergens in Qingkailing injections. The immunoassay chip used in the study of allergen, chlorogenic acid, in QingKaiLing Injection resulted in a number of patents [41–47], and the study also received recognition of its educational value in 2010.

7. Concluding Remarks

By using puerarin monoclonal antibody and immunoaffinity chromatography technology, the puerarin was deleted from *Pueraria* water extract. The puerarin immunoaffinity column prepared had strong specificity to puerarin present in the water extract and was able to delete the puerarin without affecting the remaining components in the solution. The bound puerarin was then eluted and a high purity sample of puerarin was obtained. These tools allow researchers to have a clear understanding on how to conduct a more in-depth study in CHM research. *Radix puerariae* was used here as an example for demonstrating monoclonal antibody technology using mAb deletion. There are animal and cellular models created for the purpose of studying the classic and

modern medicinal property of *Pueraria* root, which can be used to collect pharmacological data of *Pueraria* under many different experimental criteria. By comparing the pharmacological properties and pharmacodynamics of *Pueraria* extract before and after undergoing puerarin immunodepletion, the main contribution of puerarin to the overall medicinal property of *Pueraria* can then be easily evaluated, in vivo and in vitro, static and dynamic. As a result, puerarin can then be better utilized in QC, compatibility studies and pharmacokinetic studies of *Pueraria* roots, which expands the usage of *Pueraria* roots in clinical medicine in the future. And also as a result, the experimental foundation for any future CHM studies using target specific deletion techniques was established. However, the methodology introduced in this paper still has its limitations, for instance (1) it is not suitable for identifying unknown CHM components and (2) it is also incapable of complete separation of compounds that have already interacted with others. Taking everything into account, the new research strategy still holds many advantages and has proven to provide more effective and convenient ways of pursuing CHM research and help the modernisation of Chinese medicine.

Conflict of Interests

The authors declared no conflict of interests with respect to the authorship and/or publication of this paper.

Authors' Contribution

Xue-Qian Wang and Fa-Feng Cheng contributed equally to this paper.

Acknowledgment

This study was supported by the Science and Technology Major Projects for Major New Drugs (Compound transdermal delivery system-innovative drug research of the effective ingredients of Majiepingchuan poultice), no. 2012ZX09103201-007.

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Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2014, Article ID 681352, 10 pages
http://dx.doi.org/10.1155/2014/681352

Research Article

Ethyl Acetate Extract of *Artemisia anomala* S. Moore Displays Potent Anti-Inflammatory Effect

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Received 8 January 2014; Accepted 4 February 2014; Published 12 March 2014

Academic Editor: Kam-Ming Ko

Artemisia anomala S. Moore has been widely used in China to treat inflammatory diseases for hundreds of years. However, mechanisms associated with its anti-inflammatory effect are not clear. In this study, we prepared ethyl acetate, petroleum ether, *n*-BuOH, and aqueous extracts from ethanol extract of *Artemisia anomala* S. Moore. Comparing anti-inflammatory effects of these extracts, we found that ethyl acetate extract of this herb (EAFA) exhibited the strongest inhibitory effect on nitric oxide (NO) production in LPS/IFNγ-stimulated RAW264.7 cells. EAFA suppressed the production of NO in a time- and dose-dependent manner without eliciting cytotoxicity to RAW264.7 cells. To understand the molecular mechanism underlying EAFA's anti-inflammatory effect, we showed that EAFA increased total cellular anti-oxidant capacity while reducing the amount of inducible nitric oxide synthase (iNOS) in stimulated RAW264.7 cells. EAFA also suppressed the expression of IL-1β and IL-6, whereas it elevates the level of heme oxygenase-1. These EAFA-induced events were apparently associated with NF-κB and MAPK signaling pathways because the DNA binding activity of p50/p65 was impaired and the activities of both ERK and JNK were decreased in EFEA-treated cells comparing to untreated cells. Our findings suggest that EAFA exerts its anti-inflammatory effect by inhibiting the expression of iNOS.

1. Introduction

Artemisia anomala S. Moore (Nan-Liu-Ji-Nu) is a perennial herbaceous plant categorized to *Artemisia* genus Compositae family. Many species of *Artemisia* have been used as medicinal materials. In fact, *Artemisia anomala* S. Moore has been used for centuries to treat fever, empyrosis, inflammation, and dissipated liver function caused by hepatitis in China. For example, *Artemisia* oil can potently inhibit the growth of bacteria, yeasts, dermatophytes, and *Aspergillus niger* and has thus been extensively used as an anti-inflammatory agent [1]. The most well-known medicine from *Artemisia* genus is probably artemisinin and its derivatives that have the rapidest action against malaria among all antimalaria drugs. The regiment containing at least one artemisinin derivative (artemisinin-combination therapies) is the standard protocol to treat *P. falciparum* malaria worldwide [2]. The therapeutic effect of *Artemisia anomala* S. Moore is likely to be linked to its ability to counteract against inflammation, oxidation [3], and viral infection [4]. Recent studies show that dimeric guaianolides and sesquiterpenoids extracted from the aerial part of *Artemisia anomala* can suppress cyclooxygenase 2- (COX2-) associated effects [5]. Commonly used prostaglandin-like fatty acid derivatives anomalone A-D were actually isolated from *Artemisia anomala* [6]. Although sufficient evidences have demonstrated *Artemisia anomala* S. Moore as an effective anti-inflammatory agent, systematically evaluating its anti-inflammatory effects with inflammatory parameters has yet been performed.

Acute inflammatory response represents an initial protective mechanism in the body. In contrast, excessive and chronic inflammation results in severe damages of cells and tissues. Emerging evidences support the hypothesis that chronic inflammation plays a critical role in various pathological conditions, including hypertension, atherosclerosis, stroke, metabolic diseases, cancer, autoimmune disorders, and neurodegenerative diseases [7–10]. Nitric oxide (NO) is a free radical that is synthesized from L-arginine by nitric oxide synthase (NOS). There are three types of NOS: two constitutive NOS, eNOS and nNOS, and one

inducible NOS (iNOS). Constitutive NOSs generate nanomolar concentration of NO and are known to mediate various physiological functions. Contrarily, iNOS produces NO at the level of micromolar that often results in pathological consequences such as chronic inflammation. Inflammatory stimuli can induce iNOS expression through distinct signaling pathways. Proinflammatory cytokines released from inflammation-stimulated cells, for example, macrophages, can further upregulate iNOS expression and augment inflammatory responses [11, 12]. The expression of proinflammatory cytokines, including interleukin-1β (IL-1β), interleukin-6 (IL-6), is often regulated through the NF-κB and MAPK signaling pathways [13, 14]. Endogenous anti-inflammatory response is also involved in inducible heme oxygenase-1 (HO-1). Because of the ability of HO-1 to attenuate iNOS expression [15, 16], HO-1 is thought to play a protective role during inflammation [17, 18].

The objective of this study is to determine the most effective fraction of *Artemisia anomala* S. Moore that can inhibit iNOS-induced NO production. To identify such fraction, we prepared ethyl acetate, petroleum ether, *n*-BuOH, and aqueous extracts from ethanol extract of *Artemisia anomala* S. Moore. With the aid of the well-established murine macrophage RAW264.7 cell inflammation model, we found that ethyl acetate extraction of *Artemisia anomala* S. Moore (EAFA) exhibited the strongest inhibitory effect on LPS/IFNγ-induced NO production and proinflammatory cytokine expression. Since NF-κB and MAPK activities were significantly reduced in EAFA-treated cells, we suggest that EAFA exerts its inhibitory action by interfering with both NF-κB and MAPK signaling pathways.

2. Materials and Methods

2.1. Reagents. Murine recombinant IFNγ, NF-κB p50/p65 EZ-TFA transcription factor assay system, and mouse IL-6 ELISA kit were purchased from Millipore (MA, USA); lipopolysaccharide (LPS, *Escherichia coli* O111:B4), dimethyl sulfanilamine, *N*-(1-naphthyl)-ethylenediamine dihydrochloride, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoleum (MTT), L-N^6-(1-Iminoethyl)lysine hydrochloride (L-NIL), and Trolox were obtained from Sigma (St. Louis, MO). TRIzol Reagent was obtained from Invitrogen (Carlsbad, CA). Mouse IL-1beta instant ELISA was obtained from eBioscience (San Diego, CA). Takara SYBR kit and OligodT were obtained from Shanghai Invitrogen (Shanghai, China). Nuclear Extraction Kit was obtained from Biyuntian (Shanghai, China). Antibodies used in this study include murine iNOS monoclonal antibody from BD Transduction Laboratories (Lexington, KY); murine β-actin and HO-1 antibodies from Santa Cruz Biotechnology (Santa Cruz, CA); phosphor-p38, p38, phosphor-JNK, JNK, and phosphor-ERK1/2 and ERK1/2 antibodies from Cell Signaling Technology (Danvers, MA).

2.2. Herb Extraction and Fractionation. *Artemisia anomala* S. Moore was purchased from Yang-He Tang Co. (Zhangjiang High-Tech Park, Shanghai, China) and confirmed by Shanghai Institute for Food and Drug Control (SIFDC). The dried plants were first extracted with 70% ethanol at 80°C for three times (200 g raw material/1 L/60 min each time) and the obtained ethanol extract was then suspended in water followed by the constitutive partition with petroleum ether, ethyl acetate, *n*-butanol, and water. After evaporation of these partitioned solutions, five extract fractions were generated: ethanol (yield 6.73%), petroleum ether (0.17%), ethyl acetate (0.25%), *n*-butanol (0.33%), and aqueous fractions (1.64%). Each fraction was dissolved in DMSO and stored at −20°C until use.

2.3. Cell Culture. RAW264.7 cells were originally obtained from the American Tissue Culture Collection. Cells were maintained in RPMI 1640 medium supplemented with 10% FBS at 37°C in a humidified 5% CO_2 atmosphere.

2.4. Measurement of NO Production. RAW264.7 cells were plated in a 96-well plate (5×10^3 cells/well) for overnight and then serum-starved for 10 h followed by the addition of 10 U/mL IFNγ and 100 ng/mL LPS for 24 h in the presence or absence of different *Artemisia anomala* S. Moore fractions with finally concentration at 10, 100, 200 μg/mL and used L-NIL (50 μM) as positive drug control for primary screening. After obtaining the strongest fraction, the posttreat, pretreat, and simultaneous-treat of this fraction and stimulation would underprocess for secondary screening. To analyze NO production, 100 μL of supernatant was incubated with equal volume of Griess solution at room temperature for 10 min and absorbance was then read at 540 nm. Since NO content was reflected by the amount of nitrite, a calibration curve was generated using sodium nitrite. The amount of nitrite in the supernatants was calculated based on the calibration curve. The percentage inhibition of NO production is evaluated using the formula $\{1 - [(\text{nitrite amount of fraction} - \text{treated})/(\text{nitrite amount of vehicle})]\} \times 100$.

2.5. Assay for Cell Viability. Cell viability was assessed by MTT assay. Briefly, after using the 100 μL supernatants to do Griess reaction, the rest cells were incubated with 10 μL MTT (5 mg/mL in phosphate-buffered saline, pH = 7.4) for 4 h at 37°C followed by adding 50 μL 0.01 mol/L HCl buffer containing 10% SDS and 10% Isopropanol. Absorbance was measured at 540 and 630 nm in a microplate reader. The absorbance of control (untreated) cells was considered as 100% of viability.

2.6. Measurement of Total Antioxidant Capacity. Total antioxidant activity was measured by modified FRAP assay as previously described [19]. Briefly, RAW264.7 cells (2×10^6 cells/30 mm dish) were pretreated with varying concentration of EAFA (50, 100, 200 μg/mL) for 1 h followed by costimulation of 100 ng/mL LPS and 10 U/mL IFN-γ for 6 h. Cells were harvested, whereas the supernatants were collected. FRAP reagent was prepared by mixing 300 mmol/L acetate buffer (pH 3.6), 10 mmol/L 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mmol/L HCl solution and 20 mmol/L $FeCl_3$

Table 1: Primer sets for qRT-PCR.

Gene name	Forward primer	Reverse primer
iNOS	GGAGCGAGTTGTGGATTGTC	GTGAGGGCTTGGCTGAGTGAG
HO-1	CACAGATGGCGTCACTTCGTC	GTGAGGACCCACTGGAGGAG
IL-1β	GCTGTGGCAGCTACCTATGTCTTG	AGGTCGTCATCATCCCACGAG
IL-6	CCACTTCACAAGTCGGAGGCTTA	GTGCATCATCGCTGTTCATACAATC
β-actin	GCTACAGCTTCACCACCACAG	GGTCTTTACGGATGTCAACGTC

in a 10 : 1 : 1 ratio and 245 μL of freshly prepared FRAP solution was added to each well of a 96-well plate that contained 5 μL of supernatant. After 10 min incubation at room temperature, absorbance was measured at 593 nm with the aid of a microplate reader. A standard curve was prepared with various concentrations of Trolox (0.03125 to 2 mmol/L). The potency of total antioxidant capacity for each sample was determined by comparing the antioxidant capacity of 1 mM Trolox.

2.7. Detection of IL-1β and IL-6 in Supernatant. Inhibitory effects of EAFA on the cytokine IL-6 and IL-1β production from LPS plus IFN-γ treated RAW264.7 cells were detected by sandwich ELISA. The procedure was carried out under the instructions from respective kit. After preincubation of 1 h with different dosage of EAFA and stimulation with LPS plus IFN-γ on RAW264.7 cells for 24 h, supernatants were harvested and assayed for IL-1β and IL-6. Results of three independent experiments were used for statistical analysis.

2.8. RNA Isolation and Quantitative RT-PCR. Total RNA was isolated using TRIzol Reagent according to manufacturer's instruction. Quantitative RT-PCR (qRT-PCR) was performed with Takara SYBR kit using the primers sets in Table 1 as previously described [20]. The $2^{-\Delta\Delta CT}$ method was utilized to analyze the fold increase.

2.9. Nuclear Extract Preparation and NF-κB DNA Binding Assay. Nuclear extracts were prepared using Nuclear Extraction Kit according to the manufacturer's instruction. DNA binding activity of NF-κB in nuclear extracts was assessed using NF-κB p50/p65 EZ-TFA transcription factor assay kit which detects the amount of NF-κB in the nucleus.

2.10. Statistical Analysis. Student's test was used to analyze the difference between treated and untreated groups. Comparisons between multiple groups were performed with one-way ANOVA test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. EAFA Displays the Strongest Inhibitory Effect on LPS/IFNγ-Induced NO Production. The ethanol extract of *Artemisia anomala* S. Moore has been shown to exhibit inhibition of NO production in our previous screening [21]. We extracted ethanol extract of *Artemisia anomala* S. Moore further with petroleum ether, ethyl acetate, *n*-butanol, and water, and each of these obtained extracts was tested

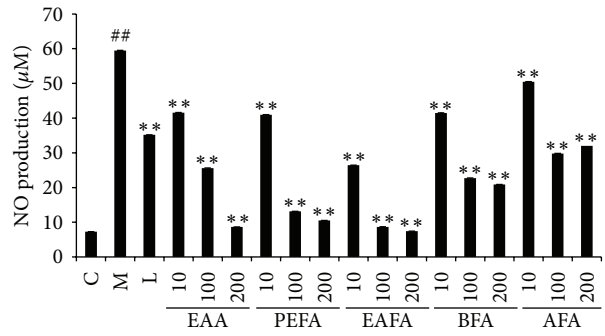

Figure 1: Effect of four solvent extracts of ethanol extract of *Artemisia anomala* S. Moore on NO production in LPS/IFNγ-stimulated RAW264.7 cells. C: control (nontreatment) group; M: LPS/IFNγ-stimulated model group; L: L-NIL (50 μM); EAA: ethanol extracted fraction (10, 100, 200 μg/mL); PEFA: petroleum ether extracted fraction (10, 100, 200 μg/mL); EAFA: ethyl acetate extracted fraction (10, 100, 200 μg/mL). BFA: *n*-butanol extracted fraction (10, 100, 200 μg/mL); AFA: queous extracted fraction (10, 100, 200 μg/mL). Data are mean ± SEM $n = 6$ per group. $^{**}P < 0.01$ (*versus* model group). $^{\#\#}P < 0.01$ (*versus* control group).

for its ability to inhibit NO production in RAW264.7 cells costimulated with LPS and IFNγ. Griess reaction assay showed that IC_{50} of original ethanol extract (EEA) was 31.07 μg/mL (Figure 1). IC_{50} of petroleum ether fraction (PEFA), *n*-butanol fraction (BFA), and aqueous fraction (AFA) from the EEA was 21.73, 39.10, and 49.25 μg/mL, respectively (Figure 1), which were similar to that of the original EEA. In contrast, IC_{50} of ethyl acetate fraction (EAFA) was 15.85 μg/mL (Figure 1), representing twice stronger inhibitory effect over the original EEA.

In a parallel experiment, we investigated the effect of EAFA on NO production in unstimulated RAW264.7 cells. Contrary to its ability to dose-dependently inhibit NO production in LPS/IFNγ-stimulated cells (Figure 2(a)), EAFA displayed little effect on NO production in unstimulated cells (Figure 2(a)). MTT assays further showed that EAFA promoted viability of LPS/IFNγ-stimulated RAW246.7 cells in dose-dependent manner while it exhibited effect on the viability of unstimulated cells (Figure 2(b)). These results suggest that EAFA selectively inhibits NO production. Since EAFA promotes cell viability in LPS/IFNγ-stimulated RAW264.7 cells (Figure 2(b)), these results also indicate that the inhibitory effect of EAFA on NO production in LPS/IFNγ-stimulated cells is not caused by LPS/IFNγ-induced cellular toxicity.

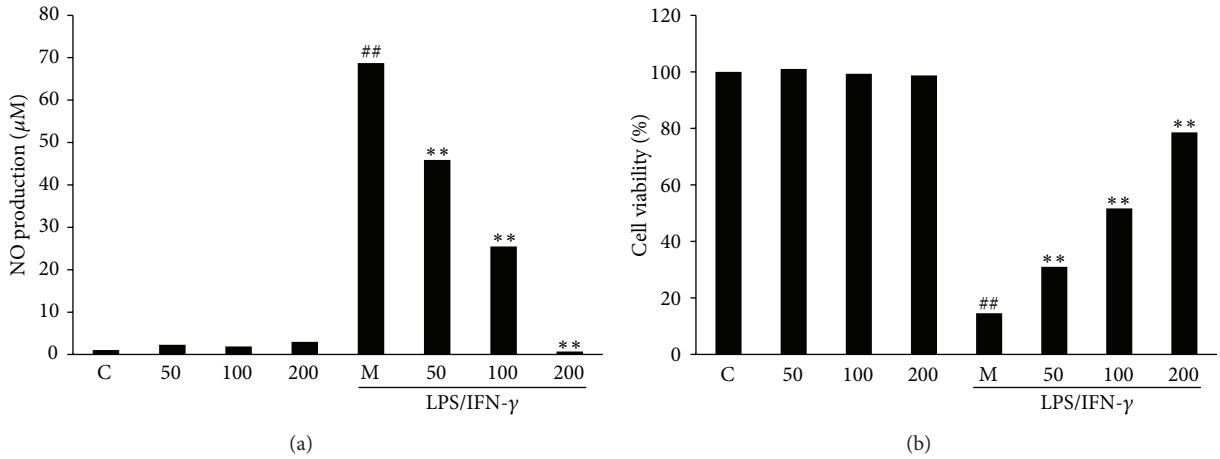

Figure 2: Effect of EAFA on NO production (a) and cell viability (b) in LPS/IFNγ-stimulated and unstimulated RAW264.7 cells. RAW264.7 cells were treated with EAFA (50–200 μg/mL) for 24 h with or without LPS/IFNγ stimulation. Nitrite concentrations in the culture medium were determined by the Griess reaction. Changes in survival are represented as percentages of the control group. Bars represent the mean ± SEM. Three independent experiments were performed. $^{\#\#}P < 0.01$; $^{\#}P < 0.05$ *versus* control group; $^{**}P < 0.01$; $^{*}P < 0.05$ *versus* model group.

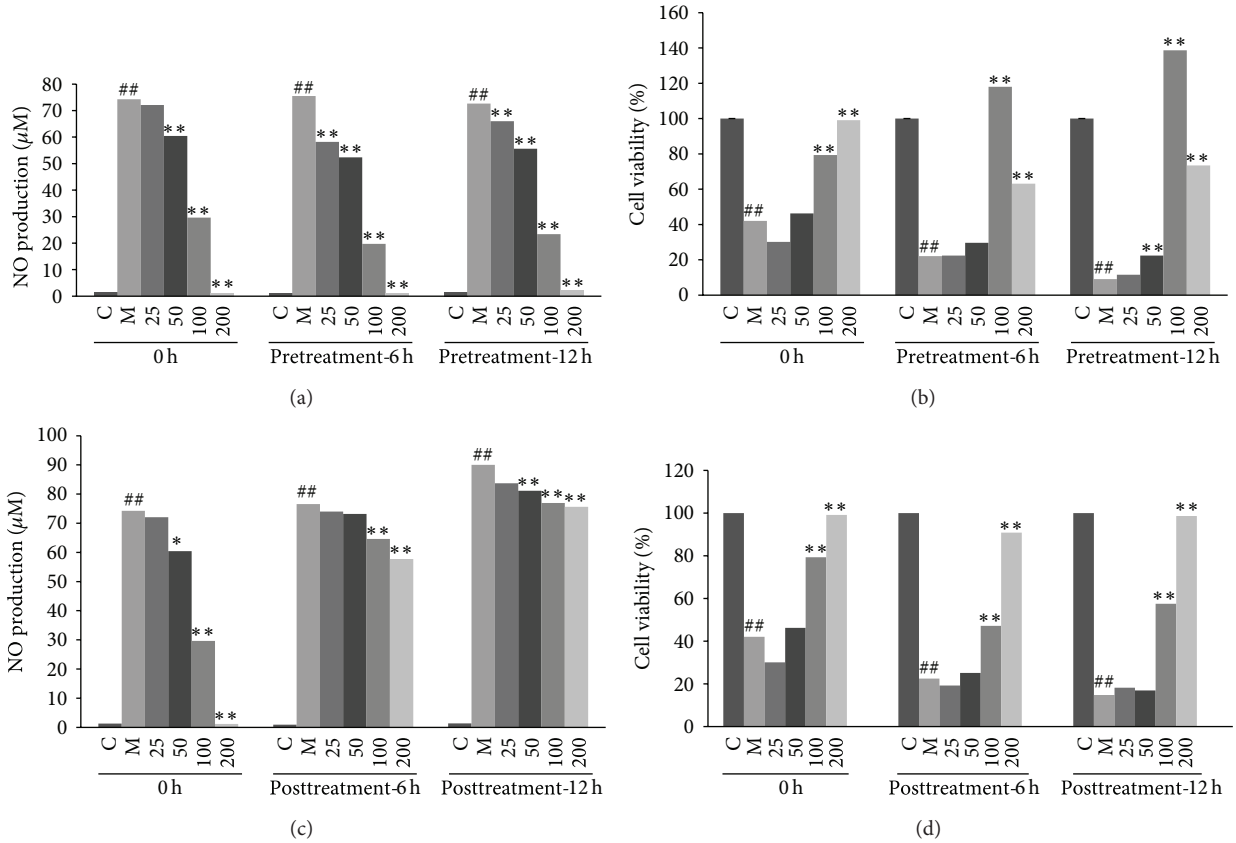

Figure 3: Effect of pretreatment and posttreatment of EAFA on NO production and cell viability. Cells were plated at a density of 1×10^5 cells/well in a 96-well plate and allowed to attach for 2 h. EAFA was added prior to (pretreatment-12 h, pretreatment-6 h), simultaneously with (0 h) (a, b) or after the treatment of the cells with IFNγ (10 U/mL) plus LPS (100 ng/mL) (posttreatment-6 h, posttreatment-12 h) (c, d). Nitric concentrations in the culture medium and cell viability were determined by the Griess reaction and MTT assay. The values (means ± SEM) were obtained from three independent experiments. $^{\#\#}P < 0.01$; $^{\#}P < 0.05$ *versus* control group; $^{**}P < 0.01$; $^{*}P < 0.05$ *versus* model group.

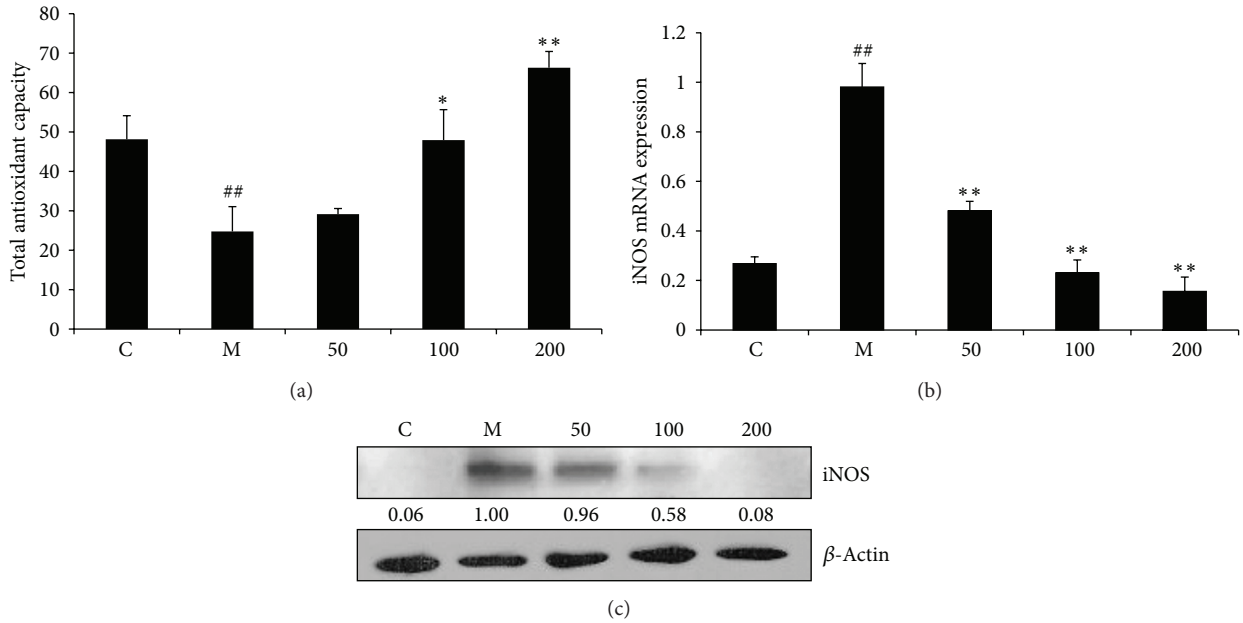

Figure 4: Effect of EAFA on total oxidant activity and iNOS expression in LPS/IFNγ-stimulated RAW264.7 cells. (a) Cells were plated at a density of 1×10^6 cells/well in 30 mm dishes and allowed to attach overnight. EAFA was added 1 h prior to the treatment of IFNγ (10 U/mL) plus LPS (100 ng/mL) for 6 h. Whole cell lysates were analyzed by FRAP assay and standardized by protein concentration. The data shown are representative of three independent experiments. $^{\#\#}P < 0.01$; $^{\#}P < 0.05$ *versus* control group; $^{**}P < 0.01$; $^{*}P < 0.05$ *versus* model group. (b) RAW264.7 cells (1×10^6 cells/dish) were pretreated with varying concentrations of EAFA for 1 h followed by LPS (100 ng/mL) and IFN-γ (10 U/mL) treatment for 4 h. Total RNA was isolated and subjected to qRT-PCR. β-actin mRNA was used as an internal control for standardization. (c) RAW264.7 cells were plated at a density of 1×10^6 cells in 30 mm dish for overnight. EAFA was added 1 h prior to the treatment of IFNγ (10 U/mL) plus LPS (100 ng/mL) for 6 h. Whole cell lysates were prepared and subjected to western blotting. The data shown are representative of three independent experiments. $^{\#\#}P < 0.01$, $^{\#}P < 0.05$ *versus* control group; $^{**}P < 0.01$, $^{*}P < 0.05$ *versus* model group.

3.2. Both Pre- and Posttreatments of EAFA Inhibit LPS/IFNγ-Induced NO Production and Cellular Toxicity in RAW264.7 Cells. To further characterize the pharmacological action by EAFA, we pretreated RAW264.7 cells with EAFA for 6 and 12 hrs followed by LPS/IFNγ stimulation. Griess reaction assays showed that EAFA pretreatment resulted in significantly better inhibitory effect on NO production in LPS/IFNγ-stimulated RAW264.7 cells than adding EAFA at the time of LPS/IFNγ stimulation (Figure 3(a)). Similarly, EAFA pretreatment promoted cell viability in a greater degree than adding EAFA simultaneously with the stimulants (Figure 3(b)). In subsequent study, RAW264.7 cells were first stimulated with LPS/IFNγ for 6 or 12 h and then treated with EAFA. Although less inhibitory effect on NO production and cell viability was detected with EAFA posttreatment compared with EAFA pretreatment, we still observed 16.22% of reduction in NO production and 98.58% of increase in cell viability in RAW264.7 cells treated with EAFA at dosage of 200 μg/mL (Figures 3(c) and 3(d)). Taken together, these results suggest than EAFA can potentially be used both as a preventive and therapeutic agent against chronic inflammation.

3.3. EAFA Pretreatment Prevents LPS/IFNγ-Suppressed Antioxidant Capacity and Inhibits iNOS Expression. NO at high concentration is often considered as oxidant stress [22]. Since EAFA can effectively reduce LPS/IFNγ-induced NO production, we hypothesized that EAFA might also possess potent antioxidant activity. To test this hypothesis, RAW264.7 cells were pretreated with varying concentrations of EAFA for 1 h followed by LPS/IFNγ stimulation for 6 h. Total ferric reducing-antioxidant power (FRAP) assay showed that LPS/IFNγ stimulation greatly reduced antioxidant capacity (TAC) in RAW264.7 cells (Figure 4(a)). However, EAFA pretreatment reversed LPS/IFNγ-caused reduction in TAC (Figure 4(a)).

The fact that iNOS is responsible for LPS/IFNγ-induced NO production indicates that EAFA might block NO production by decreasing the amount of iNOS. Because iNOS is mainly regulated at transcription level [23], we tested this possibility by determining the effect of EAFA on iNOS mRNA in LPS/IFNγ-stimulated RAW264.7 cells with the aid of quantitative RT-PCR (qRT-PCR). LPS/IFNγ stimulation elevated the level of iNOS; however, pretreatment of EAFA at 100 and 200 μg/mL led to 41.78% ($P < 0.01$) and 85.29% ($P < 0.01$) reduction in LPS/IFNγ-induced iNOS expression (Figure 4(b)). Western blot analysis also showed that EAFA pretreatment diminished LPS/IFNγ-induced iNOS protein expression in RAW264.7 cells (Figure 4(c)). Taken together, these results support the notion that EAFA is a potent preventive agent against inflammation.

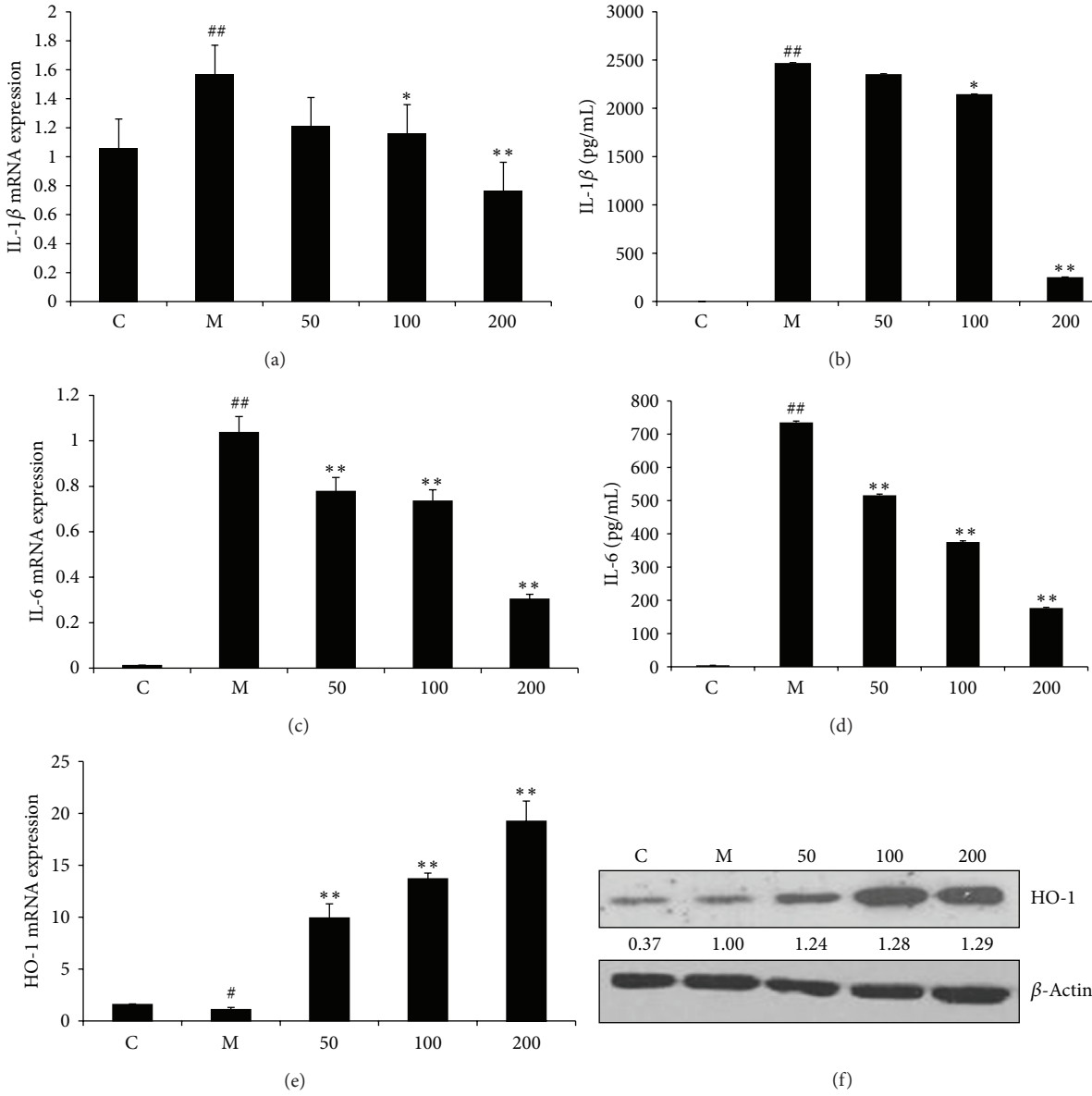

Figure 5: Effect of EAFA on proinflammatory cytokine and HO-1 expression in LPS/IFNγ-stimulated RAW264.7 cells. (a, b) RAW264.7 cells (1×10^6 cells/dish) were pretreated with varying doses of EAFA for 1 h and then stimulated with IFNγ (10 U/mL) plus LPS (100 ng/mL) for 6 h. Total RNA was isolated and subjected to qRT-PCR to determine the level of IL-1β (a) and IL-6 mRNA (b). (c, d) RAW264.7 cells were treated with IFNγ (10 U/mL) plus LPS (100 ng/mL) in the presence of varying concentrations of EAFA for 24 h. Conditioned media were collected and subjected to ELISA to determine the amount of IL-1β (c) and IL-6 (d). The values (means ± SEM) were obtained from three independent experiments. $^{\#\#}P < 0.01$, $^{\#}P < 0.05$ *versus* control group; $^{**}P < 0.01$, $^{*}P < 0.05$ *versus* model group. (e) RAW264.7 cells (1×10^6 cells/dish) were pretreated with varying concentrations of EAFA (50, 100, 200 μg/mL) for 1 h followed by LPS (100 ng/mL) plus IFNγ (10 U/mL) treatment for 18 h. Total RNA was isolated and subjected to qRT-PCR to measure HO-1 mRNA level. β-actin was used as an internal control for standardization. (f) RAW264.7 cells plated at a density of 1×10^6 cells/well in 30 mm dish for overnight were pretreated with EAFA for 1 h followed by 18 h-stimulation of IFNγ (10 U/mL) plus LPS (100 ng/mL). Whole cell lysates were prepared and subjected to western blotting to determine HO-1 protein levels. Data are the representative of three independent experiments. $^{\#\#}P < 0.01$, $^{\#}P < 0.05$ *versus* control group; $^{*}P < 0.01$, $^{**}P < 0.05$ *versus* model group.

3.4. EAFA Blocks Inflammatory Cytokines Production and Increases HO-1 Expression in RAW246.7 Cells. LPS/IFNγ costimulation has been reported to induce the expression of a plethora of proinflammatory cytokines in macrophages [24]; we thus investigated the effect of EAFA on IL-1β and IL-6 expressions in LPS/IFNγ-stimulated RAW246.7 cells. qRT-PCR showed that LPS/IFNγ stimulation led to over 1.5-fold increase in IL-1β and 104-fold increase in IL-6 expression. Pretreatment of EAFA dose-dependently abrogated LPS/IFNγ-induced IL-1β and IL-6 expression

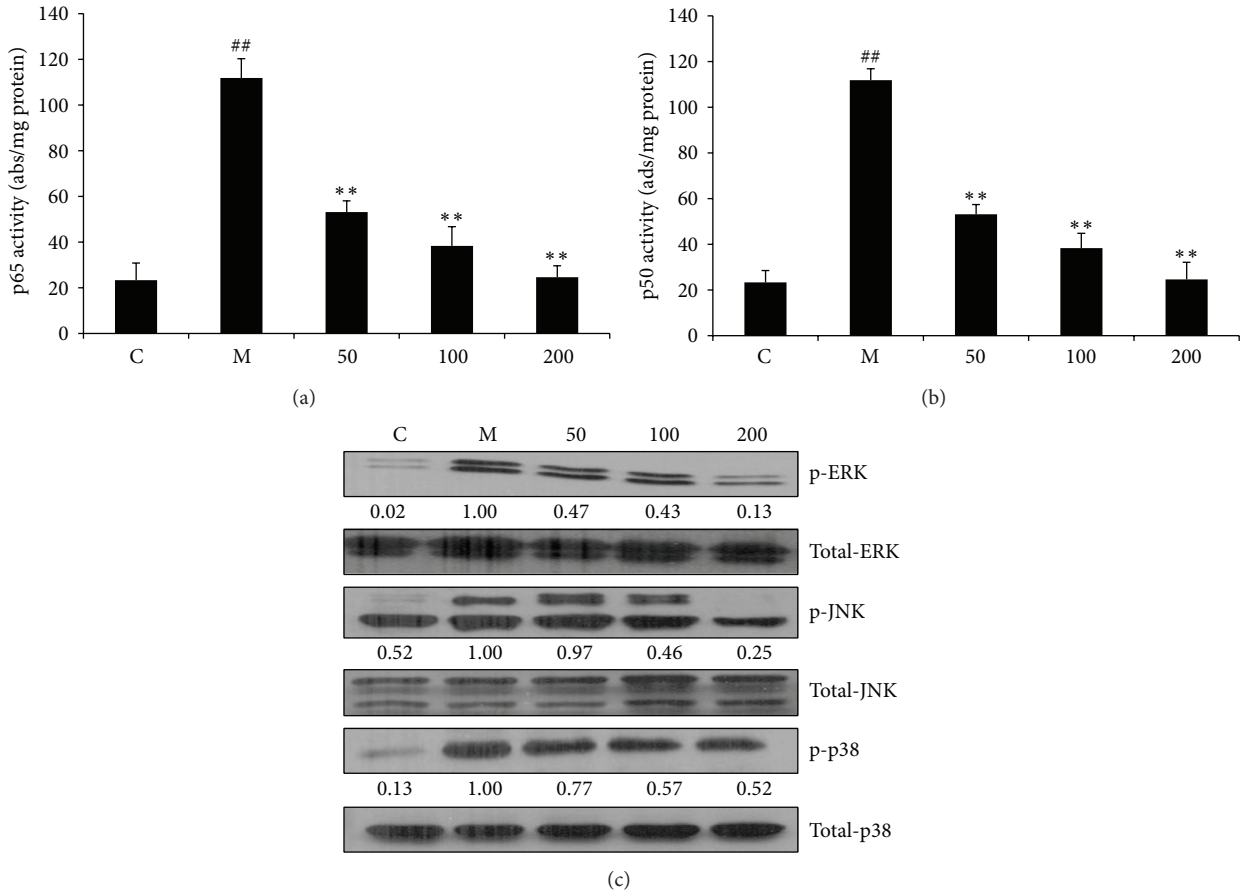

Figure 6: Effect of EAFA on NF-κB and MAPK activities in LPS/IFNγ-stimulated RAW264.7 cells. (a, b) DNA binding activity of p50 and p65 proteins in nuclear extracts was assessed using NF-κBp50/p65 EZ-TFA transcription factor assay. Absorbance was measured at 450 nm in a microplate spectrophotometer. Results were normalized to absorbance/mg protein. The data shown are representative of three independent experiments. $^{\#\#}P < 0.01$, $^{\#}P < 0.05$ *versus* control group; $^{**}P < 0.01$, $^{*}P < 0.05$ *versus* model group. (c) RAW264.7 cells were plated at a density of 1×10^6 cells/well in 30 mm dish for overnight. EAFA was added to cells for 1 h followed by 30 min stimulation of IFNγ (10 U/mL) plus LPS (100 ng/mL). Whole cell lysates were prepared and subjected to western blotting to detect phosphor-ERK, phosphor-JNK, and phosphor-p38 with the respective antibodies. Data shown are the representative of three independent experiments. $^{\#\#}P < 0.01$, $^{\#}P < 0.05$ *versus* control group; $^{**}P < 0.01$, $^{*}P < 0.05$ *versus* model group.

(Figures 5(a) and 5(c)). ELISA with the conditioned media also showed that EAFA pretreatment diminished LPS/IFNγ-induced IL-1β and IL-6 secretion by RAW246.7 cells (Figures 5(b) and 5(d)). The nature of HO-1 as a stress-inducible protein with anti-inflammatory feature [17, 18] also prompted us to determine how EAFA affected HO-1 expression in LPS/IFNγ-stimulated RAW246.7 cells. Western blot analysis showed that EAFA pretreatment upregulated HO-1 abundance while qRT-PCR revealed that EAFA increased the level of HO-1 mRNA in a dose-dependent manner (Figures 5(e) and 5(f)). These results indicate that the ability of EAFA to prevent inflammatory responses was two-folded: one is to abolish inflammatory cytokine expression and the other is to increase HO-1 expression in macrophages.

3.5. NF-κB and MAPK Signaling Pathways Are the Target of EAFA-Mediated Inhibition. NF-κB activity is known to be critical for the expression of iNOS [25–27]. To investigate how EAFA affected LPS/IFNγ-induced NF-κB activity, we analyzed the extent of p50 and p65 binding to NF-κB consensus sequence-containing oligonucleotides in nuclear extracts. LPS/IFNγ stimulation resulted in more than 5-fold increase in the amount of p50 and p65 bound to the NF-κB consensus sequence-containing oligonucleotides compared with unstimulated RAW246.7 cells (Figures 6(a) and 6(b)). However, EAFA inhibited LPS/IFNγ-induced NF-κB activation and, at 200 μg/mL, completely abolished this activation (Figures 6(a) and 6(b)). In addition to NF-κB, members of MAPK families have also been implicated to play an essential role in the inflammatory reaction. To determine the effect of EAFA on LPS/IFNγ-induced MAPK activation, western blots were performed to analyze the levels of phosphor-Erk, JNK, and p38 in RAW246.7 cells. LPS/IFNγ stimulation (30 min) evocated significant increases in the levels of phosphorylated Erk, JNK, and p38 in RAW246.7 cells. However, pretreatment of EAFA markedly inhibited the extent of Erk and JNK phosphorylation (Figure 6(c)). These results suggest that EAFA blocks inflammatory responses by the combination of blocking NF-κB, Erk, and JNK activation.

4. Discussion

Inhibition of iNOS has been shown to soothe pathological conditions characterized as inflammation. For example, iNOS-knockout mice are resistant to pleurisy and lung injury caused by carrageenan [28]. Selective inhibition of iNOS improves erosive joint disease [29], prevents experimental allergic encephalomyelitis [30], and attenuates immune dysfunction following trauma [8]. In addition, expression of iNOS has also been associated with various tumor types including brain, breast, lung, pancreas, liver, colon, and prostate cancers [9]. Selective NOS-2 inhibitors L-N6-(1-iminoethyl) lysine 5-tetrazole-amide (SC-51) and aminoguanidine (AG) actually show chemopreventive effect against the incidence of azoxymethane- (AOM-) induced colonic aberrant crypt foci [31], whereas NOS-2 blocker N-(3-(aminomethyl)benzyl) acetamidine (1400 W) is capable of suppressing tumor development in human colon adenocarcinoma DLD-1 xenograft [32]. These findings implicate the benefits of identifying novel agents targeting iNOS and its pertinent pathways. With this goal, we previously screened the ethanol extracts of 81 herbs for their ability to block LPS/IFNγ-induced inflammatory responses. Among them, we found that ethanol extract of *Artemisia anomala* S. Moore is effective to block LPS/IFNγ-induced NO production in RAW264.7 cells. In this study, we extracted ethanol extract of *Artemisia anomala* S. Moore further with four different solvents and found that ethyl acetate fraction (EAFA) had over 2-fold better potency than the original ethanol extract in the capability to inhibit NO production in LPS/IFNγ-stimulated RAW264.7 cells (Figure 1). Interestingly, EAFA also displayed significant protective effect to the viability of LPS/IFNγ-stimulated RAW264.7 cells without cytotoxicity in unstimulated cells (Figure 2). Our studies thus indicate the potential of using EAFA as anti-inflammatory effect.

Oxidative stress, which can arise from excessive ROS and/or RNS such as NO and its derivatives superoxide anions, may cause many diseases. The FRAP assay showed the antioxidative capacity of EAFA in inflammatory stimulation elicited cell damage as a cytoprotectant (Figure 4(a)). LPS/IFNγ-induced NO production is mediated by iNOS [22, 23]. We showed that EAFA blocked both iNOS mRNA and protein expression in activated macrophages (Figures 4(b) and 4(c)). HO-1 is the inducible isoform of the rate-limiting enzyme of heme degradation. Induction of HO-1 protects against the cytotoxicity of oxidative stress. HO-1 has been recognized to have anti-inflammatory properties [33]. EAFA induced the expression of HO-1 on mRNA and protein level (Figure 5). So, EAFA had dual properties in depressing the proinflammatory enzyme and inducing the anti-inflammatory enzyme.

Induction of iNOS is often accompanied with upregulation proinflammatory cytokines in macrophages [13, 14]. EAFA can also diminish the expression and secretion of IL-1β and IL-6 (Figure 5). The expression of a number of immunity and inflammatory related genes such as iNOS, IL-1β, and IL-6 was modulated by activated NF-κB [34]. Under inflammatory conditions, inhibitory protein IκBs are promptly phosphorylated and degraded from p50 and p65 subunits binding site of NF-κB; the activated NF-κB subunits migrate to the nucleus. To investigate the possible preventive capability of EAFA on NF-κB activation, we studied p50/p65 nuclear translocation by NF-κB p50/p65 EZ-TFA transcription factor assay kit. LPS/IFNγ stimulate the activation of NF-κB and induce p50/p65 movement to nucleus; EAFA repressed the amount of p50/p65 in the nucleus (Figures 6(a) and 6(b)). So EAFA displayed the interference in progress of NF-κB active heterology dimmer heading to the nucleus.

MAPKs and NF-κB signaling mechanisms have been previously linked to both iNOS and proinflammatory factor expression under inflammatory conditions. Moreover, several studies have shown that MAPKs play a critical role in the activation of NF-κB [35]. Depending on the cell system, p38, ERK, and JNK have proven to have ROS-sensitive kinase activity [36]. According to the antioxidant activity of EAFA, we investigated whether MAPK pathway was involved in attenuating inflammatory mediators express and final NO/RNS reduction. In fact, our study showed that EAFA was able to abolish LPS/IFNγ-induced activation of Erk and JNK in RAW246.7 cells (Figure 6(c)). Together, we reason that the anti-inflammatory effect of EAFA is at least partly by attenuating NF-κB and MAPKs activation.

In conclusion, our study indicates that EAFA can potently suppress inflammatory responses, and it hence warrants further identification of the effective component(s) in EAFA.

Abbreviations

EAFA: Ethyl acetate extraction of *Artemisia anomala* S. Moore
LPS: Lipopolysaccharide
IFN-γ: Interferon-γ
NO: Nitric oxide
iNOS: Inducible nitric oxide synthase
HO-1: Inducible haemoxygenase
IL-6: Interleukin-6
IL-1β: Interleukin-1β
NF-κB: Nuclear factor-kappa B
ERK: Extracellular signal-regulated kinase
JNK: c-Jun N-terminal kinase
MAPK: Mitogen-activated protein kinase.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

This work is supported by the National Science and Technology Major Project of the Ministry of Science and Technology of China (2009ZX09311-003); the Young Scientists Fund of the National Natural Science Foundation of China (81001666); Innovation Program of Shanghai Municipal Education Commission (13YZ048); the Foundation of Shanghai Education commission for Outstanding Young Teachers in University (SZY07029).

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