Review Article
Pharmacological Actions, Molecular Mechanisms, Pharmacokinetic Progressions, and Clinical Applications of Hydroxysafflor Yellow A in Antidiabetic Research

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Hydroxysafflor yellow A (HSYA), a nutraceutical compound derived from safflower (Carthamus tinctorius), has been shown as an effective therapeutic agent in cardiovascular diseases, cancer, and diabetes. Our previous study showed that the effect of HSYA on high-glucose-induced podocyte injury is related to its anti-inflammatory activities via macrophage polarization. Based on the information provided on PubMed, Scopus, and Wanfang database, we currently aim to provide an updated overview of the role of HSYA in antidiabetic research from the following points: pharmacological actions, molecular mechanisms, pharmacokinetic progressions, and clinical applications. The pharmacokinetic research of HSYA has laid foundations for the clinical applications of HSYA injection in diabetic nephropathy, diabetic retinopathy, and diabetic neuropathy. The application of HSYA as an antidiabetic oral medicament has been investigated based on its recent oral delivery system research. In vivo and in vitro pharmacological research indicated that the antidiabetic activities of HSYA were based mainly on its antioxidant and anti-inflammatory mechanisms via JNK/c-jun pathway, NOX4 pathway, and macrophage differentiation. Further anti-inflammatory exploration related to NF-κB signaling, MAPK pathway, and PI3K/Akt/mTOR pathway might deserve attention in the future. The anti-inflammatory activities of HSYA related to diabetes and diabetic complications will be a highlight in our following research.

1. Introduction

Hydroxysafflor yellow A (HSYA) is a single chalcone glycoside compound [1] which is derived from safflower (Carthamus tinctorius), a traditional Chinese herb (Figure 1) [2]. The most general and traditional method of extracting HSYA is water immersion. However, many other extraction systems have been developed such as smashing tissue extraction, microwave extraction, ultrasound extraction, and Soxhlet extraction [3]. HSYA has been commonly used in China to treat cardiovascular disease (CVD) [4]. Our recent literature research provides a number of articles and reviews describing novel applications of HSYA towards various diseases such as cancer and diabetes, beyond their conventional use against CVD (Figure 1).

According to the International Diabetes Federation, the number of adults diagnosed with diabetes has increased from 285 million in the year 2009 to 463 million in the year 2019, 95% of which are type 2 diabetes mellitus (T2DM) patients [5]. Diabetes is associated with accelerated rates of macrovascular and microvascular complications [6]. Macrovascular complications affect the heart, brain, and peripheral arteries and are termed CVD, cerebrovascular disease, and peripheral vascular disease, respectively [7]. Diabetes-related macrovascular complications are responsible for the impaired quality of life, disability, and premature death.
associated with diabetes [8, 9]. Microvascular complications affect the retina which is the inner part of the eye, the kidneys, and peripheral nerves. The resulting conditions are known as diabetic retinopathy, diabetic nephropathy (DN), and diabetic neuropathy, respectively [7]. In a study involving 689 individuals with T2DM obtained at baseline, the occurrences of microvascular complications observed during a median follow-up of 10.5 years were as follows: 206 patients had DN, 161 patients had retinopathy, and 179 patients had neuropathy [10].

Specifically, T2DM is characterized by chronic systemic inflammation alongside hyperglycemia and insulin resistance in the body [11]. Clinical data analysis showed that elevated C-reactive protein (CRP), tumor necrosis factor- (TNF-) α, and interleukin- (IL-) 6 were the most common inflammation indicators in diabetes-related angiopathies [12]. Related research suggested that HSYA could inhibit the apoptosis of pancreatic β-cells, and this might be the underlying mechanisms through which HSYA regulates glycolipid metabolism in T2DM rats [13]. Our previous study indicated that HSYA had direct protective effects on high glucose- (HG-) induced podocyte injury and indirect protective effects by regulating macrophage M1/M2 polarization [14]. These effects were related to its antioxidant and anti-inflammatory activities in vitro [15].

In this article, we first reviewed the in vivo and in vitro antidiabetic pharmacological actions and antidiabetic molecular mechanisms of HSYA. Based on signal research in the application of HSYA in the treatment of inflammation-related diseases, possible anti-inflammatory pathways involved in antidiabetic effects were discussed. The clinical applications of HSYA in diabetic macrovascular
and microvascular complications were then summarized based on its recent pharmacokinetic progression. Finally, possible application of HSYA as an antidiabetic oral medicament was investigated.

2. Antidiabetic Pharmacological Research and Related Mechanisms

2.1. In Vivo Antidiabetic Research. T2DM was induced in rats by feeding high-fat diet (HFD) for four weeks followed by intraperitoneal injection of streptozocin (STZ). The established models were treated with HSYA for eight weeks while metformin was used as positive control. The results showed that the underlying mechanisms of HSYA in T2DM rats were related to the following activities: the direct or indirect inhibition of pancreatic β-cell apoptosis, the improvement of insulin resistance, and the regulation of glycolipid metabolism [13].

Also, in the treatment of HFD- and STZ-induced T2DM rats by HSYA, renal protective effects were observed based on the improvement of renal functions including serum creatinine (Scr), blood urea nitrogen (BUN), glomerular volume, podocyte number, and cell apoptosis markers. Furthermore, in the HSYA treatment group, the levels of TNF-α and the inflammatory products, including free fatty acids (FFA) and lactic dehydrogenase (LDH), were significantly decreased. Observing oxidative stress markers, the level of superoxide dismutase (SOD) markedly increased in the HSYA treatment group, while the level of malondialdehyde (MDA) in the serum and kidney tissue evidently decreased [16].

In STZ-induced type 1 diabetes mellitus (T1DM) rats, a diabetic wound model was established by full-thickness carnosus with a biopsy punch. Topical application of HSYA excisional wounds that extended through the panniculus was needed for complete wound closure. In the treatment group, while the level of malondialdehyde (MDA) in the serum and kidney tissue evidently decreased [16].

2.2. In Vitro Antidiabetic Research. In vitro antidiabetic studies were conducted on seven different cell lines: rat INS-1 insulinoma cells [18], mice MPC-5 podocyte cells [14], human umbilical vein endothelial cells (HUVECs) [17, 19], human brain microvascular endothelial cells (HBMECs) [20], 3T3-L1 preadipocytes and adipocytes [21], and RAW264.7 macrophage cells [14, 17].

The loss of functional insulin-producing β-cells is a hallmark of diabetes; therefore, understanding the cellular biology of the pancreas is crucial. Rat insulinoma INS-1 cells are widely used to study glucose-stimulated insulin secretion [22]. DN is one of the microvascular complications of diabetes and is a main cause of end-stage nephropathy. The most common clinical feature of DN is progressive proteinuria which is related to podocyte function. Podocyte plays an important role in maintaining the integrity and function of the glomerular filtration barrier. MPC-5 cell line is also widely used to evaluate renal injury in vitro [23]. Related research showed that HG-induced apoptosis of podocytes and pancreatic β-cells was reversed by HSYA [14, 18].

In diabetic patients, hyperglycemia-induced endothelial injury results in all kinds of vascular complications [24]. In vitro research showed that HG increased NADPH oxidase apoptosis, vascular permeability, monocyte adhesion, the formation of reactive oxygen species (ROS), and the expression of NADPH oxidase 4 (NOX4) protein. The increased vascular injury by HG was attenuated by HSYA [19]. Another in vitro research showed that HSYA could inhibit methylglyoxal-induced injury in cultured HBMEC, which was associated with its antioxidation effect. Methylglyoxal is mainly formed from the degradation of glucose and glycated proteins [20].

For both diabetic wounds and DN progression, a central feature is the persistence of chronic inflammation, which is partly due to the prolonged presence of proinflammatory macrophages [25, 26]. In HG- and lipopolysaccharide-(LPS-) induced RAW264.7 macrophage cells, HSYA showed its anti-inflammatory effects by decreasing TNF-α, IL-1β, and nitric oxide (NO) levels [14, 17]. From Table 1, we could see that the main antidiabetic mechanism of HSYA is through its anti-inflammatory activity.

2.3. Anti-Inflammatory Signals in Antidiabetic Research of HSYA. So far, our review has shown that the antidiabetic mechanisms of HSYA are related to the following signals: c-jun NH2-terminal kinases/c-jun (JNK/c-jun) pathway [18], NOX4 pathway [19], macrophage polarization [14], and phosphoinositide 3-kinase/protein kinase B (PI3K/Akt) pathway [13]. HSYA also showed its ability to cause a decrease in oxidative stress factors such as ROS [18, 19] and hydrogen peroxide (H2O2) [19].

Inflammation is closely linked to the pathogenesis of diabetes, and chronic inflammation is one of the main causes of insulin resistance. Proinflammatory mediators can be related to obesity and induce insulin resistance in adipose tissue. Signaling pathways of transcription factors, particularly nuclear factor-κB (NF-κB) signaling, are involved in insulin sensitivity [27]. NF-κB plays a crucial role in the development of diabetic complications because of its involvement in the expression of genes that are responsible for the damage of organs such as the brain, liver, heart, muscles, endothelium, adipose tissue, and pancreas by inflammation, apoptosis, and oxidative stress [28].

The role of NF-κB signal in the antidiabetic activities of HSYA has not been previously reported. Figure 2 indicates that NF-κB signal plays an important role in the use of HSYA to treat other inflammatory diseases [29–32]. This might provide some research points to explore anti-inflammatory mechanisms of HSYA in the treatment of diabetes and diabetes complications.

2.4. Possible Anti-Inflammatory Mechanisms in Antidiabetic Research of HSYA. In Figure 2, organ damages treated by HSYA via the NF-κB pathway include brain damages such as ischemia reperfusion-injury, traumatic brain injury, ischemic stroke, and Alzheimer’s disease (AD) [29–32]; lung injury such as fetal lung fibroblasts, chronic obstructive pulmonary disease, and acute respiratory distress syndrome
<table>
<thead>
<tr>
<th>Disease</th>
<th>Species/strains</th>
<th>Effective dose/concentration</th>
<th>Route</th>
<th>Positive control</th>
<th>Intervention time</th>
<th>Main improved results</th>
<th>Mechanisms/pathways</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes</td>
<td>HFD- and STZ-induced T2DM rats</td>
<td>120 mg/kg</td>
<td>i.g.</td>
<td>Metformin as positive control</td>
<td>8 weeks</td>
<td>Pancreatic β-cell apoptosis, FBG, IR, TG, TC, LDLC, glycogen synthesis, hepatic glycogen↑</td>
<td>Regulation on glycolipid metabolism via PI3K/Akt pathway</td>
<td>[13]</td>
</tr>
<tr>
<td></td>
<td>HG-induced rat INS-1 insulinoma cells</td>
<td>800 μM</td>
<td></td>
<td>N-Acetylcysteine as oxidative stress scavenger control</td>
<td>72 hours</td>
<td>Pancreatic β-cell apoptosis, ROS, MDA, CAT, GSH-px, SOD↑</td>
<td>Antioxidative effects via JNK/c-jun pathway</td>
<td>[18]</td>
</tr>
<tr>
<td>Diabetic nephropathy</td>
<td>HFD- and STZ-induced T2DM rats</td>
<td>120 mg/kg</td>
<td>i.g.</td>
<td></td>
<td>8 weeks</td>
<td>Podocyte apoptosis↑</td>
<td>Anti-inflammation effects directly on podocyte cells and indirectly via macrophage polarization</td>
<td>[14]</td>
</tr>
<tr>
<td>Diabetic nephropathy</td>
<td>HG-induced mouse MPC-5 podocyte cells</td>
<td>100 μM, 200 μM</td>
<td></td>
<td>Kaempferol as positive control</td>
<td>24 hours</td>
<td>Podocyte apoptosis↑</td>
<td>Anti-inflammation effects via the NOX4 pathway</td>
<td>[19]</td>
</tr>
<tr>
<td>Diabetic vascular injury</td>
<td>HG-induced HUVECs</td>
<td>10 μM, 25 μM, 50 μM</td>
<td></td>
<td></td>
<td>24, 48, and 72 hours</td>
<td>Wound closure↑, granulation tissue formation↑, collagen deposition↑, secretion of VEGF↑, TGF-β↑</td>
<td>Antiglycation effects</td>
<td>[20]</td>
</tr>
<tr>
<td>Diabetic vascular injury</td>
<td>Methyglyoxal-induced HBMECs</td>
<td>10, 50, 100 μM</td>
<td></td>
<td></td>
<td>24 hours</td>
<td>Wound closure↑, granulation tissue formation↑, collagen deposition↑, secretion of VEGF↑, TGF-β↑</td>
<td>Antiglycation effects</td>
<td>[20]</td>
</tr>
<tr>
<td>Diabetic wound</td>
<td>STZ-induced T1DM rats</td>
<td>2 mg/mL vs ext</td>
<td></td>
<td>Hydrogel as positive control</td>
<td>30 days</td>
<td>NO production↑, HEK migration↑, HUVEC tube formation↑</td>
<td>Anti-inflammation effects</td>
<td>[17]</td>
</tr>
<tr>
<td>Diabetic wound</td>
<td>HUVECs and LPS-induced RAW264.7 cells</td>
<td>0.4, 0.8, 1.6 mM</td>
<td></td>
<td></td>
<td>60 and 96 hours</td>
<td>NO production↑, HEK migration↑, HUVEC tube formation↑</td>
<td>Anti-inflammation effects</td>
<td>[17]</td>
</tr>
<tr>
<td>Diabetic obesity</td>
<td>3T3-L1 preadipocytes and adipocytes</td>
<td>100 mg/L</td>
<td></td>
<td></td>
<td>24 hours</td>
<td>PPARγ2 promoter activities↑, PPARγ2↑</td>
<td>Increasing the expression of insulin signaling pathway-related genes</td>
<td>[21]</td>
</tr>
</tbody>
</table>

Abbreviations: T1DM: type 1 diabetes mellitus; T2DM: type 2 diabetes mellitus; HFD: high-fat diet; STZ: streptozotocin; FBG: fasting blood glucose; IR: insulin resistance; TG: triglyceride; TC: total cholesterol; LDLC: low-density lipoprotein cholesterol; DN: diabetic nephropathy; ROS: reactive oxygen species; SOD: superoxide dismutase; CAT: catalase; GSH-px: glutathione peroxidase; MDA: malondialdehyde; Scr: serum creatinine; UN: urea nitrogen; LDH: lactate dehydrogenase; FFA: free fatty acids; NOX4: NAPDH oxidase 4; H2O2: hydrogen peroxide; HG: high glucose; HBMECs: human brain microvascular endothelial cells; HUVECs: human umbilical vein endothelial cells; VCAM-1: vascular cell adhesion molecule-1; ICAM-1: intercellular adhesion molecule-1; iNOS: inducible nitric oxide synthase; TNF-α: tumor necrosis factor-α; CD206: mannose receptor; Arg-1: arginase-1; IL-1β: interleukin-1β; LPS: lipopolysaccharide; AGEs: advanced glycation end-products; VEGF: vascular growth factors; TGF-β1: transforming growth factor-β1; NO: nitric oxide; HEKs: human epithelial keratinocytes; PPARγ2: peroxisome proliferator-activated receptor-γ2.
It is reported that AD and T2DM share many common features including inflammation, oxidative stress, and neuronal degeneration [39]. β-Amyloid- (Aβ-) mediated inflammation plays a critical role in the initiation and progression of AD. HSYA protects Aβ-induced AD model by inhibiting inflammatory response, which may involve inhibiting the activation of the NF-κB pathway [32]. The NF-κB signaling pathway will be the first research point for our team in future studies on the antidiabetic mechanisms of HSYA.

Mitogen-activated protein kinase (MAPK) pathway and phosphoinositide 3-kinase/protein kinase B/mammalian target of rapamycin (PI3K/Akt/mTOR) pathway are two other important signals indicating the intervention of HSYA in the treatment of Parkinson’s disease (PD) [40], asthma [41], non-small-cell lung cancer [42], and brain microvascular endothelial injury [43]. It is reported that activated MAPK may be associated with both inflammation and energy metabolism in mice, rats, and humans fed with HFD for a short or long term [44]. A case-control study including 248 cases of T2DM and 101 controls showed that genetic variations in the PI3K/Akt/mTOR signaling pathway may be associated with increasing risk of obesity and diabetes [45]. Our previous discussion showed that HSYA could promote the activation of PI3K/Akt and inhibit the apoptosis of pancreatic β-cells in HFD- and STZ-induced T2DM rats [13]. Further research on the relationship between HSYA and PI3K/Akt/mTOR signal in diabetic complications should be conducted.

Other inflammation signals, including toll-like receptor 9 (TLR9) signal and cyclic adenosine monophosphate/protein kinase A (cAMP/PKA) signal, were observed in ischemic cortex after cerebral ischemia and reperfusion and acute lung injury [46, 47]. TLRs are a family of pattern recognition receptors that play a critical role in innate immune response. Recently, studies have reported the important role of TLR4 pathway in insulin resistance [27]. TLRs can be proposed as new targets in the intervention of HSYA in diabetes. The important roles of cAMP/PKA signal in the cognitive impairment of diabetic rats may suggest its involvement in the antidiabetic mechanism of HSYA.

3. Pharmacokinetic Progressions

3.1. Pharmacokinetics of Intravenous Administration

3.1.1. In Healthy Humans. 36 healthy volunteers were recruited in a single-center, open-label, single-dose, and multiple-dose study. It was found that the area under the curve (AUC) of plasma concentration at different time points and time to peak plasma concentration (Cmax) were linearly related to the dose ranging from 25 to 75 mg in a single administration of HSYA and the elimination half-life was about 3.91–4.18 h. When HSYA was administered for 7 d (50 mg/d) continuously, Cmax and AUC decreased significantly and the elimination half-life was prolonged from 3.91 h to 4.41 h [48].
Pharmacokinetic studies in healthy humans have shown that the metabolic process in the body after intravenous administration of HSYA conforms to the two-compartment model, indicating that HSYA can be quickly distributed in many organs including the heart, liver, spleen, lungs, brain, intestines, and kidneys [49]. The distribution of HSYA in the kidneys is more than that of the other organs [3]. The excretion rate of HSYA in urine 24 h after intravenous administration is up to 88.6% [3, 49]. The distribution of HSYA in the intestines, and kidneys [49]. The distribution of HSYA in the intestines is very low, and hence, there is the need for an improvement of its oral bioavailability.

3.1.2. In Renal Insufficient Patients. It was found that the $C_{\text{max}}$ and $AUC$ of HSYA in the diabetic impaired renal function group increased and the apparent volume of distribution and clearance rate reduced significantly after a single administration. The results showed that impaired renal function affected pharmacokinetic indicators [50].

Relevant studies have shown that after administering HSYA intravenously for 1 h, the average blood concentration of HSYA in renal insufficient patients was equivalent to 2.64 times that of patients with normal renal functions [51]. It is suggested that the dosage and frequency of administration should be adjusted according to the blood concentration when HSYA is used in DN patients.

3.1.3. In Patients with Traumatic Brain Injury. A sensitive, rapid, and reliable liquid chromatography-tandem mass spectrometry method was applied to investigate the pharmacokinetics of HSYA in patients with traumatic brain injury (TBI). The results demonstrated that some HSYA crossed the blood-brain barrier after administration. This study provides evidence to better understand the pharmacokinetics and potential clinical guidance for TBI treatment [52].

3.1.4. Clinical Antidiabetic Applications. Safflower yellow injection (SYI) containing 90% HSYA (45 mg HSYA per 50 mg SYI) has been widely used clinically [53]. In line with clinical guidelines and expert consensus [54], the use of SYI is becoming more and more standardized. Randomized controlled trials (RCTs) of SYI in the treatment of diabetes complications are summarized in Table 2 [55–61].

It can be observed from Table 2 that HSYA has effects on microvascular complications as well as macrovascular complications. Among these complications, HSYA was mostly used in the early stage of DN, and the mechanism research showed that HSYA had anti-inflammatory activity by decreasing TNF-α levels in DN patients [62]. There is no clinical anti-inflammatory research about HSYA on diabetic retinopathy, diabetic neuropathy, etc. The development of further clinical applications of HSYA may need to be carried out alongside its clinical anti-inflammatory effects.

It is said that the age of natural antioxidant compounds in the treatment of diabetic complications is coming [63]. HSYA injection has made some progress in the treatment of different diabetic complications. But from the perspective of patients, it is obvious that an oral drug is more convenient than an injection.

3.2. Pharmacokinetics of Oral Administration

3.2.1. In Healthy Humans. The pharmacokinetics of HSYA in 12 healthy volunteers after a single oral administration of HSYA was investigated. The plasma pharmacokinetics of HSYA after oral administration in the 12 healthy subjects showed that the component was absorbed quickly, with a peak time of 1 h and a short elimination half-life of approximately 2.6–3.5 h [64]. HSYA is relatively polar and easily catabolized and metabolized in the gastrointestinal tract and liver, leading to its rapid elimination, short half-life, and low bioavailability under oral or intragastric administration conditions [49]. The clinical use of HSYA as an oral preparation is being hindered by its low bioavailability, and hence, there is the need for an improvement of its oral bioavailability.
3.2.2. Oral Delivery System Research. Fortunately, research on the delivery system of HSYA made it possible to develop it into an oral medicament. Some oral delivery systems of HSYA are shown in Figure 3. They are water-in-oil microemulsion [65], self-double-emulsifying [66], hydrophobic nanoparticles [67], chitosan complex [68], solid lipid nanoparticles [69], and natural deep eutectic solvents [70].

Related research suggested that shell nanoparticles are a highly effective delivery system for resveratrol, another natural compound, due to their significant effects in increasing the bioavailability and anti-inflammatory activity [71]. We look forward to a suitable delivery system for HSYA, which will improve not only its bioavailability but also its anti-inflammatory activity in the near future.

4. Conclusion

HSYA, a major active component from safflower plant, has drawn more interest in recent years for its multiple pharmacological actions. We aim to provide an updated overview of HSYA in diabetes and diabetic complications from these four points: pharmacological actions, molecular mechanisms, pharmacokinetic progressions, and clinical applications. Anti-inflammation mechanism plays an important role in the antidiabetic pharmacological actions of HSYA. Further anti-inflammation research should pay attention to more inflammation signals such as NF-κB pathway and MAPK pathway. The pharmacokinetic properties of HSYA enhanced its wide clinical use as an injection to treat diabetic complications. Based on the development of drug delivery systems, HSYA could be expected as an oral drug with improved bioavailability and improved anti-inflammatory activity.

Data Availability

All authors declare that the readers can access the conclusions from the three figures and two tables. All of the figures and tables are summarized based on the references.

Conflicts of Interest

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

Authors’ Contributions

Xilan Zhang and Dayue Shen contributed equally to this work.

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