Oxidative Medicine and Cellular Longevity

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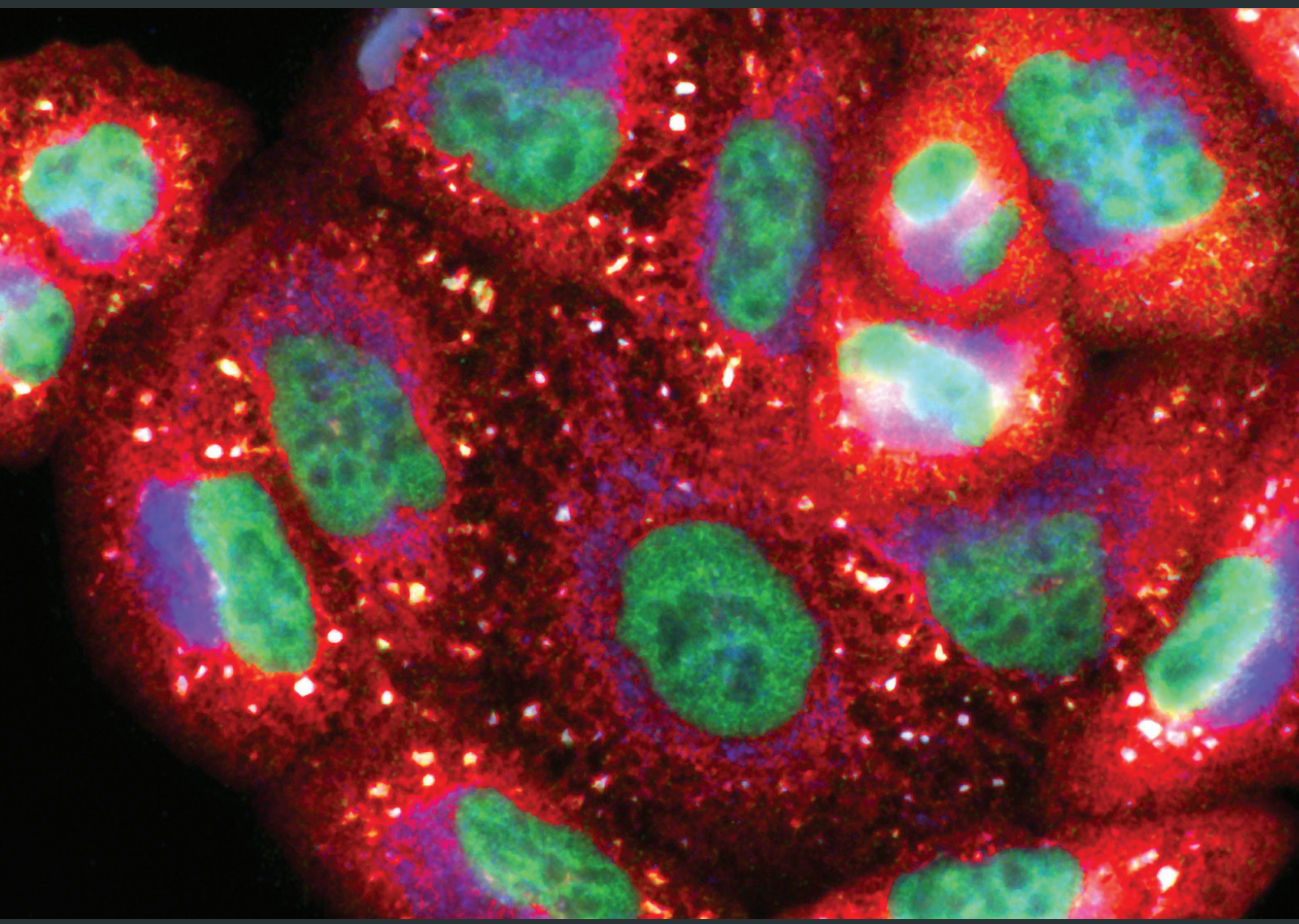

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Editorial

Role of Natural Antioxidants from Functional Foods in Neurodegenerative and Metabolic Disorders

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A food, despite its nutritional value, is defined as functional if it is appropriately shown to affect beneficially one or more target functions in the body, being a source of mental and physical well-being, and contributing to the prevention and reduction of risk factors of various diseases, or improving certain physiological functions [1]. Additionally, the ingestion of foods containing natural antioxidants such as fruits, herbs, legumes, oilseeds, whole grains, and vegetables, as well as the consumption of processed foods supplemented with natural antioxidants like vitamins C and E, carotenoids, and polyphenols, can provide the desired antioxidant status, contributing to the prevention of the neurodegenerative and metabolic disorders [2, 3]. In fact, it is noteworthy that the antioxidants play a crucial role in both food systems and in the human body to reduce oxidative processes and, consequently, neurodegenerative diseases and metabolic dysfunctions.

In this context, this special issue offers several articles reporting distinct approaches with results that corroborated the relevance of natural antioxidants in the minimization of degenerative disorders and metabolic dysfunctions. It contains six papers, and the details are presented below.

G. Li et al. investigated if the three polyphenol stilbenes (rhaponticin (RHAc), desoxyrhaponticin (dRHAc), and rhapontigenin (RHAg)) from Fenugreek (*Trigonella foenum-graecum* L.) seeds were able to improve the insulin sensitivity and mitochondrial function in 3T3-L1 adipocytes. These authors demonstrated that these compounds markedly improved the insulin sensitivity and mitochondrial function in 3T3-L1 adipocytes, RHAc being the most efficient among them.

K. S. Cho et al. reviewed the latest studies on the effects of carotenoids on neurodegenerative diseases in humans, the animal and cellular model investigations on the beneficial effects of these compounds on neurodegeneration, and the possible mechanisms and limitations of these compounds in the treatment and prevention of neurological diseases.

Novel pharmacological targets have been investigated for the treatment of diseases associated with oxidative processes and metabolic alterations by B. C. S. Santos et al. Original data revealed that methyl chavicol (MC) and its synthetic analogue 2-((4-methoxyphenyl)methyl) oxirane (MPMO) presented an antioxidant potential in trials that differ in relation to the evaluated mechanism, and MC was more effective than MPMO when the antilipase activity was evaluated, including by the molecular docking study.

G. P. F. Arrifano et al. analyzed the possible modulation of GABAergic homeostasis within synaptic clefts *in vitro* using clarified açaí (*Euterpe oleracea*) juice, to prevent seizures. According to these authors, *E. oleracea* can improve

GABAergic neurotransmission via interactions with the $GABA_A$ receptor and modulation of GABA uptake, possibly leading to the accumulation of endogenous GABA in the synaptic cleft and enhancing the inhibitory neurotransmission in the brain.

H. Zhao et al. described the antioxidant and hepatoprotective activities of hot-water-extractable polysaccharides (H-SMPS) and enzymatic-extractable polysaccharides (E-SMPS) isolated from spent mushroom substrates (SMS) of *Laetiporus sulphureus* in acute alcohol-induced mice. Their data showed that H-SMPS and E-SMPS have an antioxidant capability and potential hepatoprotective effects against alcohol-induced alcoholic liver disease.

C. G. de Souza et al. chemically studied the electroanalytical profile of the hydroalcoholic extract of "jabuticaba" (*Myrciaria cauliflora*) fruits (HEJ), their antioxidant capacity, and their effects on hypertensive animals after chronic treatment to associate the cardiovascular effects with the typical phytochemistry groups detected in this plant species. These authors observed that HEJ presents a high antioxidant potential, and the treatment with this hydroalcoholic extract attenuated the hypertension possibly improving the nitric oxide biodisponibility.

The Guest Editors hope that the readers of this special issue will find these readings attractive, delightsome, and mainly useful, making the update of this interesting and challenging subject easier than it is.

Finally, the Guest Editors are very grateful to the authors who submitted their precious research to this special issue, and they would also like to warmly acknowledge the reviewers for their excellent contribution to improve the quality of this work.

Conflicts of Interest

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

José C. T. Carvalho
Caio P. Fernandes
Julio B. Daleprane
Maria S. Alves
Didier Stien
N. P. Dhammika Nanayakkara

References

[1] V. Lobo, A. Patil, A. Phatak, and N. Chandra, "Free radicals, antioxidants and functional foods: impact on human health," *Pharmacognosy Reviews*, vol. 4, no. 8, pp. 118–126, 2010.

[2] M. Sardarodiyan and A. Mohamadi Sani, "Natural antioxidants: sources, extraction and application in food systems," *Nutrition & Food Science*, vol. 46, no. 3, pp. 363–373, 2016.

[3] B. Poljsak and I. Milisav, "Aging, oxidative stress and antioxidants," in *Oxidative Stress and Chronic Degenerative Diseases - A Role for Antioxidants, Chapter 14*, J. A. Morales-González, Ed., pp. 331–353, InTech, Rijeka, 1st edition, 2013.

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Research Article

Polyphenol Stilbenes from Fenugreek (*Trigonella foenum-graecum* L.) Seeds Improve Insulin Sensitivity and Mitochondrial Function in 3T3-L1 Adipocytes

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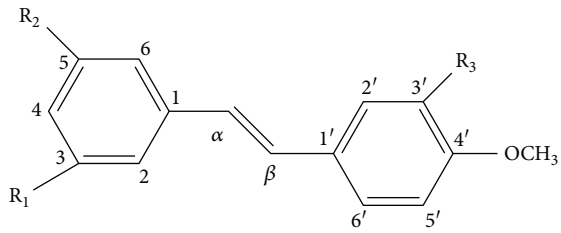
Fenugreek (*Trigonella foenum-graecum* L.) is a well-known annual plant that is widely distributed worldwide and has possessed obvious hypoglycemic and hypercholesterolemia characteristics. In our previous study, three polyphenol stilbenes were separated from fenugreek seeds. Here, we investigated the effect of polyphenol stilbenes on adipogenesis and insulin resistance in 3T3-L1 adipocytes. Oil Red O staining and triglyceride assays showed that polyphenol stilbenes differently reduced lipid accumulation by suppressing the expression of adipocyte-specific proteins. In addition, polyphenol stilbenes improved the uptake of 2-(N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-2-deoxyglucose (2-NBDG) by promoting the phosphorylation of protein kinase B (AKT) and AMP-activated protein kinase (AMPK). In present studies, it was found that polyphenol stilbenes had the ability to scavenge reactive oxygen species (ROS). Results from adenosine triphosphate (ATP) production and mitochondrial membrane potentials suggested that mitochondria play a critical role in insulin resistance and related signaling activation, such as AKT and AMPK. Rhaponticin, one of the stilbenes from fenugreek, had the strongest activity among the three compounds *in vitro*. Future studies will focus on mitochondrial biogenesis and function.

1. Introduction

Fenugreek (*Trigonella foenum-graecum* L.) is a well-known annual plant that belongs to the legume family and is widely distributed in China, India, and North African countries [1]. Fenugreek has been widely used as an edible vegetable and a medicinal plant for decades [2]. The seeds and some of its fractions have been reported to possess a wide range of biological and pharmacological effects [3, 4], including antioxidant [5, 6], hypoglycemic [7–10], hypercholesterolemia [11–13], and immunomodulatory activities [14]. Different beneficial functions of fenugreek are related to the variety of its natural components [15]. Fenugreek has been reported to contain galactomannans, nicotinic acid, alkaloids, flavonoids, salicylate, and amino acids [16]. Although several studies have shown that fenugreek seeds lowered blood glucose levels and improved lipid metabolism, the effective components are yet unknown. In our previous study, we successfully separated and purified several compounds from fenugreek seed extracts, including unsaturated fatty acids [17], flavonoids, and polyphenolic substances [18, 19]. To the best of our knowledge, it is the first report that



	R_1	R_2	R_3
Rhaponticin	Glc	OH	OH
Desoxyrhaponticin	Glc	OH	H
Rhapontigenin	OH	OH	OH

Figure 1: Chemical structure of polyphenol stilbenes from fenugreek.

polyphenol stilbenes (rhaponticin, desoxyrhaponticin, rhapontigenin) from *Trigonella foenum-graecum* L. seeds can be separated by high-speed counter-current chromatography (HSCCC) (Figure 1) [20].

Polyphenol compounds are composed of a group of substances with different chemical structures and activities and are widely present in nature [21]. Among a large variety of plant phenols, stilbenes have recently attracted extensive scientific attention. Resveratrol (3,5,4′-trihydroxy-trans-stilbene) is a well-known polyphenol compound that has widespread activities including antiobesity, antidiabetic, cardiovascular protective, and neuroprotective properties [22]. Recent studies have suggested that other polyphenol stilbenes may have a similar or even higher bioavailability compared to resveratrol [23]. Although several reports are available on the active components in fenugreek, little is known about the impact of polyphenol stilbenes on the glucose and lipid metabolism and its mechanisms of action have not yet been elucidated.

In our previous study, we demonstrated hypoglycemic effects of fenugreek extracts on streptozotocin- (STZ-) induced type 2 diabetic mice that were given with a high-fat diet. The results implied that the antidiabetic effects of stilbene extracts were related to their antioxidant effects [24]. Therefore, in the current study, we investigated the effects of polyphenol stilbenes from fenugreek seeds on both lipid accumulation and insulin resistance in 3T3-L1 adipocytes *in vitro*. To explore the underlying mechanisms of action, modulation of these stilbenes on the AMPK pathway and reactive oxygen species (ROS) was also discussed.

2. Materials and Methods

2.1. Differentiation of 3T3-L1 Preadipocytes and Induction of Insulin-Resistant Adipocytes. 3T3-L1 cells were obtained from the cell bank of the Institute of Biochemistry and Cell Biology of Shanghai (Shanghai, China). Preadipocytes were cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% calf serum (CS), 1.5 g/L sodium bicarbonate, and 1% penicillin-streptomycin solution. Cells were cultured at 37°C in a humidified atmosphere containing 5% CO_2, and medium was replaced every other day until cells were confluent. As previously described [25], 3T3-L1 cells fully differentiated to mature adipocytes and induced to insulin-resistant adipocytes [26] (Figure 2).

2.2. Cell Viability Assay. All tested compounds were obtained from the Key Laboratory of Tibetan Medicine Research, Northwest Institute of Plateau Biology, Chinese Academy of Sciences (Xining, China) and included rhaponticin (RHAc), desoxyrhaponticin (dRHAc), and rhapontigenin (RHAg). Cell viability was assessed by the lactate dehydrogenase (LDH) assay. 3T3-L1 preadipocytes were seeded in 96-well plates (0.5×10^4 cells/well) in DMEM medium without sodium pyruvate and containing 10% fetal bovine serum (FBS). Cells were incubated for 24 hr at 37°C until confluency and were separately treated with 0–100 μmol/L RHAc, dRHAc, and RHAg for 48 hr. Then, medium was transferred to 1.5 mL microcentrifuge tubes and centrifuged at 12,000 ×g and 4°C for 10 min to remove any cell debris. A total of 500 μL supernatant was added to the substrate solution, and the absorbance at 490 nm was measured using a spectrophotometer (Spectra MRTM, Dynex Technologies, Chantilly, VA, USA) according to the manufacturer's instructions of the LDH cytotoxicity assay kits (Beyotime Biotech Co., Beijing, China). The extracellular LDH activity in the media of normal control group was expressed as 100%.

2.3. Oil Red O Staining. To evaluate the effects of RHAc, dRHAc, and RHAg on lipid accumulation in 3T3-L1 adipocytes, cells were pretreated with 10 μmol/L per compound for 2 days before maturation was initiated. 3T3-L1 preadipocytes were differentiated as mentioned above on Lab-Tek® chambered cover glasses (Nalge Nunc International, Naperville, IL, USA). After induction of insulin resistance and pretreatment of the compounds, respectively, 3T3-L1 mature adipocytes were gently washed with PBS and fixed in 10% neutral formalin. Cells were permeated using 0.5% Triton-X 100 and stained with filtered Oil Red O solution (60% isopropanol and 40% water) for 30 min at room temperature. Excess Oil Red O dye was removed by washing three times with 70% EtOH. Stained oil droplets in 3T3-L1 cells were imaged using a light microscope (Olympus, Japan).

2.4. Triglyceride Assay. To evaluate the intracellular triglyceride (TG) content, 3T3-L1 preadipocytes were cultured in 12-well plates as described under Section 2.3. At confluency, cells were washed twice in ice-cold phosphate-buffered saline (PBS) and harvested in ice-cold lysis buffer. Total TG content in the lysates was measured by using TG assay kits, and cellular protein was determined using the bicinchoninic acid (BCA) protein assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

2.5. Western Blot Analysis. As described under Section 2.3, 3T3-L1 preadipocytes were gently seeded into 6-well plates (1.5×10^5 cells/well). Before maturation or inducing insulin resistance of 3T3-L1 adipocytes, cells were separately treated with RHAc, dRHAc, and RHAg for 2 days. Then, cells were washed trice with ice-cold PBS, harvested in 200 μL lysis buffer, and lysates were centrifuged at 12,000 ×g for 20 min at 4°C. The protein content of the supernatant was determined using the BCA assay kit. Cell lysates were separated

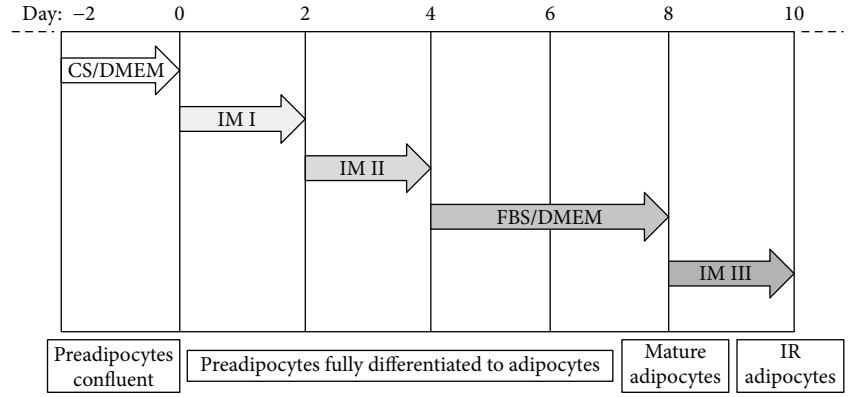


Figure 2: Timeline of 3T3-L1 differentiation and induction of insulin resistance. CS/DMEM: 10% calf serum/Dulbecco's Modified Eagle's Medium; IMI (induction media I): 10% fetal bovine serum (FBS)/DMEM + 0.5 mmol/L isobutylmethylxanthine (IBMX) + 1 μmol/L dexamethasone (Dex) + 10 μg/mL insulin; IMII (induction media II): 10% FBS/DMEM + 1 μg/mL insulin; FBS/DMEM: 10% FBS/DMEM; IMIII (induction media III): 10% FBS/DMEM + 1 μmol/L Dex.

by sodium dodecyl sulfate-polyacrylamide (SDS-PAGE) gel electrophoresis, and proteins were transferred onto a polyvinylidene fluoride (PVDF) membrane using a Bio-Rad electrophoresis equipment. Membranes were blocked with 5% skim milk in Tris-buffered saline containing 0.05% Tween-20 (TBST) for 1 hr at room temperature and incubated overnight with primary antibodies (1 : 1000, Santa Cruz Biotechnology, CA, USA) at 4°C. Membranes were washed and incubated with a horseradish peroxidase- (HRP-) conjugated secondary antibody (Boster Biotech Co. Ltd., Wuhan, China) for 2 hr at room temperature. Membranes were washed and immunoreactive proteins were visualized using an enhanced chemiluminescent (ECL) assay kit (Beyotime Biotech Co., Beijing, China) according to the manufacturer's instructions. Protein bands were analyzed using a 5200 Multi Luminescent image analyzer (Tanon Science & Technology Co. Ltd. Shanghai, China).

2.6. 2-NBDG Uptake. As described under Section 2.1, 3T3-L1 preadipocytes were gently seeded into 12-well plates (0.8×10^5 cells/well) and pretreated with RHAc, dRHAc, and RHAg for 2 days prior to induction of insulin resistance. Next, adipocytes were washed twice with PBS and the medium was changed to glucose-free DMEM containing 100 nmol/L of insulin. After 1 hr, 100 μmol/L 2-NBDG was added to the medium and cells were incubated for another 30 min. Then, cells were washed three times with PBS, trypsinized, and collected in the dark. The fluorescence was measured (excitation at 485/20 nm and emission at 540/20 nm) using a FACSAria™ flow cytometer (Becton Dickinson, San Jose, CA, USA). The data are presented as the mean fluorescent signals for 20,000 cells.

2.7. ROS Detection. As described in Section 2.6, 3T3-L1 preadipocytes were seeded into 6-well plates (1.5×10^5 cells/well) and cultured at 37°C. For induction of insulin resistance, 3T3-L1 adipocytes were treated with RHAc, dRHAc, and RHAg for 2 days. Then, cells were washed with freshly made, prewarmed PBS and incubated with 10 μmol/L of 2′,7′-dichlorofluorescein diacetate (DCFH-DA) dye for 30 min at 37°C. Then, cells were washed twice with PBS and harvested with 0.25% trypsin solution. Released intracellular ROS was detected by a FACSAria flow cytometer at wavelengths of 470/530 nm (ex/em). The data are presented as the mean fluorescent signal of 20,000 cells.

As for the detection of the mitochondrial ROS, DCFH-DA was replaced by 5 μM of MitoSOX reagent (Thermo Fisher Scientific, Beijing, China) and detected at wavelengths of 510/580 nm (ex/em).

2.8. Measurement of ATP Levels. Cells were pretreated as described in Section 2.7. After induction of insulin resistance and treatment with RHAc, dRHAc, and RHAg, cells were washed twice with ice-cold PBS and harvested with 0.25% trypsin solution. The level of ATP was determined by a spectrophotometer (Spectra MRTM, Dynex Technologies, Chantilly, VA, USA) and an ATP bioluminescence assay kit (Beyotime Biotech Co., Beijing, China) according to the manufacturer's instructions.

2.9. Monitoring of Mitochondrial Membrane Potential. Cells were pretreated as described in Section 2.8, harvested with 0.25% trypsin solution, and washed twice with PBS. Then, cells were resuspended in warm DPBS containing 10 μM of JC-1 (Sigma-Aldrich) and incubated at 37°C for 30 min. Next, cells were washed once with prewarmed PBS and centrifuged (1000 ×g, 5 min). Then, cells were gently resuspended and analyzed on a FACSAria flow cytometer with 488 nm excitation.

2.10. Statistical Analysis. Data are presented as the mean ± SD from three independent experiments. Statistical analysis was performed by one-way ANOVA or Student's *t*-test using statistical analysis software SPSS version 18.0 (SPSS, Chicago, IL, USA). $P < 0.05$ was considered statistically significant.

3. Results

3.1. Cell Viability Assay. The cytotoxicity of polyphenol stilbenes was measured by the extracellular LDH assay in the media (shown in Figure 3(a)). We found that polyphenol stilbenes did not have obvious effects on the viability of 3T3-L1 preadipocytes at the tested concentrations of 0.1–10 μmol/L for 48 hr. Thus, the concentration of three polyphenol stilbenes were confirmed as 10 μmol/L in the following experiments. And 1 μmol/L rosiglitazone was used as a positive control.

3.2. Lipogenesis and TG Assay. During the differentiation of 3T3-L1 preadipocytes to adipocytes, the formation of lipid droplets is a typical phenomenon that is used as a marker of differentiation [27]. Lipid droplets in differentiated 3T3-L1 adipocytes were stained by Oil Red O. Figure 3(b) shows that more big droplets were observed in fully differentiated 3T3-L1 cells when compared to undifferentiated control cells. This indicated that obvious lipogenesis occurred during differentiation. Treatment with both RHAc and dRHAc significantly decreased the accumulation of lipid droplets. To quantify the intracellular lipid content, TG levels were determined. The results presented in Figure 3(c) showed that during differentiation, treatment with RHAc, dRHAc, and RHAg significantly inhibited cellular TG accumulation ($P < 0.05$ or $P < 0.01$). The TG content in RHAc and dRHAc-treated cells was significantly lower compared with mature cells. These data indicated that polyphenol stilbenes differently reduced lipid accumulation during the differentiation of 3T3-L1 adipocytes.

3.3. Expression of Adipocyte-Specific Protein during 3T3-L1 Differentiation. Adipocyte differentiation from 3T3-L1 preadipocytes is associated with the expression of adipocyte-specific genes including FAS, C/EBPα, and PPARγ [28]. Therefore, we investigated the expression of these proteins (Figure 3(d)). In preadipocytes, these proteins were all expressed at a low level and were significantly increased in mature adipocytes. In general, RHAc suppressed the expression of all these proteins, and dRHAc and RHAg increased the levels of FAS and PPARγ. Together, these data indicated that the different effects of polyphenol stilbenes on the adipogenesis were tightly associated with their modulations on the expression of adipocyte-specific proteins during adipocyte differentiation.

3.4. 2-NBDG Uptake. To investigate the effects of polyphenol stilbenes on glucose uptake in insulin-resistant (IR) 3T3-L1 adipocytes, the fluorescent deoxyglucose analog (2-NBDG) was used to measure the rates of glucose uptake. 2-NBDG has been wildly used in various studies, especially for exploring cellular metabolic functions associated with GLUT systems [29]. As shown in Figure 4(a), 2-NBDG uptake in the IR group significantly decreased. Rosiglitazone (Rosi), known as a positive drug for the treatment of insulin resistance, significantly increased insulin-stimulated glucose uptake in 3T3-L1 adipocytes at the concentration of 1 μmol/L. These data suggested that establishing the IR model in this study was successful. Treatment with RHAc and dRHAc enhanced the uptake of glucose compared with the IR group.

3.5. P-AKT and P-AMPK Expression. In previous studies, it has been reported that both Akt and AMPK signaling are critical for controlling metabolic disorders, especially in the insulin signaling cascade through glucose transport [30]. We determined the expression of total Akt and phospho-Akt Ser473 as well as total AMPK and phospho-AMPK Thr172 by Western blot analysis to elucidate how polyphenol stilbenes promote glucose uptake in IR 3T3-L1 adipocytes.

Data presented in Figure 4(b) demonstrate the effects of polyphenol stilbenes on the activation of AKT and AMPK. Our results demonstrated that both the phosphorylation of AKT and AMPK were significantly decreased in IR adipocytes. Insulin at 100 nmol/L significantly stimulated Akt and AMPK activity. Rosi (1 μmol/L), a well-known AMPK activator used in this study significantly reversed the reduction of AMPK phosphorylation. Moreover, RHAc treatment showed a more remarkable effect than the other two compounds. As expected, we demonstrated that treatment with polyphenol stilbenes increased ATP levels in IR 3T3-L1 adipocytes (Figure 5(a)).

3.6. Detection of ROS Levels. Recent studies have suggested that several naturally derived active components could prevent metabolic diseases. During adipocyte proliferation and differentiation, the generation of ROS was related to the activation of AMPK [31]. Therefore, in this study, we evaluated the production of ROS by using DCFH-DA dye. As shown in Figure 5(a), intracellular ROS was rapidly upregulated in IR adipocytes. Under the same conditions, treatment with different compounds had different effects on the production of ROS. For example, Rosi and RHAc significantly reduced the expression of ROS ($P < 0.01$), whereas RHAg did not have obvious effects on intracellular ROS.

Mitochondria are the main providers but also the main scavengers of cell oxidative stress. Therefore, we monitored mitochondrial ROS by measuring the fluorescence of MitoSOX. Figure 5(b) showed that mitochondrial ROS of IR adipocytes was significantly elevated by approximately 40% when compared with mature adipocytes. Treatment with Rosi and polyphenol stilbenes had obvious effects on the scavenging of mitochondrial ROS ($P < 0.01$ or $P < 0.05$). Taken together, RHAc had the most significant effect on inhibiting the generation of ROS, no matter it is intracellular or mitochondrial.

3.7. ATP Production and Mitochondrial Membrane Potential. Mitochondria are the "power houses" of eukaryotic cells. The main function of mitochondria is to produce the cellular energy source, ATP. Thus, ATP production and the membrane potential can be used to evaluate mitochondrial function. The membrane-permeant JC-1 dye is widely used to monitor mitochondrial health. The JC-1 dye produces potential-dependent accumulation in mitochondria, as indicated by a shift in fluorescence emission from green (~529 nm) to red (~590 nm). Consequently, mitochondrial depolarization is indicated by a decrease in the ratio of

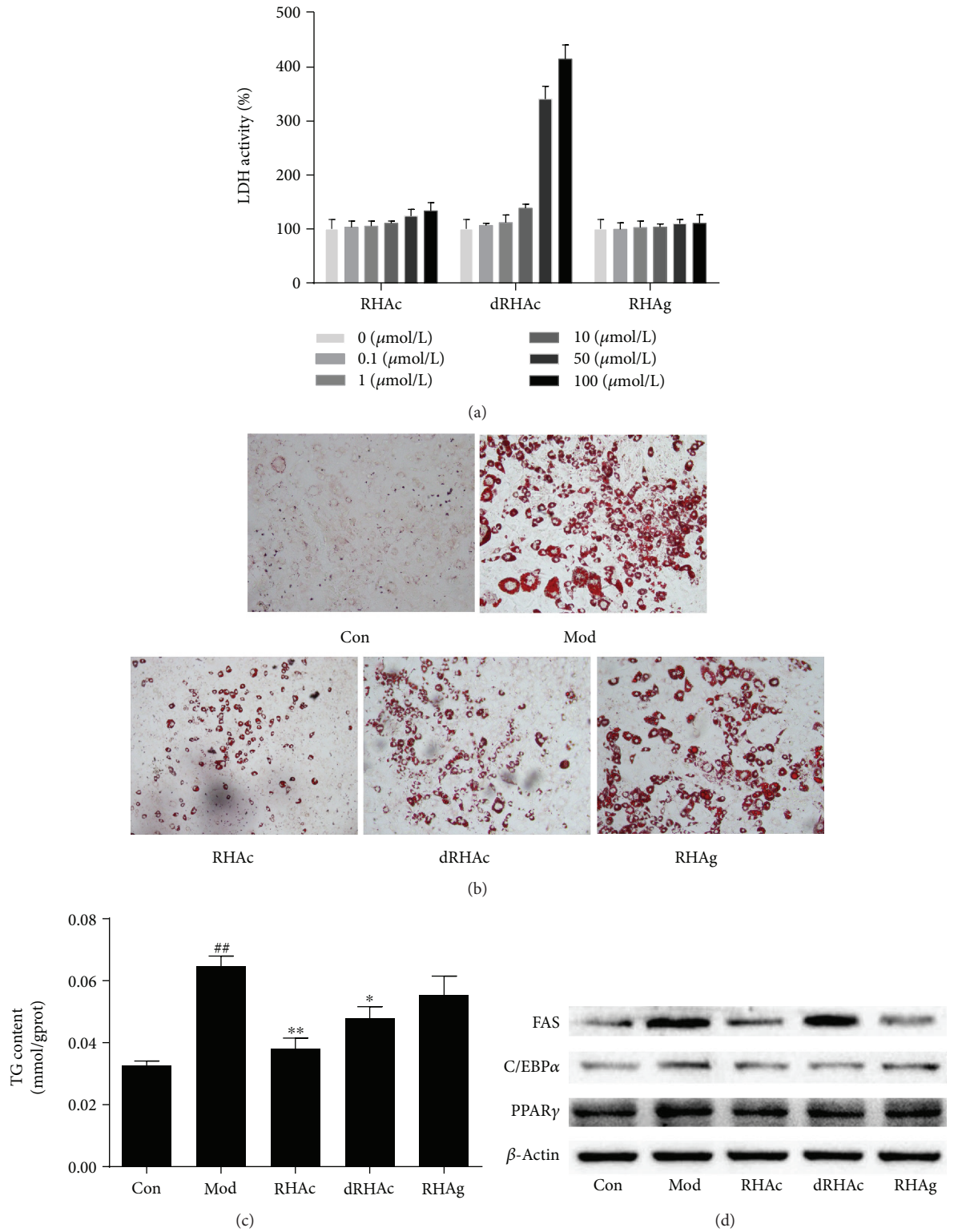


Figure 3: Effects of polyphenol stilbenes on viability and differentiation of 3T3-L1 adipocytes. (a) 3T3-L1 preadipocytes were incubated with different concentrations of polyphenol stilbenes for 48 hr after which cell viability was determined by extracellular LDH assay in the media. Data are expressed as the mean ± SD of three independent experiments. (b) 3T3-L1 preadipocytes were induced to full differentiation for 8 days as described in Figure 2. Lipid droplets were stained by Oil Red O. Con: undifferentiated 3T3-L1 preadipocytes; Mod: fully differentiated 3T3-L1 adipocytes. (c) On day 8, the cellular triglyceride (TG) content was measured and normalized against total protein. Data are presented as the mean ± SD of three independent experiments ($n = 3$). $^{\#\#}P < 0.01$ mature adipocytes versus preadipocytes; $^{*}P < 0.05$, $^{**}P < 0.01$ compound-treated adipocytes versus mature adipocytes. (d) Adipocyte-specific protein expression on day 8 was determined by Western blot analysis.

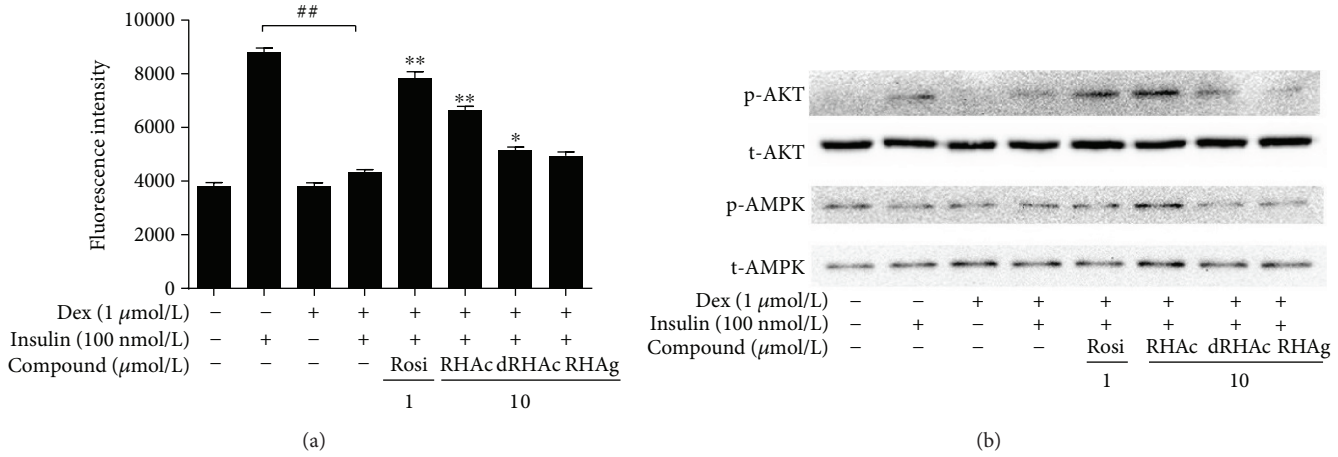


Figure 4: Effects of polyphenol stilbenes on 2-NBDG uptake and p-AKT and p-AMPK expression in 3T3-L1 adipocytes. (a) Results are presented as the means ± SD of three independent experiments ($n = 3$). $^{\#\#}p < 0.01$ Insulin-resistant (IR) adipocytes versus mature adipocytes; $^{*}P < 0.05$, $^{**}P < 0.01$ compound-treated adipocytes versus IR adipocytes. (b) p-AKT and p-AMPK expression in 3T3-L1 adipocytes was determined by Western blot analysis.

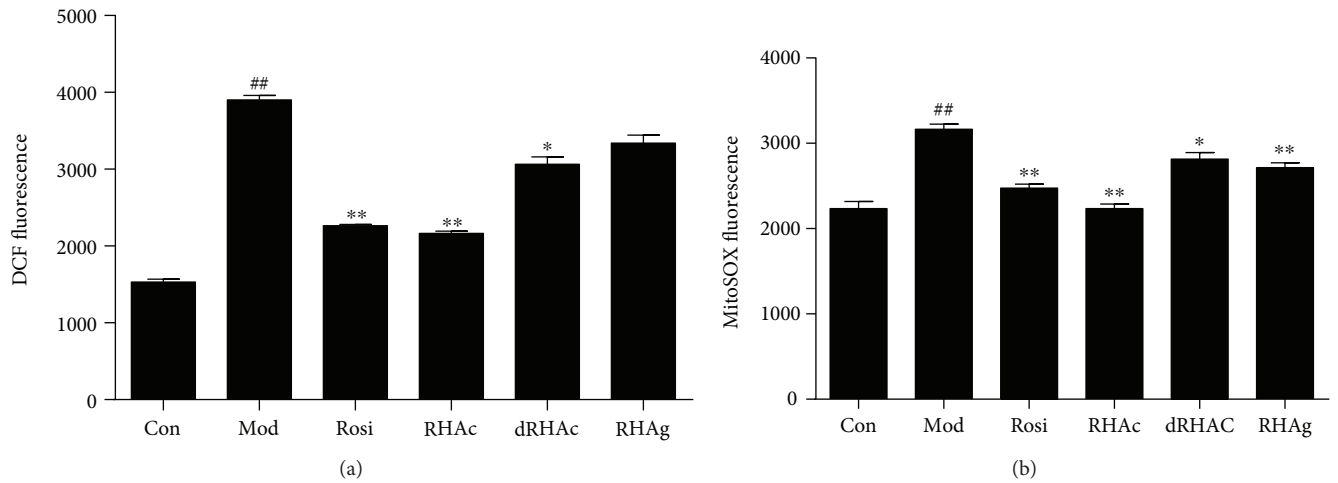


Figure 5: Effects of polyphenol stilbenes on ROS production in 3T3-L1 adipocytes. 1 μmol/L of Rosi and 10 μmol/L of the three polyphenol stilbenes were used in the experiments. Results are shown as the mean ± SD of three independent experiments ($n = 3$). $^{\#\#}P < 0.01$, IR adipocytes versus mature adipocytes; $^{*}P < 0.05$, $^{**}P < 0.01$ compound-treated adipocytes versus IR adipocytes.

red/green fluorescence intensity [32]. The data presented in Figure 6 suggested that the mitochondrial function of IR adipocytes was significantly damaged ($P < 0.01$). Both Rosi and RHAc could significantly increase ATP production and the mitochondrial membrane potential. However, the effects of RHAc were better compared to that of Rosi. These findings were consistent with the ROS scavenging ability of the compounds evaluated previously, which had been shown in Figure 5.

4. Discussion

Type 2 diabetes mellitus (T2DM) has become one of the world's most important public health problems. Insulin resistance seems to be attributed to the progressive failure of T2DM and metabolic syndrome [33]. Adipocytes play a central role in maintaining lipid homeostasis and energy balance [34]. Induced 3T3-L1 preadipocytes can differentiate into mature adipocytes, which are widely used to investigate glucose and lipid metabolism in adipocytes *in vitro* [35]. To fight against metabolic disorders, scientific research is focused on effective functional natural molecules from herbals, fruits, and vegetables [31]. Among these molecules, stilbenes, a group of polyphenols have received great interest in recent years. In our previous study, we demonstrated that extracts from stilbenes from fenugreek have beneficial hypoglycemic effects in diabetic mice, and three polyphenol stilbenes (RHAc, dRHAc, and RHAg) were separated from fenugreek seeds by HSCCC [20]. Therefore, in the current study, we further explored the effects of these polyphenol stilbenes on lipogenesis and glucose uptake in IR 3T3-L1 adipocytes.

The data presented here showed that these stilbenes could inhibit lipid accumulation during 3T3-L1 differentiation by suppressing adipocyte-specific proteins at a concentration

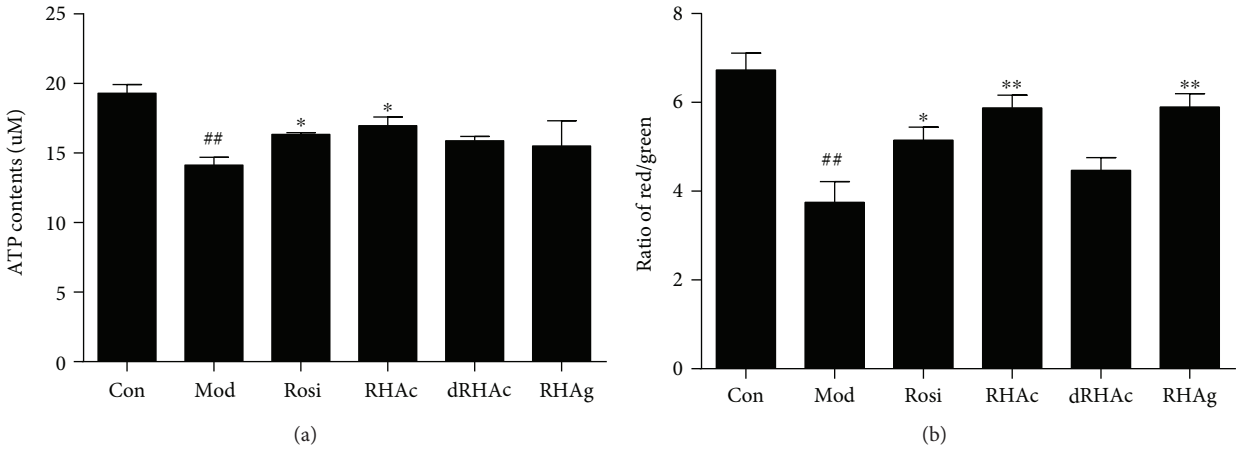


Figure 6: Effects of polyphenol stilbenes on ATP production (a) and mitochondrial membrane potential (b) in 3T3-L1 adipocytes. 1 μmol/L of Rosi and 10 μmol/L of the three polyphenol stilbenes were used in the experiments. Results are presented as the mean ± SD of three independent experiments ($n = 3$). $^{\#\#}P < 0.01$, IR adipocytes versus mature adipocytes; $^{*}P < 0.05$, $^{**}P < 0.01$, compound-treated adipocytes *versus* IR adipocytes.

of 10 μmol/L. In addition, glucose uptake was differently improved after treatment with the three compounds. When comparing the effects of the compounds used, RHAc had the best effect. RHAc significantly stimulated the activation of Akt and AMPK in IR 3T3-L1 adipocytes. However, RHAg had the lowest activity despite its structure contains 3 phenolic hydroxyl groups.

It has been shown that the antiradical and the hydrogen peroxide scavenging activities by phenolic compounds positively correlate with both the position and number of hydroxyl groups bound with the aromatic ring [36, 37]. Moreover, structure-activity relationship analysis indicated that the therapeutic effects of natural phenols involve the reduction of ROS and include polyphenol stilbenes [38]. After the hypoglycemic activity of these stilbenes was confirmed, we further investigated both the intracellular and mitochondrial ROS production in IR 3T3-L1 adipocytes (Figure 5). Oxidative stress is closely linked with the development of T2DM [39] and ROS can suppress the insulin response and contribute to the development of insulin resistance, a key pathological feature of T2DM [40]. Our results regarding ROS measurements supported this conclusion. It seemed likely that the activation of AKT and AMPK was related to the production of ROS [30]. With regard to the polyphenol stilbenes, ROS generation in the RHAc-treated group was significantly reduced. This is consistent with the structure-activity relationship of these compounds in ROS scavenging. Moreover, compounds with a strong ROS clearing ability may also activate the AMPK and other related pathways [41]. Eventually, this would lead to inefficient modulation of the glucose and lipid metabolism, as demonstrated by RHAc in this study. Lipid accumulation and impaired glucose uptake are related to the weakened metabolic capacity of mitochondria in IR adipocytes. Several natural active products, such as RHAc improved mitochondrial function, enhanced insulin sensitivity, and promoted the metabolism of carbohydrates and fatty acids.

In conclusion, our results demonstrated that polyphenol stilbenes isolated from fenugreek seeds showed marked improvement in insulin sensitivity and mitochondrial function in 3T3-L1 adipocytes. RHAc possessed the best effects *in vitro*. Moreover, the results strongly indicated that mitochondria play a major role in insulin resistance and related signaling activation. In the future, we will focus on mitochondrial biogenesis and function to explore the underlying mechanism of action.

Conflicts of Interest

The authors report no competing interests.

Authors' Contributions

Gang Li and Guangxiang Luan contributed equally to this work.

Acknowledgments

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References

[1] S. N. Acharya, J. E. Thomas, and S. K. Basu, "Fenugreek, an alternative crop for semiarid regions of North America," *Crop Science*, vol. 48, no. 3, pp. 841–853, 2008.

[2] K. Srinivasan, "Fenugreek (*Trigonella foenum-graecum*): a review of health beneficial physiological effects," *Food Reviews International*, vol. 22, no. 2, pp. 203–224, 2006.

[3] M. Akbari, H. Rasouli, and T. Bahdor, "Physiological and pharmaceutical effect of fenugreek: a review," *IOSR Journal of Pharmacy (IOSRPHR)*, vol. 2, no. 4, pp. 49–53, 2012.

[4] U. C. S. Yadav and N. Z. Baquer, "Pharmacological effects of *Trigonella foenum-graecum* L. in health and disease," *Pharmaceutical Biology*, vol. 52, no. 2, pp. 243–254, 2014.

[5] O. Belguith-Hadriche, M. Bouaziz, K. Jamoussi, M. S. J. Simmonds, A. El Feki, and F. Makni-Ayedi, "Comparative study on hypocholesterolemic and antioxidant activities of various extracts of fenugreek seeds," *Food Chemistry*, vol. 138, no. 2-3, pp. 1448–1453, 2013.

[6] H. Pandey and P. Awasthi, "Effect of processing techniques on nutritional composition and antioxidant activity of fenugreek (*Trigonella foenum-graecum*) seed flour," *Journal of Food Science and Technology*, vol. 52, no. 2, pp. 1054–1060, 2015.

[7] T. Zia, S. N. Hasnain, and S. K. Hasan, "Evaluation of the oral hypoglycaemic effect of *Trigonella foenum-graecum* L. (methi) in normal mice," *Journal of Ethnopharmacology*, vol. 75, no. 2-3, pp. 191–195, 2001.

[8] W. L. Xue, X. S. Li, J. Zhang, Y. H. Liu, Z. L. Wang, and R. J. Zhang, "Effect of *Trigonella foenum-graecum* (fenugreek) extract on blood glucose, blood lipid and hemorheological properties in streptozotocin-induced diabetic rats," *Asia Pacific Journal of Clinical Nutrition*, vol. 16, Supplement 1, pp. 422–426, 2007.

[9] A. Eidi, M. Eidi, and M. Sokhteh, "Effect of fenugreek (*Trigonella foenum-graecum* L) seeds on serum parameters in normal and streptozotocin-induced diabetic rats," *Nutrition Research*, vol. 27, no. 11, pp. 728–733, 2007.

[10] N. Hamza, B. Berke, C. Cheze et al., "Preventive and curative effect of Trigonella foenum-graecum L. seeds in C57BL/6J models of type 2 diabetes induced by high-fat diet," *Journal of Ethnopharmacology*, vol. 142, no. 2, pp. 516–522, 2012.

[11] R. D. Sharma, T. C. Raghuram, and N. S. Rao, "Effect of fenugreek seeds on blood glucose and serum lipids in type I diabetes," *European Journal of Clinical Nutrition*, vol. 44, no. 4, pp. 301–306, 1990.

[12] A. Stark and Z. Madar, "The effect of an ethanol extract derived from fenugreek (Trigonella foenum-graecum) on bile acid absorption and cholesterol levels in rats," *British Journal of Nutrition*, vol. 69, no. 1, pp. 277–287, 1993.

[13] S. Kaviarasan, P. Viswanathan, and C. V. Anuradha, "Fenugreek seed (*Trigonella foenum graecum*) polyphenols inhibit ethanol-induced collagen and lipid accumulation in rat liver," *Cell Biology and Toxicology*, vol. 23, no. 6, pp. 373–383, 2007.

[14] B. Bin-Hafeez, R. Haque, S. Parvez, S. Pandey, I. Sayeed, and S. Raisuddin, "Immunomodulatory effects of fenugreek (*Trigonella foenum graecum* L.) extract in mice," *International Immunopharmacology*, vol. 3, no. 2, pp. 257–265, 2003.

[15] S. Nathiya, M. Durga, and T. Devasena, "Therapeutic role of Trigonella foenum-graecum [fenugreek]–a review," *International Journal of Pharmaceutical Sciences Review and Research*, vol. 27, no. 2, pp. 74–80, 2014.

[16] A. Mehrafarin, A. Qaderi, S. H. Rezazadeh, H. Naghdi Badi, G. H. Noormohammadi, and E. S. Zand, "Bioengineering of important secondary metabolites and metabolic pathways in fenugreek (*Trigonella foenum-graecum* L.)," *Journal of Medicinal Plants*, vol. 9, no. 35, pp. 1–18, 2010.

[17] R. Yang, H. Wang, G. Li, Y. Suo, and J. You, "Optimization of ultrasonic-assisted extraction of flavonoids from fenugreek (Trigonella foenum-graecum L.) using response surface methodology and HPLC-DAD-MS analysis," *Asian Journal of Chemistry*, vol. 26, no. 15, pp. 4830–4834, 2014.

[18] Y. He, H. Lv, X. Wang, Y. Suo, and H. Wang, "Isolation and purification of six bioactive compounds from the seeds of *Trigonella foenum-graecum* L. using high-speed counter-current chromatography," *Separation Science and Technology*, vol. 49, no. 4, pp. 580–587, 2014.

[19] Y. He, C. Ding, X. Wang, H. Wang, and Y. Suo, "Using response surface methodology to optimize countercurrent chromatographic separation of polyphenol compounds from fenugreek (*Trigonella foenum-graecum* L.) seeds," *Journal of Liquid Chromatography & Related Technologies*, vol. 38, no. 1, pp. 29–35, 2014.

[20] Y. He, X. Wang, Y. Suo, C. Ding, and H. Wang, "Efficient protocol for isolation of rhaponticin and rhapontigenin with consecutive sample injection from fenugreek (*Trigonella foenum-graecum* L.) by HSCCC," *Journal of Chromatographic Science*, vol. 54, no. 3, pp. 479–485, 2016.

[21] D. Pereira, P. Valentão, J. Pereira, and P. Andrade, "Phenolics: from chemistry to biology," *Molecules*, vol. 14, no. 6, pp. 2202–2211, 2009.

[22] M. Reinisalo, A. Kårlund, A. Koskela, K. Kaarniranta, and R. O. Karjalainen, "Polyphenol stilbenes: molecular mechanisms of defence against oxidative stress and aging-related diseases," *Oxidative Medicine and Cellular Longevity*, vol. 2015, Article ID 340520, 24 pages, 2015.

[23] J. Chang, A. Rimando, M. Pallas et al., "Low-dose pterostilbene, but not resveratrol, is a potent neuromodulator in aging and Alzheimer's disease," *Neurobiology of Aging*, vol. 33, no. 9, pp. 2062–2071, 2012.

[24] X. Y. Li, S. S. Lu, H. L. Wang et al., "Effects of the fenugreek extracts on high-fat diet-fed and streptozotocin-induced type 2 diabetic mice," *Animal Models and Experimental Medicine*, vol. 1, no. 1, pp. 68–73, 2018.

[25] G. Luan, Y. Wang, Z. Wang et al., "Flavonoid glycosides from fenugreek seeds regulate glycolipid metabolism by improving mitochondrial function in 3T3-L1 adipocytes in vitro," *Journal of Agricultural and Food Chemistry*, vol. 66, no. 12, pp. 3169–3178, 2018.

[26] K. L. Anil Kumar and A. R. Marita, "Troglitazone prevents and reverses dexamethasone induced insulin resistance on glycogen synthesis in 3T3 adipocytes," *British Journal of Pharmacology*, vol. 130, no. 2, pp. 351–358, 2000.

[27] E. D. Rosen, C. J. Walkey, P. Puigserver, and B. M. Spiegelman, "Transcriptional regulation of adipogenesis," *Genes & Development*, vol. 14, no. 11, pp. 1293–1307, 2000.

[28] M. Lee, H. H. Lee, J. K. Lee, S. K. Ye, S. H. Kim, and S. H. Sung, "Anti-adipogenic activity of compounds isolated from *Idesia polycarpa* on 3T3-L1 cells," *Bioorganic & Medicinal Chemistry Letters*, vol. 23, no. 11, pp. 3170–3174, 2013.

[29] C. Zou, Y. Wang, and Z. Shen, "2-NBDG as a fluorescent indicator for direct glucose uptake measurement," *Journal of Biochemical and Biophysical Methods*, vol. 64, no. 3, pp. 207–215, 2005.

[30] S. M. Schultze, B. A. Hemmings, M. Niessen, and O. Tschopp, "PI3K/AKT, MAPK and AMPK signalling: protein kinases in

glucose homeostasis," *Expert Reviews in Molecular Medicine*, vol. 14, p. e1, 2012.

[31] P. V. A. Babu, D. Liu, and E. R. Gilbert, "Recent advances in understanding the anti-diabetic actions of dietary flavonoids," *The Journal of Nutritional Biochemistry*, vol. 24, no. 11, pp. 1777–1789, 2013.

[32] S. Perry, J. Norman, J. Barbieri, E. Brown, and H. Gelbard, "Mitochondrial membrane potential probes and the proton gradient: a practical usage guide," *BioTechniques*, vol. 50, no. 2, pp. 98–115, 2011.

[33] J. Szendroedi, E. Phielix, and M. Roden, "The role of mitochondria in insulin resistance and type 2 diabetes mellitus," *Nature Reviews Endocrinology*, vol. 8, no. 2, pp. 92–103, 2011.

[34] G. Frühbeck, J. Gómez-Ambrosi, F. J. Muruzábal, and M. A. Burrell, "The adipocyte: a model for integration of endocrine and metabolic signaling in energy metabolism regulation," *American Journal of Physiology-Endocrinology and Metabolism*, vol. 280, no. 6, pp. E827–E847, 2001.

[35] M. J. Thomson, M. G. Williams, and S. C. Frost, "Development of insulin resistance in 3T3-L1 adipocytes," *Journal of Biological Chemistry*, vol. 272, no. 12, pp. 7759–7764, 1997.

[36] Z. Sroka and W. Cisowski, "Hydrogen peroxide scavenging, antioxidant and anti-radical activity of some phenolic acids," *Food and Chemical Toxicology*, vol. 41, no. 6, pp. 753–758, 2003.

[37] Y.-Z. Cai, M. Sun, J. Xing, Q. Luo, and H. Corke, "Structure–radical scavenging activity relationships of phenolic compounds from traditional Chinese medicinal plants," *Life Sciences*, vol. 78, no. 25, pp. 2872–2888, 2006.

[38] T. Perecko, V. Jancinova, K. Drabikova, R. Nosal, and J. Harmatha, "Structure-efficiency relationship in derivatives of stilbene. Comparison of resveratrol, pinosylvin and pterostilbene," *Neuroendocrinology Letters*, vol. 29, no. 5, pp. 802–805, 2008.

[39] J. L. Rains and S. K. Jain, "Oxidative stress, insulin signaling, and diabetes," *Free Radical Biology and Medicine*, vol. 50, no. 5, pp. 567–575, 2011.

[40] T. Tiganis, "Reactive oxygen species and insulin resistance: the good, the bad and the ugly," *Trends in Pharmacological Sciences*, vol. 32, no. 2, pp. 82–89, 2011.

[41] J.-a. Kim, Y. Wei, and J. R. Sowers, "Role of mitochondrial dysfunction in insulin resistance," *Circulation Research*, vol. 102, no. 4, pp. 401–414, 2008.

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Review Article

Recent Advances in Studies on the Therapeutic Potential of Dietary Carotenoids in Neurodegenerative Diseases

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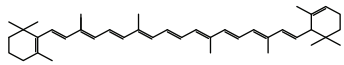
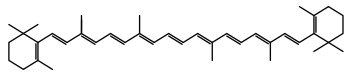
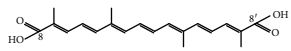
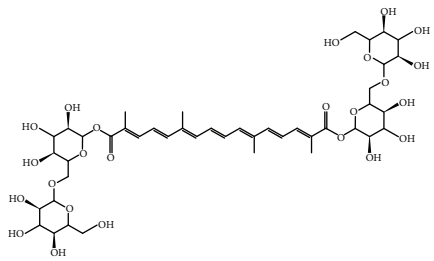
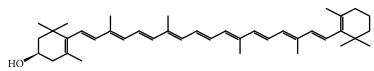
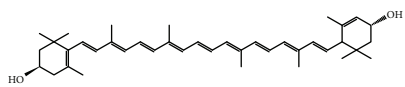
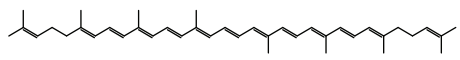
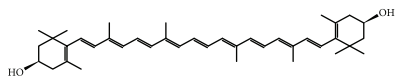
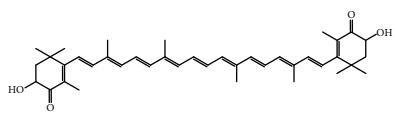
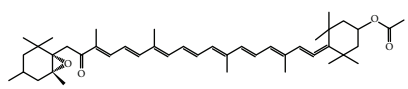
Carotenoids, symmetrical tetraterpenes with a linear C40 hydrocarbon backbone, are natural pigment molecules produced by plants, algae, and fungi. Carotenoids have important functions in the organisms (including animals) that obtain them from food. Due to their characteristic structure, carotenoids have bioactive properties, such as antioxidant, anti-inflammatory, and autophagy-modulatory activities. Given the protective function of carotenoids, their levels in the human body have been significantly associated with the treatment and prevention of various diseases, including neurodegenerative diseases. In this paper, we review the latest studies on the effects of carotenoids on neurodegenerative diseases in humans. Furthermore, animal and cellular model studies on the beneficial effects of carotenoids on neurodegeneration are also reviewed. Finally, we discuss the possible mechanisms and limitations of carotenoids in the treatment and prevention of neurological diseases.

1. Introduction

Carotenoids are natural pigments present in various organisms, such as plants, animals, and microorganisms. For example, the orange color of carrots and the red color of tomatoes are due to their carotenoid components [1]. Plant, algae, and fungi produce >600 different types of carotenoids. Animals obtain carotenoids from food since they cannot synthesize them. As pigment molecules, carotenoids play a role in the process of photosynthesis, either in photoprotection or light collection [2]. Carotenoids confer photoprotection by dissipating light energy that is not used directly for photosynthesis, and they contribute to photosynthesis through light collection, during which they pass light through to the chloroplast [2]. Carotenoids also act as antioxidants that reduce reactive by-products, such as reactive oxygen species (ROS), during photosynthesis [3]. As a result, carotenoids protect the photosynthetic apparatus from oxidative damage. In addition, carotenoids play various other roles in nature, including the development and oxidative stress signaling in plants, sex-related coloration patterns, and as a precursor for vitamin A in many species [3, 4].

Carotenoids, also known as tetraterpenoids, are C40 hydrocarbons that have isoprenoids as building units (Table 1). The C40 carbon skeleton of carotenoids is produced by the linkage of two C20 geranylgeranyl diphosphate molecules; all of the carotenoid variants are derived from the skeleton [4]. Carotenoids can be divided into two groups according to their polarity: xanthophylls (polar carotenoids such as astaxanthin, β-cryptoxanthin, lutein, and zeaxanthin) and carotene (nonpolar carotenoids such as α-carotene, β-carotene, and lycopene) [5]. The distinctive structural feature of carotenoids is the long, alternating double and single bond system, which is associated with light absorption and oxidation [4].

Table 1: Representative food-derived carotenoids and their structures.

Carotenoid	Structure	Food source	Reference
α-Carotene		Banana, butternut, carrot, pumpkin	[11, 153–156]
β-Carotene		Apricots, banana, broccoli, cantaloupe, carrot, dairy products, honeydew, kale, mango, nectarine, peach, pumpkin, spinach, sweet potato, tomato	[1, 11, 13, 153, 154]
Crocetin		Gardenia fruit, saffron stigma	[157, 158]
Crocin		Gardenia fruit, saffron stigma	[159, 160]
β-Cryptoxanthin		Apple, broccoli, celery, chili, crustaceans, grape, green beans, papaya, pea, peach, peppers, salmonid fish, squashes, tangerine	[11, 12, 14, 153, 161]
Lutein		Apple, basil, broccoli, celery, crustaceans, cucumber, dairy products, grapes, green pepper, kale, kiwi, maize, parsley, pea, pumpkin, salmonid fish, spinach, squash	[1, 11, 12, 162, 163]
Lycopene		Grapefruit, guava, tomato, watermelon	[1, 11, 153, 164, 165]
Zeaxanthin		Basil, crustaceans, cucumber, dairy products, honeydew, kale, maize, mango, orange, parsley, salmonid fish, spinach	[1, 11, 12, 162]
Marine			
Astaxanthin		Crustaceans, algae, salmonid fish	[12, 14, 15, 36]
Fucoxanthin		Brown seaweeds	[16, 17, 166]

The major sources of carotenoids in the human diet are fruits and vegetables, which have various colors, such as green, red, orange, and yellow [6]. Humans consume approximately 40 carotenoids from common fruits and vegetables (Table 1) [7]. Dark green vegetables, such as broccoli, coriander, kale, and spinach, contain a large number of chloroplasts, in which most carotenoids exist; therefore, they possess high concentrations of carotenoids [8]. As chloroplasts generally contain the most consistent carotenoid composition [9], the distribution of carotenoids is similar among different plant species in this group [7]. On the other hand, in red-, orange-, or yellow-colored fruits and vegetables, carotenoids are mainly accumulated in chromoplasts, which are usually converted from chloroplasts during ripening [10]. As chromoplasts in different plant species contain various carotenoids, the carotenoid distribution in this group is diverse [6]. Some seafood and animal foods also contain carotenoids. Animals cannot synthesize carotenoids; instead, they ingest carotenoids through foods and accumulate these molecules in their bodies. As a result, some animal foods contain carotenoids. For example, high concentrations of lutein and zeaxanthin accumulate in egg yolks [11]. Milk and dairy products, salmonid fish, and crustaceans also provide various carotenoids [12]. The main carotenoid in bovine milk is β-carotene [13], whereas the major carotenoids in salmonid fish and crustaceans are astaxanthin and canthaxanthin [12, 14, 15]. In addition, some edible brown seaweeds contain fucoxanthin as a major carotenoid [16, 17].

Carotenoids are differentially distributed in various organs of the human body. Interestingly, xanthophylls account for 66–77% of the total carotenoids in the frontal and occipital lobes of the human brain [18], whereas less than 40% of the total carotenoids in most tissues and plasma are reported to be xanthophylls [19–21]. It was reported that

the human brain contains sixteen carotenoids, with the major carotenoids being anhydrolutein, α-carotene, α-cryptoxanthin, *cis*- and *trans*-β-carotene, β-cryptoxanthin, lutein, *cis*- and *trans*-lycopene, and zeaxanthin [18]. Given their property of protecting tissues from oxidative stress and their localization in the brain, the role of carotenoids in preventing or treating oxidative stress-associated diseases, including neurodegenerative diseases, is of interest.

As carotenoids have various physiological activities, such as antioxidant activity, the amount of carotenoid in the human body is important for health. Therefore, the intake of carotenoids through the diet is associated with the prevention and treatment of various diseases, including age-related macular degeneration [22], cancer [23, 24], cardiovascular diseases [25], and neurodegenerative diseases [5]. In the present paper, we review the latest studies that show the effects of dietary carotenoids on neurodegenerative diseases, and discuss the prospect of the use of carotenoids in the prevention and treatment of these diseases.

2. Bioactivities of Carotenoids

As stated above, most carotenoids have a symmetrical tetraterpene structure with a linear C40 hydrocarbon backbone (Table 1). These highly unsaturated fatty chains are susceptible to modifications, such as *cis-trans* isomerization or cyclization, and result in the characteristic coloration induced by light absorption. Owing to their highly lipophilic structures, carotenoids are found in the lipid membrane. Nonpolar carotenes reside in the inner part of the membrane, whereas polar xanthophylls are located across the bilayer, tilted ~40° from the axis normal to the membrane plane [26, 27]. Inserted carotenoids may affect the physical properties of the lipid bilayer; however, their exact function in the membrane remains unclear besides in their prevention of the oxidative damage of lipids [27]. Significant evidence has shown that carotenoids can reduce oxidative damage by scavenging ROS and exert anti-inflammatory effects *in vivo* (Table 2) [28].

2.1. Antioxidant Activity. Carotenoids have been demonstrated to be one of the most potent natural singlet oxygen scavengers, with a fast quenching rate ($\cong 10^{10}\ M^{-1}\ s^{-1}$) [29]. They can effectively neutralize ROS and other free radicals to provide protection against oxidation in both photosynthetic and nonphotosynthetic organisms [6, 16, 29–37]. However, each carotenoid shows different antioxidant activities, owing to the presence of functional groups with increasing polarities as well as the number of conjugated double bonds [31]. The antioxidant property of carotenoids has inspired many epidemiological and clinical studies that have investigated if these pigment molecules are able to prevent various ROS-mediated disorders such as cancer, inflammation, retinal degeneration, and neurodegeneration. In the case of cancer, many studies have shown that carotenoid consumption is correlated with a reduced risk of several types of cancer; however, other studies have shown that the cancer-preventive effects of carotenoids are negligible or even that they are carcinogenic [38, 39].

Lutein is a xanthophyll and the most abundant carotenoid in the human retina and brain [18, 40]. The Age-Related Eye Disease Study (AREDS) showed that a formulation consisting of vitamins C, E, β-carotene, and zinc is beneficial for the prevention of age-related macular degeneration (AMD) [41]. In a second study, although primary analysis from the AREDS2 did not reveal a benefit of daily supplementation with lutein/zeaxanthin on AMD progression [42], secondary exploratory analyses suggested that lutein/zeaxanthin were helpful in reducing this risk [43]. In addition, given that increased oxidative stress and inflammation are observed in age-related macular degeneration [44], lutein supplementation may improve visual function through antioxidant activity.

In addition to their antioxidant activities, carotenoids can protect cells from the oxidative stress induced by some stressors via activation of endogenous antioxidant enzymatic activities and a reduction in DNA damage. Crocetin, a pharmacologically active metabolite of *Crocus sativus* L., exerts cardioprotective effects by increasing superoxide dismutase (SOD) and glutathione peroxidase activities in cardiac hypertrophy induced by norepinephrine in rats [35]. Crocin, another component of *Crocus sativus* L., has also been shown to increase SOD activity to prevent the death of PC-12 cells during serum/glucose deprivation [34]. Recent studies have demonstrated that marine carotenoids such as astaxanthin and fucoxanthin also display antioxidant properties by activating the antioxidant network, including SOD and catalase [45, 46]. In addition, β-cryptoxanthin protects human cells from H_2O_2-induced damage by stimulating the repair of damage caused by DNA oxidation as well as by its antioxidant activity [36]. Lycopene and β-carotene also provide protection against DNA damage at low concentrations [32]. However, opposite effects have been shown at higher concentrations in cells with oxidative damage [32].

2.2. Antineuroinflammation. Neuroinflammation is a local response of the nervous system during neurodegeneration, trauma, and autoimmune disorders. A variety of cell types, including astrocytes, microglia, vascular cells, neutrophils, and macrophages, are involved in neuroinflammation [47]. Growing evidence suggests that neuroinflammation is one of the pathological features of many neurodegenerative disorders and autoimmune diseases, such as multiple sclerosis [44, 47, 48]. In the last decade, some carotenoids have been shown to have antineuroinflammatory effects *in vivo*. Among the polar xanthophylls, the ability of lutein to suppress inflammation has been demonstrated in murine retinal cells [49–51] and in a clinical trial studying retinal health in preterm infants [52]. In addition, it has been shown that lutein reduces lipid peroxidation and proinflammatory cytokine release by suppressing the activation of the nuclear factor-κB (NF-κB) pathway in the presence of a variety of oxidative stressors [53–56]. It has also been demonstrated that crocin and crocetin are able to suppress the production of proinflammatory cytokines and nitric oxide by lipopolysaccharide, interferon γ, and β-amyloid (Aβ) stimulation in microglial cells [57]. Astaxanthin, a member of the xanthophyll family that confers the pink color in flamingos, has an anti-inflammatory effect and antioxidant activity similar to other

Table 2: Bioactivities of representative food-derived carotenoids and their implications in neurodegenerative diseases.

Carotenoid	Bioactivity	Reference	Implication in ND	Reference
α-Carotene	Antioxidant	[6, 31]	AD: human	[130]
			PD: human	[137, 138]
β-Carotene	Antioxidant	[6, 30, 31]	AD: human	[130, 134, 135]
			PD: rodent	[137, 138]
			ALS: cell	[113]
Crocetin	Antioxidant	[35]	AD: cell	[121, 125]
	Antineuroinflammation	[54]		
Crocin	Antioxidant	[34]	AD: rodent	[115]
	Antineuroinflammation	[57]	Cell	[114]
			PD: rodent	[117, 118]
	Autophagy	[71]	*Drosophila*	[119]
			Cell	[116]
β-Cryptoxanthin	Antioxidant	[6, 36]	AD: human	[130]
Lutein	Antioxidant	[6, 31]	AD: human	[130–133]
	Antineuroinflammation	[49–51, 53]	PD: rodent	[167]
	Autophagy	[68–70]	HD: rodent	[168]
Lycopene	Antioxidant	[6, 31]	AD: human	[130, 133]
	Antineuroinflammation	[64, 66]	Rodent	[36, 89, 91]
	Autophagy	[72–74]	*C. elegans*	[104]
			Cell	[100–102]
			PD: human	[138]
			Rodent	[92–95]
			Cell	[103]
			HD: rodent	[96–99]
Zeaxanthin	Antioxidant	[6, 31]	AD: human	[132, 133]
Marine				
Astaxanthin	Antioxidant	[6, 31, 33]	AD: human	[134]
	Antineuroinflammation	[58, 59, 62]	Cell	[107, 108]
	Autophagy	[75, 76]	PD: rodent	[110]
			Cell	[109–112]
			ALS: cell	[113]
Fucoxanthin	Antioxidant	[16]	AD: rodent	[106]
	Antineuroinflammation	[63]	Cell	[105]
	Autophagy	[77]		

carotenoids [58, 59]. Furthermore, astaxanthin has also been found to reduce hippocampal and retinal inflammation in streptozotocin-induced diabetic rats, alleviating cognitive deficits, retinal oxidative stress, and depression [60–62]. Fucoxanthin, another member of the marine xanthophylls, exerts anti-inflammatory effects against various stimuli through Akt, NF-κB, and mitogen-activated protein kinase pathways [63].

Lycopene, one of the carotenes present in large amounts in tomatoes, has been demonstrated to reduce neuroinflammatory phenotypes, depression-like behaviors, and inflammation-induced cognitive function defects in murine models [64–66]. As a whole, cellular and animal models have revealed that carotenoids are potent anti-inflammatory agents in the nervous system and act through the suppression of inflammation pathways.

2.3. Modulation of Autophagy. Autophagy, a catabolic process necessary for the cleanup of damaged organelles, protein complexes, and even single proteins, as well as for the recycling of nutritional building blocks, has been implicated in numerous disorders and conditions such as aging, cancer, and neurodegeneration. A growing amount of evidence strongly suggests that autophagy removes misfolded or aggregated proteins, the main features of most neurodegenerative diseases, for example, tau fibrils in Alzheimer's disease (AD) and Lewy bodies in Parkinson's disease (PD) [67]. Recent studies have shown that some carotenoids are able to modulate autophagy in cellular and animal models. It has been recently demonstrated that lutein attenuates cobalt chloride-induced autophagy via the mTOR pathway in rat Müller cells [68], whereas it induces autophagy through the upregulation of Beclin-1 in retinal pigment epithelial cells

[69, 70]. Crocin has also been shown to have a paradoxical effect on autophagy. The induction of autophagy by crocin occurs during hypoxia, and the inhibition of autophagy by crocin occurs during reperfusion [71]. Lycopene has also been shown to be involved in autophagy [72–74]. Astaxanthin has been found to attenuate autophagy in hepatic cells [75, 76]. In a model of murine traumatic brain injury, fucoxanthin has the ability to protect neuronal cells from death through the activation of autophagy and the nuclear factor erythroid 2-related factor pathway [77]. The modulation of autophagy by carotenoids remains a controversial topic, and the precise molecular mechanism of this modulation remains unclear.

3. Beneficial Effects of Carotenoids on Neurodegenerative Diseases

3.1. Neurodegenerative Diseases. Neurodegenerative diseases are neuronal disorders that feature a progressive loss of neurons and are associated with protein aggregates [78]. The major neurodegenerative diseases include AD, PD, Huntington's disease (HD), and amyotrophic lateral sclerosis (ALS), all of which have disease-specific causative factors and pathological features. For examples, senile plaques with Aβ aggregates and fibrillary tangles with hyperphosphorylated tau are hallmarks of AD [79]. Similarly, aggregation of α-synuclein, huntingtin, and TAR DNA-binding protein 43 is associated with PD, HD, and ALS, respectively [80–82].

Although these neurodegenerative diseases have different causative factors, they share common features that might be closely related to the onset and progress of disease by the induction of neuronal cell death. One of the shared features is oxidative stress due to elevated ROS production during disease progression [78]. ROS are reactive chemicals with oxygen that can attack and damage the macromolecules, such as lipids, DNA, and proteins, of living cells [83]. In neuronal cells of patients with neurodegenerative diseases, ROS levels are increased by various cellular events, including mitochondrial insults and release of redox metals that interact with oxygen [84, 85], which result in neuronal cell death [85]. In addition, the pathological environment of neurodegenerative diseases, such as the increase in protein aggregates, results in sustained inflammation due to microglia activation [86]. Although the inducers of inflammation vary among different diseases, chronic inflammation is induced in neurons through largely common mechanisms [87]. Once neuroinflammation is chronically activated, cytokines and chemokines are released by long-standing activated microglia and oxidative stress is increased, which may be detrimental to neurons [88].

Given that oxidative damage and increased neuroinflammation are critically related with the pathogenesis of and late-onset massive neuronal loss in neurodegenerative diseases, the neuroprotective effect of carotenoids has been of specific interest in the search for effective treatments for these diseases. To provide an update on the latest advances in this field, we have reviewed the papers published in recent years in the following paragraphs.

3.2. Animal and Cellular Model Studies on the Beneficial Effects of Carotenoids on Neurodegenerative Diseases. Controlled animal model or cell culture studies allow for the accurate assessment of the sole influence of carotenoid administration on neurodegenerative diseases that human studies do not. Indeed, numerous experimental studies have recently highlighted the beneficial effects of carotenoids on neurodegenerative diseases (Table 2). Notably, most of these recent experimental studies have focused on either AD or PD.

In the case of well-studied lycopene, administration of lycopene in murine models of AD leads to the attenuation of mitochondrial oxidative damage [89] and inhibition of NF-κB activity and related expression of proinflammatory cytokines in the brain [64], which together may contribute to the suppression of Aβ formation [90] and improvement of memory retention [64, 89]. In a recent study that used a tau transgenic mouse model for AD, dietary lycopene supplementation was shown to improve cognitive performance [91]. Similarly, in the context of PD, lycopene-rich tomato powder intake successfully prevented the decline in striatal dopamine levels and degeneration of nigral dopaminergic neurons in rodent models of PD [92, 93]. Consistently, in more recent studies, administration of lycopene was shown to protect against rotenone-induced oxidative stress, neurobehavioral impairments [94], and depletion of dopamine and its metabolites by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine [95]. Moreover, the effect of lycopene was also experimentally assessed *in vivo* in the context of HD. Administration of lycopene alone [96–98], in combination with epigallocatechin-3-gallate [96], or with quercetin and poloxamer 188 [99] showed protective effects against HD-like symptoms induced by 3-nitropropionic acid in rodent models. Consistent with the results obtained from animal model studies, lycopene treatment was also recently shown to be very effective in attenuating neuropathic phenotypes in cultured cell models of AD [100–102] and PD [103]. Interestingly, the beneficial effects of lycopene were also confirmed in a study using a *Caenorhabditis elegans* model for AD [104].

In addition to lycopene, other dietary carotenoids such as fucoxanthin, astaxanthin, crocin, and crocetin have recently begun to be investigated experimentally for their potential beneficial effects. The beneficial effect of fucoxanthin was recently assessed in the context of AD [105, 106]. Moreover, astaxanthin has been shown to protect neurons in the context of various neurodegenerative diseases, including AD [107, 108], PD [109–112], and ALS [113]. Similarly, crocin was recently shown to be beneficial in both AD [114, 115] and PD [116–119]. Other recent studies on crocetin also support the beneficial effects of carotenoids on AD [120–122]. Of note, Tiribuzi et al. [122] used monocytes derived from patients with AD for analysis, and concluded that *trans*-crocetin improved the clearance of Aβ *in vitro* through the involvement of cathepsin B.

3.3. Human Studies on the Beneficial Effects of Carotenoids on Neurodegenerative Diseases. Consistent with the results obtained from animal and cell culture model studies showing the beneficial effects of carotenoid treatment on

neurodegenerative diseases, a number of epidemiological studies have also linked the consumption of a carotenoid-rich diet with a decreased risk of neurodegenerative diseases in humans (Table 2) [123–126].

The epidemiological correlation between disease risk and carotenoid intake (or its level in the blood) is evident in various neurodegenerative diseases (Table 2). In the case of AD, the most investigated neurodegenerative disease, several studies have reported lower concentrations of carotenoids such as β-carotene, lutein, and vitamin A in the blood plasma of AD patients than in control subjects [127–129]. A very recent case-control study showed that the concentration of six major carotenoids (α-carotene, β-carotene, β-cryptoxanthin, lutein, lycopene, and zeaxanthin) in serum was significantly lower in patients with AD than in cognitively normal control subjects [130]. The results of a study comparing the levels of plasma carotenoids between patients with AD and normal subjects suggested that maintaining a high level of lutein in relation to plasma lipids can reduce the risk of AD [131]. Consistently, Nolan et al. [132] concluded that the serum concentrations of lutein, zeaxanthin, and *meso*-zeaxanthin were significantly lower in AD patients than in control subjects. Similarly, high concentrations of lutein, lycopene, and zeaxanthin in the serum were associated with a lower risk of death from AD [133].

In addition to blood carotenoid levels, carotenoid intake has also been epidemiologically associated with a reduced risk of AD and decreased rates of cognitive decline [123–125]. Additional studies have suggested that there are potential beneficial effects of providing carotenoid supplementation to patients with AD [126]. Kiko et al. [134] and de Oliveira et al. [135] reported that the supply of antioxidant supplements, including astaxanthin and a vitamin complex containing α-tocopherol, ascorbic acid, and β-carotene, reduced Aβ contents in red blood cells and ROS generation in the cells of AD patients, respectively. Moreover, a potentially related result was also recently published in an elderly Chinese population, and showed carotenoids to be one of the most highly protective factors against mild cognitive impairment in a cross-sectional study based on a 33-item food frequency questionnaire collected from 2892 elderly subjects [136]. However, the sole influence of carotenoid consumption on the risk or progression of AD has not yet been clearly established in humans.

Unlike in AD, there have been inconsistencies on the association between carotenoid intake and the reduced risk of PD until very recently. Although a number of epidemiological studies have proposed a possible association between increased intake of both provitamin A and nonprovitamin A carotenoid species and the reduced risk of PD, the risk reduction was small and did not always reach statistical significance [6]. To clarify the inconsistencies observed in human studies, a recent systematic review that analyzed pooled data from relevant papers published between 1990 and 2013 raised the possibility that both α- and β-carotene levels might be inversely proportional to PD risk [137]. Following this systematic study, a very recent paper by Kim et al. [138] reported that the serum levels of some carotenoids (α-carotene, β-carotene, and lycopene) were significantly lower in PD patients, and that these carotenoids were inversely correlated with clinical variables representing disease progression. On the contrary, another study reported that the consumption of vitamin E and carotenoids was not associated with the risk of PD [139]. Thus, more studies are required to draw a solid conclusion on the relationship between carotenoid intake and PD risk reduction.

In the case of ALS, Fitzgerald et al. [140] analyzed pooled results from five published cohort studies on the association between carotenoids and ALS, and suggested that the ingestion of carotenoid-rich foods can prevent or delay the onset of ALS. Consistently, a recent paper published in *JAMA Neurology* [141] reported that a greater intake of antioxidant nutrients and foods high in carotenoids seems to be associated with more beneficial effects in ALS around the time of diagnosis. Lastly, in the case of HD, there have been no epidemiological studies on carotenoids published in recent years, unlike for the other representative neurodegenerative diseases described above.

Unlike animal model studies, the human studies conducted thus far have focused on the statistical assessment of the epidemiological correlation between the risk of disease and carotenoid intake (or its level in the blood), rather than clinical trials directly measuring the beneficial effects of carotenoid supplementation on the treatment of disease symptoms. However, as can be expected from the *in vivo* and *in vitro* bioactivities of carotenoids, a number of studies have shown that various carotenoids have beneficial effects on neurodegenerative diseases.

3.4. Possible Molecular Mechanisms of the Effects of Carotenoids on Neurodegenerative Diseases. Extensive studies suggest that carotenoids may inhibit the onset of neurodegenerative diseases through a variety of mechanisms. The effects of carotenoids have already been studied in different cellular contexts [28, 126, 142, 143] that may have the same working mechanisms as in neurodegenerative diseases. In the case of AD, it has been shown that, through ROS quenching, upregulation of antioxidant enzyme systems, hypocholesterolemic properties, antineuroinflammatory effects, antiamyloid aggregation activity, and regulation of amyloid oligomer-induced signaling, carotenoids may ameliorate mitochondrial dysfunction, oxidative stress, sustained neuroinflammation, impaired lipid metabolism, Aβ aggregation, and Aβ neurotoxicity, all of which are critically associated with the pathogenesis of AD [63, 105, 106, 144]. In neurodegenerative disease states, the various mechanisms of action of carotenoids are likely to occur simultaneously. For example, administration of lycopene resulted in the concomitant reduction of Aβ-induced mitochondrial dysfunction, inflammatory cytokine mediators, and caspase-3 activity in a rat model of AD [64]. Furthermore, astaxanthin treatment reduced Aβ-induced damage in a cultured cell model through several mechanisms including downregulation of apoptotic factors, inhibition of inflammatory cytokine mediating action, and simultaneous reduction of ROS [106].

How can a single substance exhibit these various effects? The functional diversity may be due to the strong antioxidant properties of carotenoids that regulate ROS, key regulators of

various biological activities. ROS induces functional modification of macromolecules, including lipids, DNA, and proteins, in the aging brains and brains of patients with neurodegenerative diseases [78]. These modifications may affect cellular processes by altering gene expression and signal transduction [145]. For example, the oxidation of PTEN, a lipid phosphatase and suppressor of PI3-kinase pathway, via oxidative stress results in the activation of the NF-κB pathway via IκB kinase (IKK) activation [54]. Since NF-κB regulates the expression of many genes, including oxidative stress-responsive and inflammation-related genes [146], a sustained increase in ROS in the brains of patients with neurodegenerative diseases may lead to an inflammatory response. Therefore, powerful antioxidants, such as carotenoids, can lower the level of ROS to mitigate cellular damage and simultaneously inhibit inflammatory responses by lowering the activity of NF-κB. Indeed, recent studies have shown that various carotenoids suppress inflammation via inhibition of NF-κB activity [56, 63, 147–152]. Given that oxidative stress and neuroinflammation are crucial to the onset and progress of various neurodegenerative diseases, it is expected that similar working mechanisms may be commonly applied to other neurodegenerative diseases.

In addition to these well-characterized cellular mechanisms of carotenoid functions, the recently proposed carotenoid-mediated regulation of autophagy described above also has a strong potential for protecting neurons in neurodegenerative disease conditions through the reduction of toxic disease proteins conferring neuronal toxicity. However, the role of autophagy in the effects of carotenoids on neurodegenerative diseases remains unclear. Further studies are needed to determine the role that carotenoid-regulated autophagy plays in neuroprotection.

4. Conclusion

In this article, we reviewed the recent updates on the beneficial effects of dietary carotenoids on neurodegenerative diseases. An increasing number of papers have demonstrated that dietary carotenoids protect neurons in the context of neurodegenerative diseases through several mechanisms, such as ROS quenching, upregulation of antioxidant enzyme systems, and antineuroinflammatory effects. Indeed, the number of research papers studying the link between carotenoids and neurodegenerative diseases has steadily increased to date. Notably, animal and cell culture model studies have recently begun to be actively conducted, and these model studies strongly support the hypothesis that carotenoid intake may have therapeutic potential in either preventing or ameliorating various neurodegenerative diseases. AD and PD have been more thoroughly studied in this regard than other types of rare neurodegenerative diseases (e.g., ALS and HD). In the rodent models of these diseases, administration of certain types of carotenoids, including lycopene, successfully attenuated not only cellular-level phenotypes such as mitochondrial oxidative damage and increased neuroinflammation, but also organism-level phenotypes such as memory impairment and locomotive defects. Of note, the beneficial effects of dietary carotenoids such as astaxanthin, crocin, crocetin, and fucoxanthin on neurodegenerative diseases have been recently studied in animal model systems, broadening our understanding of the association between carotenoid uptake and neurodegenerative diseases.

Although many of the studies presented in this paper demonstrate the beneficial effects of carotenoids as food nutrients on neurodegenerative diseases, some aspects of the carotenoid effect need to be clarified for medical use beyond food nutrients. First, the results of many studies have lacked an accurate analysis of the mechanisms by which carotenoids exert neuroprotective effects. The strong antioxidant properties of carotenoids can explain the neuroprotective effects of carotenoids in that one of the characteristic pathologies in neurodegenerative diseases is increased oxidative stress. However, the mechanisms by which carotenoids inhibit neuroinflammation and activate autophagy have not been thoroughly studied. In addition, many cell studies have shown that some carotenoids regulate the expression of antioxidant and inflammatory proteins, and it is interesting to note how they regulate gene expression. Second, clinical application studies in human patients are required. The causal relationship of the carotenoid effect in human patients can only be clarified by studies of this type. It may also be possible to infer the correlation between carotenoid intake and the onset of disease through comparative studies of races that eat different foods. Finally, in terms of the complexity in the pathogenic mechanisms underlying these diseases, it seems likely that simply increasing the dietary intake of carotenoids may exert only limited protective effects to neurons. For this reason, we expect that future studies determining other neuroprotective reagents/treatments that confer synergistic effects in combination with carotenoids in neurodegenerative diseases will be essential in finding effective treatments.

Abbreviations

Aβ: β-Amyloid
AD: Alzheimer's disease
ALS: Amyotrophic lateral sclerosis
HD: Huntington's disease
PD: Parkinson's disease
ROS: Reactive oxygen species.

Disclosure

Sunhong Kim and Sung Bae Lee are cocorresponding authors.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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References

[1] N. I. Krinsky and E. J. Johnson, "Carotenoid actions and their relation to health and disease," *Molecular Aspects of Medicine*, vol. 26, no. 6, pp. 459–516, 2005.

[2] B. Demmig-Adams, A. M. Gilmore, and W. Adams, "Carotenoids 3: *in vivo* function of carotenoids in higher plants," *The FASEB Journal*, vol. 10, no. 4, pp. 403–412, 1996.

[3] N. Nisar, L. Li, S. Lu, N. C. Khin, and B. J. Pogson, "Carotenoid metabolism in plants," *Molecular Plant*, vol. 8, no. 1, pp. 68–82, 2015.

[4] G. Britton, "Structure and properties of carotenoids in relation to function," *The FASEB Journal*, vol. 9, no. 15, pp. 1551–1558, 1995.

[5] J. Widomska, M. Zareba, and W. K. Subczynski, "Can xanthophyll-membrane interactions explain their selective presence in the retina and brain?," *Foods*, vol. 5, no. 4, p. 7, 2016.

[6] J. Guest and R. Grant, "Carotenoids and neurobiological health," *The Benefits of Natural Products for Neurodegenerative Diseases*, vol. 12, pp. 199–228, 2016.

[7] F. Khachik, G. R. Beecher, M. B. Goli, and W. R. Lusby, "Separation, identification, and quantification of carotenoids in fruits, vegetables and human plasma by high performance liquid chromatography," *Pure and Applied Chemistry*, vol. 63, no. 1, pp. 71–80, 1991.

[8] H. E. Khoo, K. N. Prasad, K. W. Kong, Y. Jiang, and A. Ismail, "Carotenoids and their isomers: color pigments in fruits and vegetables," *Molecules*, vol. 16, no. 2, pp. 1710–1738, 2011.

[9] F. Khachik, G. R. Beecher, and N. F. Whittaker, "Separation, identification, and quantification of the major carotenoid and chlorophyll constituents in extracts of several green vegetables by liquid chromatography," *Journal of Agricultural and Food Chemistry*, vol. 34, no. 4, pp. 603–616, 1986.

[10] L. Li and H. Yuan, "Chromoplast biogenesis and carotenoid accumulation," *Archives of Biochemistry and Biophysics*, vol. 539, no. 2, pp. 102–109, 2013.

[11] O. Sommerburg, J. E. E. Keunen, A. C. Bird, and F. J. G. M. van Kuijk, "Fruits and vegetables that are sources for lutein and zeaxanthin: the macular pigment in human eyes," *British Journal of Ophthalmology*, vol. 82, no. 8, pp. 907–910, 1998.

[12] R. Schweiggert and R. Carle, "Carotenoid deposition in plant and animal foods and its impact on bioavailability," *Critical Reviews in Food Science and Nutrition*, vol. 57, no. 9, pp. 1807–1830, 2017.

[13] P. J. M. Hulshof, T. van Roekel-Jansen, P. van de Bovenkamp, and C. E. West, "Variation in retinol and carotenoid content of milk and milk products in the Netherlands," *Journal of Food Composition and Analysis*, vol. 19, no. 1, pp. 67–75, 2006.

[14] B. Bjerkeng, "Carotenoids in aquaculture: fish and crustaceans," *Carotenoids*, vol. 4, pp. 237–254, 2008.

[15] B. S. Dissing, M. E. Nielsen, B. K. Ersbøll, and S. Frosch, "Multispectral imaging for determination of astaxanthin concentration in salmonids," *PLoS One*, vol. 6, no. 5, article e19032, 2011.

[16] X. Yan, Y. Chuda, M. Suzuki, and T. Nagata, "Fucoxanthin as the major antioxidant in *Hijikia fusiformis*, a common edible seaweed," *Bioscience, Biotechnology, and Biochemistry*, vol. 63, no. 3, pp. 605–607, 1999.

[17] H. Kanda, Y. Kamo, S. Machmudah, and M. Goto, "Extraction of fucoxanthin from raw macroalgae excluding drying and cell wall disruption by liquefied dimethyl ether," *Marine Drugs*, vol. 12, no. 5, pp. 2383–2396, 2014.

[18] N. Craft, T. Haitema, K. Garnett, K. Fitch, and C. Dorey, "Carotenoid, tocopherol, and retinol concentrations in elderly human brain," *The Journal of Nutrition, Health & Aging*, vol. 8, no. 3, pp. 156–162, 2004.

[19] L. Kaplan, J. Lau, and E. Stein, "Carotenoid composition, concentrations, and relationships in various human organs," *Clinical Physiology and Biochemistry*, vol. 8, no. 1, pp. 1–10, 1990.

[20] D. W. Nierenberg and S. L. Nann, "A method for determining concentrations of retinol, tocopherol, and five carotenoids in human plasma and tissue samples," *The American Journal of Clinical Nutrition*, vol. 56, no. 2, pp. 417–426, 1992.

[21] K. J. Yeum, S. L. Booth, J. A. Sadowski et al., "Human plasma carotenoid response to the ingestion of controlled diets high in fruits and vegetables," *The American Journal of Clinical Nutrition*, vol. 64, no. 4, pp. 594–602, 1996.

[22] J. M. Seddon, U. A. Ajani, R. D. Sperduto et al., "Dietary carotenoids, vitamins A, C, and E, and advanced age-related macular degeneration," *JAMA*, vol. 272, no. 18, pp. 1413–1420, 1994.

[23] R. G. Ziegler, "A review of epidemiologic evidence that carotenoids reduce the risk of cancer," *The Journal of Nutrition*, vol. 119, no. 1, pp. 116–122, 1989.

[24] E. Giovannucci, A. Ascherio, E. B. Rimm, M. J. Stampfer, G. A. Colditz, and W. C. Willett, "Intake of carotenoids and retino in relation to risk of prostate cancer," *Journal of the National Cancer Institute*, vol. 87, no. 23, pp. 1767–1776, 1995.

[25] S. Voutilainen, T. Nurmi, J. Mursu, and T. H. Rissanen, "Carotenoids and cardiovascular health," *The American Journal of Clinical Nutrition*, vol. 83, no. 6, pp. 1265–1271, 2006.

[26] D. Augustynska, M. Jemioła-Rzemińska, K. Burda, and K. Strzałka, "Influence of polar and nonpolar carotenoids on structural and adhesive properties of model membranes," *Chemico-Biological Interactions*, vol. 239, pp. 19–25, 2015.

[27] W. Grudzinski, L. Nierzwicki, R. Welc et al., "Localization and orientation of xanthophylls in a lipid bilayer," *Scientific Reports*, vol. 7, no. 1, p. 9619, 2017.

[28] J. Fiedor and K. Burda, "Potential role of carotenoids as antioxidants in human health and disease," *Nutrients*, vol. 6, no. 2, pp. 466–488, 2014.

[29] R. Edge, D. McGarvey, and T. Truscott, "The carotenoids as anti-oxidants—a review," *Journal of Photochemistry and Photobiology B: Biology*, vol. 41, no. 3, pp. 189–200, 1997.

[30] G. W. Burton and K. Ingold, "Beta-carotene: an unusual type of lipid antioxidant," *Science*, vol. 224, no. 4649, pp. 569–573, 1984.

[31] N. J. Miller, J. Sampson, L. P. Candeias, P. M. Bramley, and C. A. Rice-Evans, "Antioxidant activities of carotenes and xanthophylls," *FEBS Letters*, vol. 384, no. 3, pp. 240–242, 1996.

[32] G. M. Lowe, L. A. Booth, A. J. Young, and R. F. Bilton, "Lycopene and β-carotene protect against oxidative damage in HT29 cells at low concentrations but rapidly lose this capacity at higher doses," *Free Radical Research*, vol. 30, no. 2, pp. 141–151, 1999.

[33] Y. M. Naguib, "Antioxidant activities of astaxanthin and related carotenoids," *Journal of Agricultural and Food Chemistry*, vol. 48, no. 4, pp. 1150–1154, 2000.

[34] T. Ochiai, S. Ohno, S. Soeda, H. Tanaka, Y. Shoyama, and H. Shimeno, "Crocin prevents the death of rat pheochromyctoma (PC-12) cells by its antioxidant effects stronger than those of α-tocopherol," *Neuroscience Letters*, vol. 362, no. 1, pp. 61–64, 2004.

[35] X. C. Shen and Z. Y. Qian, "Effects of crocetin on antioxidant enzymatic activities in cardiac hypertrophy induced by norepinephrine in rats," *Die Pharmazie-An International Journal of Pharmaceutical Sciences*, vol. 61, no. 4, pp. 348–352, 2006.

[36] Y. Lorenzo, A. Azqueta, L. Luna, F. Bonilla, G. Dominguez, and A. R. Collins, "The carotenoid β-cryptoxanthin stimulates the repair of DNA oxidation damage in addition to acting as an antioxidant in human cells," *Carcinogenesis*, vol. 30, no. 2, pp. 308–314, 2008.

[37] J. Fiedor, A. Sulikowska, A. Orzechowska, L. Fiedor, and K. Burda, "Antioxidant effects of carotenoids in a model pigment-protein complex," *Acta Biochimica Polonica*, vol. 59, no. 1, pp. 61–64, 2012.

[38] L. E. Voorrips, R. A. Goldbohm, H. A. Brants et al., "A prospective cohort study on antioxidant and folate intake and male lung cancer risk," *Cancer Epidemiology, Biomarkers & Prevention*, vol. 9, no. 4, pp. 357–365, 2000.

[39] K. Athreya and M. F. Xavier, "Antioxidants in the treatment of cancer," *Nutrition and Cancer*, vol. 69, no. 8, pp. 1099–1104, 2017.

[40] E. J. Johnson, "Role of lutein and zeaxanthin in visual and cognitive function throughout the lifespan," *Nutrition Reviews*, vol. 72, no. 9, pp. 605–612, 2014.

[41] Age-Related Eye Disease Study Research Group, "The relationship of dietary carotenoid and vitamin A, E, and C intake with age-related macular degeneration in a case-control study: AREDS report no. 22," *Archives of Ophthalmology*, vol. 125, no. 9, pp. 1225–1232, 2007.

[42] Age-Related Eye Disease Study 2 (AREDS2) Research Group, E. Y. Chew, J. P. San Giovanni et al., "Lutein/zeaxanthin for the treatment of age-related cataract: AREDS2 randomized trial report no. 4," *JAMA Ophthalmology*, vol. 131, no. 7, pp. 843–850, 2013.

[43] The Age-Related Eye Disease Study 2 (AREDS2) Research Group, E. Y. Chew, T. E. Clemons et al., "Secondary analyses of the effects of lutein/zeaxanthin on age-related macular degeneration progression: AREDS2 report no. 3," *JAMA Ophthalmology*, vol. 132, no. 2, pp. 142–149, 2014.

[44] F. Parmeggiani, M. R. Romano, C. Costagliola et al., "Mechanism of inflammation in age-related macular degeneration," *Mediators of Inflammation*, vol. 2012, Article ID 435607, 16 pages, 2012.

[45] M. A. Gammone, G. Riccioni, and N. D'Orazio, "Marine carotenoids against oxidative stress: effects on human health," *Marine Drugs*, vol. 13, no. 10, pp. 6226–6246, 2015.

[46] C. Galasso, C. Corinaldesi, and C. Sansone, "Carotenoids from marine organisms: biological functions and industrial applications," *Antioxidants*, vol. 6, no. 4, p. 96, 2017.

[47] R. Masgrau, C. Guaza, R. M. Ransohoff, and E. Galea, "Should we stop saying "glia" and "neuroinflammation"?," *Trends in Molecular Medicine*, vol. 23, no. 6, pp. 486–500, 2017.

[48] P. L. McGeer, E. McGeer, and K. Yasojima, "Alzheimer disease and neuroinflammation," in *Advances in Dementia Research*, K. Jellinger, R. Schmidt, and M. Windisch, Eds., pp. 53–57, Springer, Vienna, Austria, 2000.

[49] M. Sasaki, Y. Ozawa, T. Kurihara et al., "Neuroprotective effect of an antioxidant, lutein, during retinal inflammation," *Investigative Ophthalmology & Visual Science*, vol. 50, no. 3, pp. 1433–1439, 2009.

[50] Q. Bian, S. Gao, J. Zhou et al., "Lutein and zeaxanthin supplementation reduces photooxidative damage and modulates the expression of inflammation-related genes in retinal pigment epithelial cells," *Free Radical Biology & Medicine*, vol. 53, no. 6, pp. 1298–1307, 2012.

[51] S. Y. Li, F. K. Fung, Z. J. Fu, D. Wong, H. H. L. Chan, and A. C. Y. Lo, "Anti-inflammatory effects of lutein in retinal ischemic/hypoxic injury: *in vivo* and *in vitro* studies," *Investigative Ophthalmology & Visual Science*, vol. 53, no. 10, pp. 5976–5984, 2012.

[52] L. P. Rubin, G. M. Chan, B. M. Barrett-Reis et al., "Effect of carotenoid supplementation on plasma carotenoids, inflammation and visual development in preterm infants," *Journal of Perinatology*, vol. 32, no. 6, pp. 418–424, 2012.

[53] J. E. Kim, R. M. Clark, Y. Park, J. Lee, and M. L. Fernandez, "Lutein decreases oxidative stress and inflammation in liver and eyes of guinea pigs fed a hypercholesterolemic diet," *Nutrition Research and Practice*, vol. 6, no. 2, pp. 113–119, 2012.

[54] J. H. Kim, H. J. Na, C. K. Kim et al., "The non-provitamin A carotenoid, lutein, inhibits NF-κB-dependent gene expression through redox-based regulation of the phosphatidylinositol 3-kinase/PTEN/Akt and NF-κB-inducing kinase pathways: role of H_2O_2 in NF-κB activation," *Free Radical Biology & Medicine*, vol. 45, no. 6, pp. 885–896, 2008.

[55] W. Wu, Y. Li, Y. Wu, Y. Zhang, Z. Wang, and X. Liu, "Lutein suppresses inflammatory responses through Nrf2 activation and NF-κB inactivation in lipopolysaccharide-stimulated BV-2 microglia," *Molecular Nutrition & Food Research*, vol. 59, no. 9, pp. 1663–1673, 2015.

[56] T. Liu, W. H. Liu, J. S. Zhao, F. Z. Meng, and H. Wang, "Lutein protects against β-amyloid peptide-induced oxidative stress in cerebrovascular endothelial cells through modulation of Nrf-2 and NF-κB," *Cell Biology and Toxicology*, vol. 33, no. 1, pp. 57–67, 2017.

[57] K. N. Nam, Y. M. Park, H. J. Jung et al., "Anti-inflammatory effects of crocin and crocetin in rat brain microglial cells," *European Journal of Pharmacology*, vol. 648, no. 1–3, pp. 110–116, 2010.

[58] S. Franceschelli, M. Pesce, A. Ferrone et al., "Astaxanthin treatment confers protection against oxidative stress in U937 cells stimulated with lipopolysaccharide reducing O_2^- production," *PLoS One*, vol. 9, no. 2, article e88359, 2014.

[59] X. S. Zhang, X. Zhang, Q. Wu et al., "Astaxanthin offers neuroprotection and reduces neuroinflammation in experimental subarachnoid hemorrhage," *Journal of Surgical Research*, vol. 192, no. 1, pp. 206–213, 2014.

[60] X. Zhou, F. Zhang, X. Hu et al., "Inhibition of inflammation by astaxanthin alleviates cognition deficits in diabetic mice," *Physiology & Behavior*, vol. 151, pp. 412–420, 2015.

[61] P. T. Yeh, H. W. Huang, C. M. Yang, W. S. Yang, and C. H. Yang, "Astaxanthin inhibits expression of retinal oxidative stress and inflammatory mediators in streptozotocin-induced diabetic rats," *PLoS One*, vol. 11, no. 1, article e0146438, 2016.

[62] X. Y. Zhou, F. Zhang, X. T. Hu et al., "Depression can be prevented by astaxanthin through inhibition of hippocampal inflammation in diabetic mice," *Brain Research*, vol. 1657, pp. 262–268, 2017.

[63] D. Zhao, S. H. Kwon, Y. S. Chun, M. Y. Gu, and H. O. Yang, "Anti-neuroinflammatory effects of fucoxanthin via inhibition of Akt/NF-κB and MAPKs/AP-1 pathways and activation of PKA/CREB pathway in lipopolysaccharide-activated

BV-2 microglial cells," *Neurochemical Research*, vol. 42, no. 2, pp. 667–677, 2017.

[64] A. K. Sachdeva and K. Chopra, "Lycopene abrogates Aβ (1–42)-mediated neuroinflammatory cascade in an experimental model of Alzheimer's disease," *The Journal of Nutritional Biochemistry*, vol. 26, no. 7, pp. 736–744, 2015.

[65] F. Zhang, Y. Fu, X. Zhou et al., "Depression-like behaviors and heme oxygenase-1 are regulated by lycopene in lipopolysaccharide-induced neuroinflammation," *Journal of Neuroimmunology*, vol. 298, pp. 1–8, 2016.

[66] B. Zhao, B. Ren, R. Guo et al., "Supplementation of lycopene attenuates oxidative stress induced neuroinflammation and cognitive impairment via Nrf2/Nf-κB transcriptional pathway," *Food and Chemical Toxicology*, vol. 109, Part 1, pp. 505–516, 2017.

[67] M. A. Rahman and H. Rhim, "Therapeutic implication of autophagy in neurodegenerative diseases," *BMB Reports*, vol. 50, no. 7, pp. 345–354, 2017.

[68] F. K. Fung, B. Y. Law, and A. C. Lo, "Lutein attenuates both apoptosis and autophagy upon cobalt (II) chloride-induced hypoxia in rat Műller cells," *PLoS One*, vol. 11, no. 12, article e0167828, 2016.

[69] C. J. Chang, J. F. Lin, C. Y. Hsiao et al., "Lutein induces autophagy via beclin-1 upregulation in IEC-6 rat intestinal epithelial cells," *The American Journal of Chinese Medicine*, vol. 45, no. 6, pp. 1273–1291, 2017.

[70] S. J. Sheu, J. L. Chen, Y. S. Bee, Y. A. Chen, S. H. Lin, and C. W. Shu, "Differential autophagic effects of vital dyes in retinal pigment epithelial ARPE-19 and photoreceptor 661W cells," *PLoS One*, vol. 12, no. 3, article e0174736, 2017.

[71] C. Zeng, H. Li, Z. Fan et al., "Crocin-elicited autophagy rescues myocardial ischemia/reperfusion injury via paradoxical mechanisms," *The American Journal of Chinese Medicine*, vol. 44, no. 3, pp. 515–530, 2016.

[72] M. Buyuklu, F. Kandemir, M. Ozkaraca et al., "Beneficial effects of lycopene against contrast medium-induced oxidative stress, inflammation, autophagy, and apoptosis in rat kidney," *Human & Experimental Toxicology*, vol. 34, no. 5, pp. 487–496, 2015.

[73] F. Chen, Z. W. Sun, L. F. Ye, G.-S. Fu, Y. Mou, and S.-J. Hu, "Lycopene protects against apoptosis in hypoxia/reoxygenation-induced H9C2 myocardioblast cells through increased autophagy," *Molecular Medicine Reports*, vol. 11, no. 2, pp. 1358–1365, 2015.

[74] F. Zhang, S. Xing, and Z. Li, "Antagonistic effects of lycopene on cadmium-induced hippocampal dysfunctions in autophagy, calcium homeostatis and redox," *Oncotarget*, vol. 8, no. 27, pp. 44720–44731, 2017.

[75] M. Shen, K. Chen, J. Lu et al., "Protective effect of astaxanthin on liver fibrosis through modulation of TGF-β1 expression and autophagy," *Mediators of Inflammation*, vol. 2014, Article ID 954502, 14 pages, 2014.

[76] J. Li, F. Wang, Y. Xia et al., "Astaxanthin pretreatment attenuates hepatic ischemia reperfusion-induced apoptosis and autophagy via the ROS/MAPK pathway in mice," *Marine Drugs*, vol. 13, no. 6, pp. 3368–3387, 2015.

[77] L. Zhang, H. Wang, Y. Fan et al., "Fucoxanthin provides neuroprotection in models of traumatic brain injury via the Nrf2-ARE and Nrf2-autophagy pathways," *Scientific Reports*, vol. 7, article 46763, 2017.

[78] K. J. Barnham, C. L. Masters, and A. I. Bush, "Neurodegenerative diseases and oxidative stress," *Nature Reviews Drug Discovery*, vol. 3, no. 3, pp. 205–214, 2004.

[79] J. Hardy and D. J. Selkoe, "The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics," *Science*, vol. 297, no. 5580, pp. 353–356, 2002.

[80] M. G. Spillantini, M. L. Schmidt, V. M. Y. Lee, J. Q. Trojanowski, R. Jakes, and M. Goedert, "α-Synuclein in Lewy bodies," *Nature*, vol. 388, no. 6645, pp. 839-840, 1997.

[81] M. Neumann, D. M. Sampathu, L. K. Kwong et al., "Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis," *Science*, vol. 314, no. 5796, pp. 130–133, 2006.

[82] F. O. Walker, "Huntington's disease," *The Lancet*, vol. 369, no. 9557, pp. 218–228, 2007.

[83] B. Halliwell, "Reactive oxygen species in living systems: source, biochemistry, and role in human disease," *The American Journal of Medicine*, vol. 91, no. 3, pp. S14–S22, 1991.

[84] M. T. Lin and M. F. Beal, "Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases," *Nature*, vol. 443, no. 7113, pp. 787–795, 2006.

[85] B. Uttara, A. V. Singh, P. Zamboni, and R. Mahajan, "Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options," *Current Neuropharmacology*, vol. 7, no. 1, pp. 65–74, 2009.

[86] T. Wyss-Coray and L. Mucke, "Inflammation in neurodegenerative disease—a double-edged sword," *Neuron*, vol. 35, no. 3, pp. 419–432, 2002.

[87] C. K. Glass, K. Saijo, B. Winner, M. C. Marchetto, and F. H. Gage, "Mechanisms underlying inflammation in neurodegeneration," *Cell*, vol. 140, no. 6, pp. 918–934, 2010.

[88] T. C. Frank-Cannon, L. T. Alto, F. E. McAlpine, and M. G. Tansey, "Does neuroinflammation fan the flame in neurodegenerative diseases?," *Molecular Neurodegeneration*, vol. 4, no. 1, p. 47, 2009.

[89] A. Prakash and A. Kumar, "Implicating the role of lycopene in restoration of mitochondrial enzymes and BDNF levels in β-amyloid induced Alzheimer's disease," *European Journal of Pharmacology*, vol. 741, pp. 104–111, 2014.

[90] S. Katayama, H. Ogawa, and S. Nakamura, "Apricot carotenoids possess potent anti-amyloidogenic activity *in vitro*," *Journal of Agricultural and Food Chemistry*, vol. 59, no. 23, pp. 12691–12696, 2011.

[91] L. Yu, W. Wang, W. Pang, Z. Xiao, Y. Jiang, and Y. Hong, "Dietary lycopene supplementation improves cognitive performances in tau transgenic mice expressing P301L mutation via inhibiting oxidative stress and tau hyperphosphorylation," *Journal of Alzheimer's Disease*, vol. 57, no. 2, pp. 475–482, 2017.

[92] H. Suganuma, T. Hirano, Y. Arimoto, and T. Inakuma, "Effect of tomato intake on striatal monoamine level in a mouse model of experimental Parkinson's disease," *Journal of Nutritional Science and Vitaminology*, vol. 48, no. 3, pp. 251–254, 2002.

[93] V. di Matteo, M. Pierucci, G. Di Giovanni et al., "Intake of tomato-enriched diet protects from 6-hydroxydopamine-induced degeneration of rat nigral dopaminergic neurons," *Birth, Life and Death of Dopaminergic Neurons in the Substantia Nigra*, vol. 73, pp. 333–341, 2009.

[94] H. Kaur, S. Chauhan, and R. Sandhir, "Protective effect of lycopene on oxidative stress and cognitive decline in rotenone induced model of Parkinson's disease," *Neurochemical Research*, vol. 36, no. 8, pp. 1435–1443, 2011.

[95] A. Prema, U. Janakiraman, T. Manivasagam, and A. J. Thenmozhi, "Neuroprotective effect of lycopene against MPTP induced experimental Parkinson's disease in mice," *Neuroscience Letters*, vol. 599, pp. 12–19, 2015.

[96] P. Kumar and A. Kumar, "Effect of lycopene and epigallocatechin-3-gallate against 3-nitropropionic acid induced cognitive dysfunction and glutathione depletion in rat: a novel nitric oxide mechanism," *Food and Chemical Toxicology*, vol. 47, no. 10, pp. 2522–2530, 2009.

[97] P. Kumar, H. Kalonia, and A. Kumar, "Lycopene modulates nitric oxide pathways against 3-nitropropionic acid-induced neurotoxicity," *Life Sciences*, vol. 85, no. 19-20, pp. 711–718, 2009.

[98] R. Sandhir, A. Mehrotra, and S. S. Kamboj, "Lycopene prevents 3-nitropropionic acid-induced mitochondrial oxidative stress and dysfunctions in nervous system," *Neurochemistry International*, vol. 57, no. 5, pp. 579–587, 2010.

[99] D. Jain and A. Gangshettiwar, "Combination of lycopene, quercetin and poloxamer 188 alleviates anxiety and depression in 3-nitropropionic acid-induced Huntington's disease in rats," *Journal of Intercultural Ethnopharmacology*, vol. 3, no. 4, pp. 186–191, 2014.

[100] M. Qu, L. Li, C. Chen et al., "Protective effects of lycopene against amyloid β-induced neurotoxicity in cultured rat cortical neurons," *Neuroscience Letters*, vol. 505, no. 3, pp. 286–290, 2011.

[101] M. Qu, Z. Jiang, Y. Liao, Z. Song, and X. Nan, "Lycopene prevents amyloid (beta)-induced mitochondrial oxidative stress and dysfunctions in cultured rat cortical neurons," *Neurochemical Research*, vol. 41, no. 6, pp. 1354–1364, 2016.

[102] S. Hwang, J. W. Lim, and H. Kim, "Inhibitory effect of lycopene on amyloid-β-induced apoptosis in neuronal cells," *Nutrients*, vol. 9, no. 12, p. 883, 2017.

[103] F. Yi, X. He, and D. Wang, "Lycopene protects against MPP^+-induced cytotoxicity by maintaining mitochondrial function in SH-SY5Y cells," *Neurochemical Research*, vol. 38, no. 8, pp. 1747–1757, 2013.

[104] W. Chen, L. Mao, H. Xing et al., "Lycopene attenuates Aβ1–42 secretion and its toxicity in human cell and *Caenorhabditis elegans* models of Alzheimer disease," *Neuroscience Letters*, vol. 608, pp. 28–33, 2015.

[105] J. Lin, J. Yu, J. Zhao et al., "Fucoxanthin, a marine carotenoid, attenuates β-amyloid oligomer-induced neurotoxicity possibly via regulating the PI3k/Akt and the ERK pathways in SH-SY5Y cells," *Oxidative Medicine and Cellular Longevity*, vol. 2017, Article ID 6792543, 10 pages, 2017.

[106] S. Xiang, F. Liu, J. Lin et al., "Fucoxanthin inhibits β-amyloid assembly and attenuates β-amyloid oligomer-induced cognitive impairments," *Journal of Agricultural and Food Chemistry*, vol. 65, no. 20, pp. 4092–4102, 2017.

[107] C. H. Chang, C. Y. Chen, J. Y. Chiou, R. Y. Peng, and C. H. Peng, "Astaxanthine secured apoptotic death of PC12 cells induced by β-amyloid peptide 25–35: its molecular action targets," *Journal of Medicinal Food*, vol. 13, no. 3, pp. 548–556, 2010.

[108] P. Lobos, B. Bruna, A. Cordova et al., "Astaxanthin protects primary hippocampal neurons against noxious effects of Aβ-oligomers," *Neural Plasticity*, vol. 2016, Article ID 3456783, 13 pages, 2016.

[109] Y. Ikeda, S. Tsuji, A. Satoh, M. Ishikura, T. Shirasawa, and T. Shimizu, "Protective effects of astaxanthin on 6-hydroxydopamine-induced apoptosis in human neuroblastoma SH-SY5Y cells," *Journal of Neurochemistry*, vol. 107, no. 6, pp. 1730–1740, 2008.

[110] D. H. Lee, C. S. Kim, and Y. J. Lee, "Astaxanthin protects against MPTP/MPP^+-induced mitochondrial dysfunction and ROS production *in vivo* and *in vitro*," *Food and Chemical Toxicology*, vol. 49, no. 1, pp. 271–280, 2011.

[111] Q. Ye, B. Huang, X. Zhang, Y. Zhu, and X. Chen, "Astaxanthin protects against MPP^+-induced oxidative stress in PC12 cells via the HO-1/NOX2 axis," *BMC Neuroscience*, vol. 13, no. 1, p. 156, 2012.

[112] Q. Ye, X. Zhang, B. Huang, Y. Zhu, and X. Chen, "Astaxanthin suppresses MPP^+-induced oxidative damage in PC12 cells through a Sp1/NR1 signaling pathway," *Marine Drugs*, vol. 11, no. 12, pp. 1019–1034, 2013.

[113] R. Isonaka, H. Hiruma, T. Katakura, and T. Kawakami, "Inhibition of superoxide dismutase selectively suppresses growth of rat spinal motor neurons: comparison with phosphorylated neurofilament-containing spinal neurons," *Brain Research*, vol. 1425, pp. 13–19, 2011.

[114] A. Ghahghaei, S. Z. Bathaie, H. Kheirkhah, and E. Bahraminejad, "The protective effect of crocin on the amyloid fibril formation of Aβ42 peptide *in vitro*," *Cellular & Molecular Biology Letters*, vol. 18, no. 3, pp. 328–339, 2013.

[115] F. Asadi, A. H. Jamshidi, F. Khodagholi et al., "Reversal effects of crocin on amyloid β-induced memory deficit: modification of autophagy or apoptosis markers," *Pharmacology Biochemistry and Behavior*, vol. 139, pp. 47–58, 2015.

[116] G. F. Zhang, Y. Zhang, and G. Zhao, "Crocin protects PC12 cells against MPP^+-induced injury through inhibition of mitochondrial dysfunction and ER stress," *Neurochemistry International*, vol. 89, pp. 101–110, 2015.

[117] M. Hosseini, Z. Rajaei, H. Alaei, and M. Tajadini, "The effects of crocin on 6-OHDA-induced oxidative/nitrosative damage and motor behaviour in hemiparkinsonian rats," *The Malaysian Journal of Medical Sciences*, vol. 23, no. 6, pp. 35–43, 2016.

[118] Z. Rajaei, M. Hosseini, and H. Alaei, "Effects of crocin on brain oxidative damage and aversive memory in a 6-OHDA model of Parkinson's disease," *Arquivos de Neuro-Psiquiatria*, vol. 74, no. 9, pp. 723–729, 2016.

[119] S. V. Rao, S. C. Yenisetti, and P. S. Rajini, "Evidence of neuroprotective effects of saffron and crocin in a *Drosophila* model of parkinsonism," *Neurotoxicology*, vol. 52, pp. 230–242, 2016.

[120] J. H. Ahn, Y. Hu, M. Hernandez, and J. R. Kim, "Crocetin inhibits beta-amyloid fibrillization and stabilizes beta-amyloid oligomers," *Biochemical and Biophysical Research Communications*, vol. 414, no. 1, pp. 79–83, 2011.

[121] Y. Kong, L. P. Kong, T. Luo et al., "The protective effects of crocetin on Aβ1–42-induced toxicity in Ht22 cells," *CNS & Neurological Disorders-Drug Targets*, vol. 13, no. 9, pp. 1627–1632, 2014.

[122] R. Tiribuzi, L. Crispoltoni, V. Chiurchiù et al., "*trans*-Crocetin improves amyloid-β degradation in monocytes from Alzheimer's disease patients," *Journal of the Neurological Sciences*, vol. 372, pp. 408–412, 2017.

[123] Q. Dai, A. R. Borenstein, Y. Wu, J. C. Jackson, and E. B. Larson, "Fruit and vegetable juices and Alzheimer's disease: the Kame Project," *The American Journal of Medicine*, vol. 119, no. 9, pp. 751–759, 2006.

[124] F. J. Li, L. Shen, and H. F. Ji, "Dietary intakes of vitamin E, vitamin C, and β-carotene and risk of Alzheimer's disease: a meta-analysis," *Journal of Alzheimer's Disease*, vol. 31, no. 2, pp. 253–258, 2012.

[125] E. Kesse-Guyot, V. A. Andreeva, V. Ducros et al., "Carotenoid-rich dietary patterns during midlife and subsequent cognitive function," *British Journal of Nutrition*, vol. 111, no. 5, pp. 915–923, 2014.

[126] N. M. Honarvar, A. Saedisomeolia, M. Abdolahi et al., "Molecular anti-inflammatory mechanisms of retinoids and carotenoids in Alzheimer's disease: a review of current evidence," *Journal of Molecular Neuroscience*, vol. 61, no. 3, pp. 289–304, 2017.

[127] Z. Zaman, S. Roche, P. Fielden, P. G. Frost, D. C. Niriella, and A. C. D. Cayley, "Plasma concentrations of vitamins A and E and carotenoids in Alzheimer's disease," *Age and Ageing*, vol. 21, no. 2, pp. 91–94, 1992.

[128] F. J. Jiménez-Jiménez, J. A. Molina, F. Bustos et al., "Serum levels of β-carotene, α-carotene and vitamin A in patients with Alzheimer's disease," *European Journal of Neurology*, vol. 6, no. 4, pp. 495–497, 1999.

[129] W. Wang, L. Shinto, W. E. Connor, and J. F. Quinn, "Nutritional biomarkers in Alzheimer's disease: the association between carotenoids, n-3 fatty acids, and dementia severity," *Journal of Alzheimer's Disease*, vol. 13, no. 1, pp. 31–38, 2008.

[130] K. Mullan, M. A. Williams, C. R. Cardwell et al., "Serum concentrations of vitamin E and carotenoids are altered in Alzheimer's disease: a case-control study," *Alzheimer's & Dementia: Translational Research & Clinical Interventions*, vol. 3, no. 3, pp. 432–439, 2017.

[131] C. Feart, L. Letenneur, C. Helmer et al., "Plasma carotenoids are inversely associated with dementia risk in an elderly French cohort," *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, vol. 71, no. 5, pp. 683–688, 2016.

[132] J. M. Nolan, E. Loskutova, A. N. Howard et al., "Macular pigment, visual function, and macular disease among subjects with Alzheimer's disease: an exploratory study," *Journal of Alzheimer's Disease*, vol. 42, no. 4, pp. 1191–1202, 2014.

[133] J. Y. Min and K. B. Min, "Serum lycopene, lutein and zeaxanthin, and the risk of Alzheimer's disease mortality in older adults," *Dementia and Geriatric Cognitive Disorders*, vol. 37, no. 3-4, pp. 246–256, 2014.

[134] T. Kiko, K. Nakagawa, A. Satoh et al., "Amyloid β levels in human red blood cells," *PLoS One*, vol. 7, no. 11, article e49620, 2012.

[135] B. F. de Oliveira, C. A. Veloso, J. A. Nogueira-Machado et al., "Ascorbic acid, alpha-tocopherol, and beta-carotene reduce oxidative stress and proinflammatory cytokines in mononuclear cells of Alzheimer's disease patients," *Nutritional Neuroscience*, vol. 15, no. 6, pp. 244–251, 2012.

[136] Y. Lu, Y. An, J. Guo et al., "Dietary intake of nutrients and lifestyle affect the risk of mild cognitive impairment in the Chinese elderly population: a cross-sectional study," *Frontiers in Behavioral Neuroscience*, vol. 10, article 00229, 2016.

[137] A. Takeda, O. P. Nyssen, A. Syed, E. Jansen, B. Bueno-de-Mesquita, and V. Gallo, "Vitamin A and carotenoids and the risk of Parkinson's disease: a systematic review and meta-analysis," *Neuroepidemiology*, vol. 42, no. 1, pp. 25–38, 2014.

[138] J. H. Kim, J. Hwang, E. Shim, E. J. Chung, S. H. Jang, and S. B. Koh, "Association of serum carotenoid, retinol, and tocopherol concentrations with the progression of Parkinson's disease," *Nutrition Research and Practice*, vol. 11, no. 2, pp. 114–120, 2017.

[139] K. C. Hughes, X. Gao, I. Y. Kim et al., "Intake of antioxidant vitamins and risk of Parkinson's disease," *Movement Disorders*, vol. 31, no. 12, pp. 1909–1914, 2016.

[140] K. C. Fitzgerald, É. J. O'Reilly, E. Fondell et al., "Intakes of vitamin C and carotenoids and risk of amyotrophic lateral sclerosis: pooled results from 5 cohort studies," *Annals of Neurology*, vol. 73, no. 2, pp. 236–245, 2013.

[141] J. W. Nieves, C. Gennings, P. Factor-Litvak et al., "Association between dietary intake and function in amyotrophic lateral sclerosis," *JAMA Neurology*, vol. 73, no. 12, pp. 1425–1432, 2016.

[142] S. A. Paiva and R. M. Russell, "β-Carotene and other carotenoids as antioxidants," *Journal of the American College of Nutrition*, vol. 18, no. 5, pp. 426–433, 1999.

[143] W. Stahl and H. Sies, "Antioxidant activity of carotenoids," *Molecular Aspects of Medicine*, vol. 24, no. 6, pp. 345–351, 2003.

[144] J. Lakey-Beitia, D. Doens, D. J. Kumar et al., "Anti-amyloid aggregation activity of novel carotenoids: implications for Alzheimer's drug discovery," *Clinical Interventions in Aging*, vol. Volume 12, pp. 815–822, 2017.

[145] T. Finkel and N. J. Holbrook, "Oxidants, oxidative stress and the biology of ageing," *Nature*, vol. 408, no. 6809, pp. 239–247, 2000.

[146] E. M. Conner and M. B. Grisham, "Inflammation, free radicals, and antioxidants," *Nutrition*, vol. 12, no. 4, pp. 274–277, 1996.

[147] S. Li, X. Liu, J. Lei, J. Yang, P. Tian, and Y. Gao, "Crocin protects podocytes against oxidative stress and inflammation induced by high glucose through inhibition of NF-κB," *Cellular Physiology and Biochemistry*, vol. 42, no. 4, pp. 1481–1492, 2017.

[148] A. G. Mazumder, P. Sharma, V. Patial, and D. Singh, "Crocin attenuates kindling development and associated cognitive impairments in mice via inhibiting reactive oxygen species-mediated NF-κB activation," *Basic and Clinical Pharmacology and Toxicology*, vol. 120, no. 5, pp. 426–433, 2017.

[149] K. Sahin, C. Orhan, F. Akdemir et al., "β-Cryptoxanthin ameliorates metabolic risk factors by regulating NF-κB and Nrf2 pathways in insulin resistance induced by high-fat diet in rodents," *Food and Chemical Toxicology*, vol. 107, Part A, pp. 270–279, 2017.

[150] F. Wang, N. Wang, Y. Gao et al., "β-Carotene suppresses osteoclastogenesis and bone resorption by suppressing NF-κB signaling pathway," *Life Sciences*, vol. 174, pp. 15–20, 2017.

[151] M. Zhang, Z. Cui, H. Cui, Y. Wang, and C. Zhong, "Astaxanthin protects astrocytes against trauma-induced apoptosis through inhibition of NKCC1 expression via the NF-κB signaling pathway," *BMC Neuroscience*, vol. 18, no. 1, p. 42, 2017.

[152] X. Zhou, S. Wang, X. Ding et al., "Zeaxanthin improves diabetes-induced cognitive deficit in rats through activating

PI3K/AKT signaling pathway," *Brain Research Bulletin*, vol. 132, pp. 190–198, 2017.

[153] A. R. Mangels, J. M. Holden, G. R. Beecher, M. R. Forman, and E. Lanza, "Carotenoid content of fruits and vegetables: an evaluation of analytic data," *Journal of the American Dietetic Association*, vol. 93, no. 3, pp. 284–296, 1993.

[154] L. Englberger, I. Darnton-Hill, T. Coyne, M. H. Fitzgerald, and G. C. Marks, "Carotenoid-rich bananas: a potential food source for alleviating vitamin A deficiency," *Food and Nutrition Bulletin*, vol. 24, no. 4, pp. 303–318, 2003.

[155] L. M. J. de Carvalho, L. de Azevedo Sarmet Moreira Smiderle, J. L. V. de Carvalho, F. de Souza Neves Cardoso, and M. G. B. Koblitz, "Assessment of carotenoids in pumpkins after different home cooking conditions," *Food Science and Technology*, vol. 34, no. 2, pp. 365–370, 2014.

[156] F. Zaccari and G. Galietta, "α-Carotene and β-carotene content in raw and cooked pulp of three mature stage winter squash "type butternut"," *Food*, vol. 4, no. 4, pp. 477–486, 2015.

[157] I. A. Lee, J. H. Lee, N. I. Baek, and D. H. Kim, "Antihyperlipidemic effect of crocin isolated from the fructus of *Gardenia jasminoides* and its metabolite crocetin," *Biological and Pharmaceutical Bulletin*, vol. 28, no. 11, pp. 2106–2110, 2005.

[158] M. Giaccio, "Crocetin from saffron: an active component of an ancient spice," *Critical Reviews in Food Science and Nutrition*, vol. 44, no. 3, pp. 155–172, 2004.

[159] T. Q. Pham, F. Cormier, E. Farnworth, V. H. Tong, and M. R. Van Calsteren, "Antioxidant properties of crocin from *Gardenia jasminoides* Ellis and study of the reactions of crocin with linoleic acid and crocin with oxygen," *Journal of Agricultural and Food Chemistry*, vol. 48, no. 5, pp. 1455–1461, 2000.

[160] H. Hosseinzadeh, H. R. Sadeghnia, T. Ziaee, and A. Danaee, "Protective effect of aqueous saffron extract (*Crocus sativus* L.) and crocin, its active constituent, on renal ischemia-reperfusion-induced oxidative damage in rats," *Journal of Pharmacy & Pharmaceutical Sciences*, vol. 8, no. 3, pp. 387–393, 2005.

[161] D. E. Breithaupt and A. Bamedi, "Carotenoid esters in vegetables and fruits: a screening with emphasis on β-cryptoxanthin esters," *Journal of Agricultural and Food Chemistry*, vol. 49, no. 4, pp. 2064–2070, 2001.

[162] E. S. M. Abdel-Aal, H. Akhtar, K. Zaheer, and R. Ali, "Dietary sources of lutein and zeaxanthin carotenoids and their role in eye health," *Nutrients*, vol. 5, no. 4, pp. 1169–1185, 2013.

[163] I. Jaswir, N. Shahidan, R. Othman, Y. Z. Has-Yun Hashim, F. Octavianti, and M. N. . Salleh, "Effects of season and storage period on accumulation of individual carotenoids in pumpkin flesh *(Cucurbita moschata)*," *Journal of Oleo Science*, vol. 63, no. 8, pp. 761–767, 2014.

[164] U. Chandrika, K. Fernando, and K. Ranaweera, "Carotenoid content and *in vitro* bioaccessibility of lycopene from guava *(Psidium guajava)* and watermelon *(Citrullus lanatus)* by high-performance liquid chromatography diode array detection," *International Journal of Food Sciences and Nutrition*, vol. 60, no. 7, pp. 558–566, 2009.

[165] D. P. S. Oberoi and D. S. Sogi, "Utilization of watermelon pulp for lycopene extraction by response surface methodology," *Food Chemistry*, vol. 232, pp. 316–321, 2017.

[166] K. Miyashita, S. Nishikawa, F. Beppu, T. Tsukui, M. Abe, and M. Hosokawa, "The allenic carotenoid fucoxanthin, a novel marine nutraceutical from brown seaweeds," *Journal of the Science of Food and Agriculture*, vol. 91, no. 7, pp. 1166–1174, 2011.

[167] J. Nataraj, T. Manivasagam, A. J. Thenmozhi, and M. M. Essa, "Lutein protects dopaminergic neurons against MPTP-induced apoptotic death and motor dysfunction by ameliorating mitochondrial disruption and oxidative stress," *Nutritional Neuroscience*, vol. 19, no. 6, pp. 237–246, 2016.

[168] Y. Binawade and A. Jagtap, "Neuroprotective effect of lutein against 3-nitropropionic acid-induced Huntington's disease-like symptoms: possible behavioral, biochemical, and cellular alterations," *Journal of Medicinal Food*, vol. 16, no. 10, pp. 934–943, 2013.

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Research Article

Methyl Chavicol and Its Synthetic Analogue as Possible Antioxidant and Antilipase Agents Based on the *In Vitro* and In Silico Assays

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This study investigated the *in vitro* and in silico biological properties of the methyl chavicol (MC) and its analogue 2-[(4-methoxyphenyl)methyl]oxirane (MPMO), emphasizing the antioxidant and antilipase effects. MPMO was synthesized from MC that reacted with *meta*-chloroperbenzoic acid and, after separation and purification, was identified by ^{1}H and ^{13}C NMR and GC-MS. The antioxidant activity was investigated by DPPH, cooxidation β-carotene/linoleic acid, and thiobarbituric acid assays. With the use of colorimetric determination, the antilipase effect on the pancreatic lipase was tested, while the molecular interaction profiles were evaluated by docking molecular study. MC (IC_{50} = 312.50 ± 2.28 μg/mL) and MPMO (IC_{50} = 8.29 ± 0.80 μg/mL) inhibited the DPPH free radical. The inhibition of lipid peroxidation (%) was 73.08 ± 4.79 and 36.16 ± 4.11 to MC and MPMO, respectively. The malonaldehyde content was significantly reduced in the presence of MC and MPMO. MC and MPMO inhibited the pancreatic lipase in 58.12 and 26.93%, respectively. MC and MPMO (−6.1 kcal·mol^{-1}) produced a binding affinity value lower than did diundecylphosphatidylcholine (−5.6 kcal·mol^{-1}). These findings show that MC and MPMO present antioxidant and antilipase activities, which may be promising molecular targets for the treatment of diseases associated with oxidative damage and lipid metabolism.

1. Introduction

The imbalance between "prooxidant" and "antioxidant" chemical species produces oxidative stress, which causes lipidic peroxidation, aggression to proteins, and damage to DNA and RNA and triggers mechanisms associated with inflammatory, cardiovascular, and neuro-degenerative diseases; allergies; accelerated aging; hemorrhage; cataracts; immunological dysfunctions; and cancer [1, 2]. Among these disorders, the metabolic syndrome includes risk factors such as visceral obesity, endothelial dysfunction, dyslipidemia, and hypertension and is related to the development of type 2 diabetes mellitus with a high cardiovascular risk and mortality [3]. In addition, obesity and type 2 diabetes mellitus promote the increase of disease due to oxidative damage to proteins, lipids, DNA, and inflammatory process with

generation of free radicals (FR) and deficiency in cell detoxification and repairs of damaged molecules [4]. In these pathways of metabolism, lipid peroxidation (LPO) is one of the triggered processes, since it forms lipid hydroperoxide by the incorporation of molecular oxygen to one of the polyunsaturated fatty acids. However, antioxidants can prevent the formation of FR or inhibit auto-oxidation, while the antilipase agents, such as orlistat, used in the treatment of obesity, are able to inhibit digestive lipases and reduce the absorption of fats from food, reducing cardiovascular risks [2, 5, 6]. These aspects interact with each other, and the search for new drugs that are capable of inhibiting oxidative and disease-associated mechanisms may be a great strategy for the treatment of different pathologies.

Methyl chavicol (MC), chemically known as 1-methoxy-4-prop-2-enylbenzene, estragole, or *p*-allylanisole, is a special metabolite belonging to the class of phenylpropanoids found in essential oils of medicinal and food plants [7]. The chemical structure consists of a benzene ring in the presence of a methoxy group ($-OCH_3$) and a propenyl ($-CH_3CHCH_2$) at the 1 and 4 positions, respectively. The insecticidal activity of *Ocimum* spp. essential oils was attributed to this constituent against *Anopheles braziliensis*, a transmitter of malaria, dengue, and yellow fever [8]. This compound blocks voltage-activated sodium channels [9], and the anti-inflammatory activity is due to inhibition of leukocyte migration and stimulation of macrophages phagocytosis [10]. Pattnaik et al. [11] also revealed that MC showed a weak antimicrobial activity and the essential oil of *Ocimum basilicum* was cytotoxic against tumor cell lines such as Caco2 (colon cancer), HepG2 (hepatocellular cancer), and MCF-7 (breast adenocarcinoma).

Elevated doses of MC have hepatocarcinogenic potential, and the carcinogenicity is linked to 1′-hydroxy-methyl chavicol, a genotoxic metabolite catalyzed by cytochromes P4501A2 and P4502A6 [12–15]. In addition, the phase I metabolism includes O-demethylation, epoxidation, and 3′-hydroxylation reactions with formation of 4-allylphenol, methyl chavicol-2′, 3′-oxide, and 3′-hydroxyanethole, in this order [16–21]. The sulfonation reaction of 1′-hydroxy-methyl chavicol generates a carcinogenic metabolite, which is capable of reacting with DNA [14, 19, 22, 23].

As described above, biological properties of essential oils of medicinal and food plants have been attributed to MC, and the oxidative processes involve different mechanisms and pathological responses. In this sense, the understanding of antioxidant actions of promising compounds has been a strategy for the development of new therapeutic options for the treatment of metabolic disorders. Based on this principle, the present study aimed to synthesize an analogue from MC and evaluate the antioxidant activity and the inhibitory capacity on the pancreatic lipase using *in vitro* and in silico methods.

2. Materials and Methods

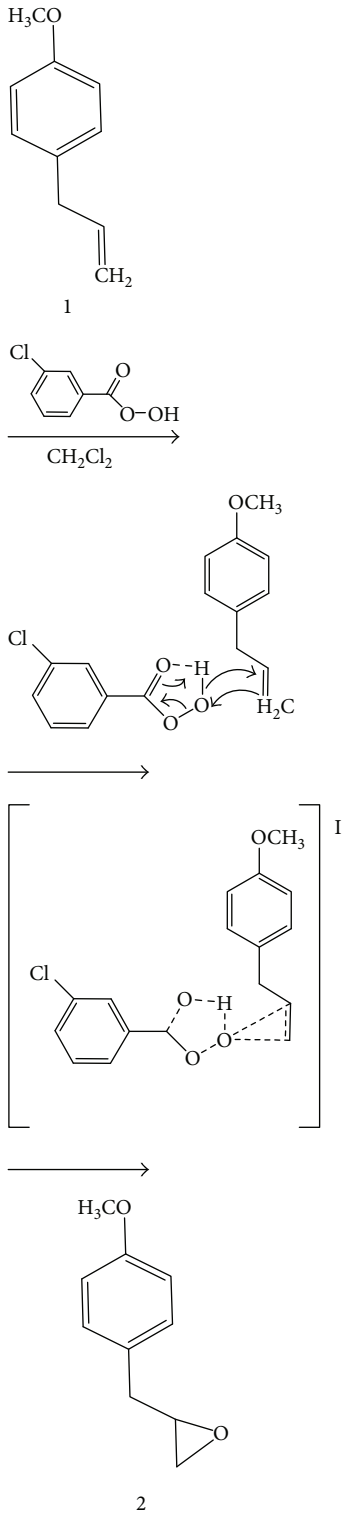
2.1. Chemicals and Reagents. The analytical products used for the development of this study were as follows: methyl chavicol (≥93.63%), *meta*-chloroperbenzoic acid (≥77%), and deuterated chloroform ($CDCl_3$ 99.8%) (Sigma-Aldrich®); hexane (≥98%), ethyl ether (≥98%), chloroform (≥99%), hydrochloric acid (≥37%), methanol (≥98%), dichloromethane (≥98%), sulfuric acid (≥95%), sodium chloride (≥99.5%), sodium hydroxide (≥99%), pyridine (≥98%), acetic anhydride (≥95%), and potassium permanganate (≥98%) (Merck®); sodium bicarbonate (≥95%) and ethanol (≥99%) (BIOTEC®); iodine (≥95%) (Synth®); sodium sulfite (≥98%) and sodium thiosulfate (≥98%) (Reagen®); and anhydrous sodium sulfate (≥99%) (Quimex®).

2.2. Synthesis and Characterization of 2-[(4-Methoxyphenyl)methyl]oxirane (MPMO). Methyl chavicol (**1**) (0.674 mmol/mL in dichloromethane) was reacted with *meta*-chloroperbenzoic acid (MCPBA) (0.35 mmol/mL in dichloromethane) for 40 minutes at temperature of 0°C (ice bath) and maintained at room temperature for 24 hours. After this time, 60 mL of 10% sodium sulfite (in water) was added to the reaction with stirring for one hour to separate the aqueous and organic phases. The aqueous phase was treated with dichloromethane, and, at the end of the separation, the organic phase was combined and washed with 5% sodium bicarbonate, saturated with sodium chloride solution, and subjected to anhydrous sodium sulfate to remove water residues. The solvent was evaporated, and the product was purified using silica gel chromatography column (70–230 mesh ASTM; Sigma-Aldrich) eluted in hexane/ethyl acetate (8 : 2). The yield of this reaction was 75% (Scheme 1).

^1H and ^{13}C nuclear magnetic resonance spectra of MC (Supplementary 1 and 2) and MPMO (Supplementary 4 and 5) were obtained at 500 MHz and 75 MHz, respectively, on a Bruker Avance DRX/500 spectrometer. As an internal reference, tetramethylsilane (TMS) or the residual hydrogen of the deuterated solvent was used. The chemical shift values (δ) were reported in parts per million (ppm) and the coupling constants (J) in hertz (Hz). The peak areas were acquired by electronic integration and their multiplicities described as follows: s = singlet; D = doublet; T = triplet; Tdd = triplet of double doublet; Dd = double doublet; Ddd = double doublet; and M = multiplet. MC spectral data were reported in Supplementary 1, 2, and 3 as described by the manufacturer.

MC and MPMO were analyzed by gas chromatography coupled to mass spectroscopy (GC-MS). The substances were diluted in 1% ethyl acetate (v/v), and 1.0 μL was injected with flow division (1 : 20) into a gas chromatograph, model Shimadzu® GCMS-QP2010 Plus, capillary column type Rtx-5 (5% phenyl, 95% dimethylpolysiloxane). Helium was used as entrainment gas with a flow rate of 1.0 mL/min. The temperature was programmed from 60 to 240°C at a heating rate of 8°C/min. The mass detector was operated in the electron ionization mode (70 eV). The percentage composition of the synthesized products was obtained by normalization and integration of the peak areas.

For the chromatography column, silica gel 60G 0.063–0.200 mm (70–230 mesh ASTM, Sigma-Aldrich) was used, while for thin-layer chromatography (TLC), precoated



Scheme 1: Oxidation reaction of methyl chavicol to obtain 2-[(4-methoxyphenyl)methyl]oxirane.

alumina plates F_{254} (Sigma-Aldrich) and solvent systems containing hexane/ethyl acetate (9 : 1, 8 : 2, 7 : 3, 6 : 4, and 1 : 1) were used. Ultraviolet (UV) lamp at 254 nm and iodine vapors were used as developers.

2.3. DPPH Radical Sequestration Method. The antioxidant activity was determined by the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) method as described by Mensor et al. [24]. From the stock solutions (750 mg/mL) of MC and MPMO and 3,5-di-*tert*-butyl-4-hydroxy toluene (BHT, 1 mg/mL) in ethanol PA, dilutions were prepared to obtain different concentrations. 2.5 mL was transferred, in triplicate, to test tubes, followed by addition of 1 mL of DPPH solution (0.03 mM). The antioxidant capacity was determined by reaction kinetics in the categories: rapid (reaction time < 30 minutes), medium (reaction time > 30 and <60 minutes), and slow (reaction time > 60 minutes) kinetics. The absorbances were plotted between zero time and 210 minutes (t_0, t_{15}, t_{30}, t_{45}, t_{60}, t_{75}, t_{90}, t_{120}, t_{150}, t_{180}, and t_{210}) in an interval of 15 minutes [25]. After this time, the ability of the samples to reduce DDPH to 2,2-diphenyl-1-picryl hydrazine was observed by spectrophotometry (Shimadzu, UV-1800®) at 518 nm [24]. The blank (samples and BHT) consisted of 2.5 mL of solutions and 1.0 mL of ethanol. The negative control was composed of 2.5 mL of ethanol and 1.0 mL of DPPH solution, whose auto-zero was only performed with ethanol. From the absorbances (Abs), the percentage of antioxidant activity (% AA) was determined using the following equation:

$$\%\mathrm{AA} = 100 - \frac{\mathrm{Abs}_{\mathrm{sample}} - \mathrm{Abs}_{\mathrm{sample\,blank}}}{\mathrm{Abs}_{\mathrm{control}} - \mathrm{Abs}_{\mathrm{control\,blank}}} \times 100. \quad (1)$$

After linear regression analysis by least-squares method, the half maximal inhibitory concentration (IC_{50}) was determined.

2.4. Cooxidation of the β-Carotene/Linoleic Acid Method. The antioxidant activity of MC and MPMO was determined by the cooxidation β-carotene/linoleic acid method described by Koleva et al. [26]. One milliliter of β-carotene (0.2 mg/mL in chloroform), 25 μL of linoleic acid, and 200 mg of Tween 40 were placed into a rotavaporation flask. After that, the solvent was removed, and 50 mL of distilled water was slowly added, under constant stirring with bubbling oxygenation, to form an emulsion. In a microplate, 30 μL of the samples and BHT (positive control) at 25 μg/mL were added, in triplicate, followed by 250 μL of the emulsion. The negative control was composed of 30 μL of ethanol and 250 μL of the emulsion. The blank was composed of 280 μL of ethanol. The assay consisted of microplate readings between zero and 105 minutes (t_0, t_{15}, t_{30}, t_{45}, t_{60}, t_{75}, t_{90}, and t_{105}) at 15-minute intervals after incubation in an oven at 50°C. Absorbances were measured on a microplate reader (ThermoPlate®, TP-Reader) at 492 nm. The graph of the decay absorbances (Abs) as a function of time was elaborated, and the percentage of inhibition of the lipid peroxidation (% I) was determined from the following equation:

$$\%I = 100 - \frac{\mathrm{Abs}_{\mathrm{control}} - \mathrm{Abs}_{\mathrm{sample}}}{\mathrm{Abs}_{\mathrm{control}}} \times 100, \quad (2)$$

where $\mathrm{Abs}_{\mathrm{control}} = \mathrm{Abs}_{t_0} - \mathrm{Abs}_{t_{105}}$ and $\mathrm{Abs}_{\mathrm{sample}} = \mathrm{Abs}_{t_0} - \mathrm{Abs}_{t_{105}} \cdot \mathrm{Abs}_{\mathrm{control}}$: negative control and $\mathrm{Abs}_{\mathrm{sample}}$: MC, MPMO, and BHT.

2.5. Thiobarbituric Acid Method. The lipid peroxidation method using thiobarbituric acid as described by Zeb and Ullah [27], with modifications, was applied to determine the antioxidant activity. This test consists in the analysis of the malonaldehyde and derivative substances from lipid peroxidation through the detection of the chromogenic complex [28]. Homogenates were prepared with 25 g of low-fat ground beef, 17 mL of distilled water, and 7.5, 15, and 30 mg of the samples in 200 μL of methanol. The homogenates were heated until the meat was cooked. After this procedure, distilled water was added to complete 100 mL, and the homogenate was mixed, transferred to amber vials, and stored under refrigeration. In triplicate, the test was performed with 500 mg of each homogenate, 50 μL of 4% BHT in ethanol, 2.5 mL of 1% phosphoric acid, and 1.25 mL of 1% thiobarbituric acid in 0.05 M sodium hydroxide. The tubes were boiled during 15 minutes, followed by cooling in an ice bath for 10 minutes. After cooling, 3.0 mL of butanol was added to each tube with stirring slowly under inversion and centrifuged at 4000 rpm for 5 minutes. The supernatant was used in the spectrophotometric reading at 535 nm (Shimadzu, UV-1800). The concentration of the thiobarbituric-malonaldehyde acid complex was calculated from the standard malonaldehyde (MDA) curve. Butanol was used as blank, BHT as positive control, and methanol as negative control.

2.6. Inhibitory Capacity on the Pancreatic Lipase Enzyme. The assay to determine the inhibitory capacity against pancreatic lipase was performed by spectrophotometric method with some modifications [29]. MC and MPMO were prepared at the concentration of 10 mg/mL in dimethyl sulfoxide (DMSO). From this solution, the assay was performed using a 10 g/L swine pancreatic lipase solution in 0.05 mol/L Tris-HCl buffer, pH 8.0, containing 0.010 mol/L calcium chloride and 0.025 mol/L sodium chloride. The substrate *p*-nitrophenol palmitate (8 mmol/L) was dissolved in 0.5% Triton X-100. In triplicate, 50 μL of the sample solution, 100 μL of the enzyme, and 50 μL of the substrate were placed into microtubes and incubated in a 37°C water bath at the times of 10, 20, 30, and 40 minutes. After this period, the reaction was stopped with an ice bath and 1.0 mL of 0.05 mol/L Tris-HCl buffer. For each time, the controls were used without enzyme (substrate blank) and without substrate (enzyme blank). As a positive control, 1 mg/mL orlistat was used. The absorbances of lipase products (*p*-nitrophenol) were determined using a spectrophotometer (Shimadzu, UV-1800) at 410 nm. After the absorbances were obtained, linear regression analysis by the least-squares method was performed to acquire the straight equation and angular coefficients (slope of the line), and inhibition of pancreatic lipase was determined. The percent inhibition (% I) of pancreatic lipase was calculated according to the following equation:

$$\%I = 100 \times \frac{(A-a)-(B-b)}{(A-a)}, \quad (3)$$

where A is the absorbance in the absence of the possible inhibitor, which corresponds to the control enzyme assay; a is the absorbance in the absence of the sample and enzyme (blank substrate); B is the absorbance in the presence of the possible inhibitor with the enzyme and substrate; and b is the absorbance in the absence of the enzyme.

2.7. Molecular Docking Study. The three-dimensional structure of the ligands was generated in the MarvinSketch 16.7.4 program [30]. Then, the geometry of ligands was refined by semiempirical calculations using Parametric Method 7 (PM7) [31] implemented in MOPAC2012 software using the *Octopus* workflow [32]. The crystallographic coordinates of the three-dimensional structure of the protein were obtained from the Protein Data Bank (PDB) under code 1LPA for pancreatic lipase [33]. The validation of the crystallographic ligands obtained from PDB was done by a redocking procedure that consisted of reproducing a crystallographic protein-binder complex with root-mean-square deviation (RMSD) of less than 2 Å.

The molecular docking was performed by AutoDock Vina 1.1.2 program [34]. In addition, a grid box was generated with dimensions of $30 \times 30 \times 30$ Å for molecular targets, and the coordinates of grid box were centered on crystallographic ligand with x 6.309, y 27.567, and z 48.586 Å using MGLTools software [35]. The analyses of the molecular recognition interactions were performed through the Discovery Studio v. 4.5 2016 program [36, 37].

2.8. Statistical Analyses. The results were expressed as mean ± standard error mean (SEM). Analysis of variance (ANOVA) followed by Tukey's HSD (honest significant difference) test was applied to measure the degree of significance for $P < 0.05$. The GraphPad Prism® program was used in these analyses.

3. Results

3.1. Synthesis of 2-[(4-Methoxyphenyl)methyl]oxirane. 2-[(4-Methoxyphenyl)methyl]oxirane (MPMO) was synthesized from methyl chavicol, which showed the physical appearance of a brown oily liquid of molecular formula $C_{10}H_{12}O_2$ and molecular mass 164.204 g·mol^{-1}. The yield of the reaction was 75% with purity of 99% when analyzed by gas chromatography (GC) (Supplementary 7).

The spectral data obtained were the following: ^{1}H NMR, 500 Hz, ($CDCl_3$): δ (ppm): 6.85 (d, 2H, J = 2.14 Hz); 6.74 (d, 2H, J = 2.14 Hz); 3.89 (s, 3H, J = 5.53, 5.53, 3.89, 2.75 Hz); 2.70 (m, 3H); 2.56 (dd, 1H, J = 5.04, 2.59 Hz); and 2.11 (m, 3H) (Supplementary 4). ^{13}C NMR, 75 Hz, ($CDCl_3$): δ (ppm): 171.395; 145.782; 145.608; 130.553; 120.612; 115.412; 110.935; 60.610; 56.193; and 52.774 (Supplementary 5). MS: m/z = 164 (M^+); 121; 108; 91; 77; and 65 (Supplementary 6).

3.2. DPPH Radical Sequestration Method. The kinetic profile showed that MC has a slow antioxidant capacity with reaction time greater than one hour. The percentage of unreacted DPPH radicals with MC in relation to the time is shown in Figure 1. When the steady state was reached, about 90 minutes (methyl chavicol) and 15

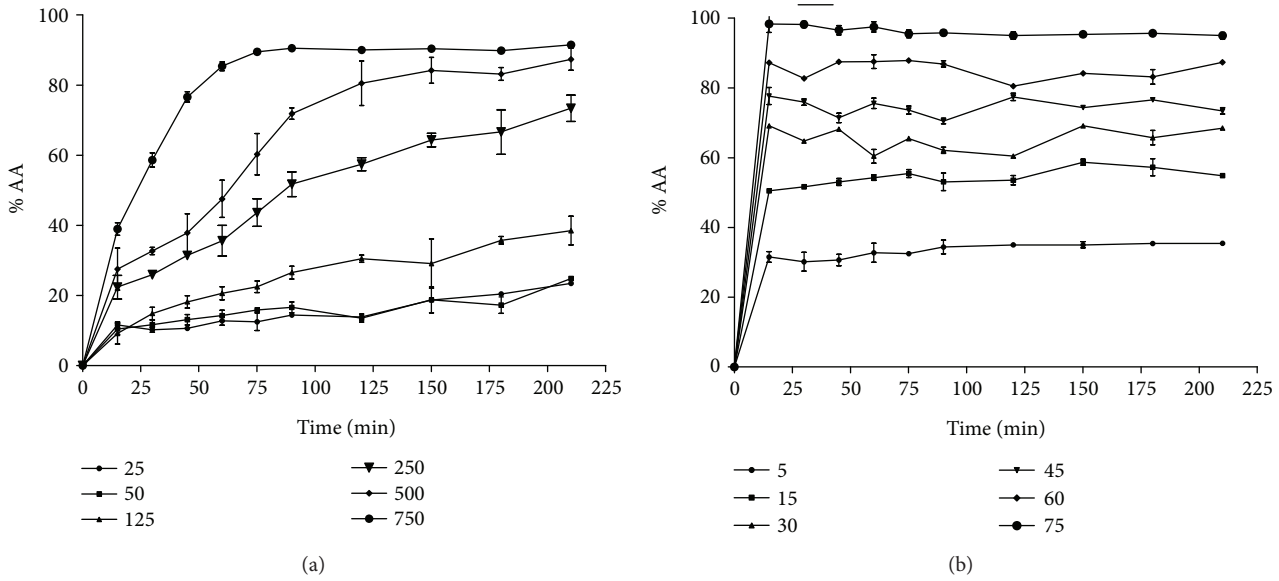


Figure 1: Kinetic profile of the methyl chavicol and 2-[(4-methoxyphenyl)methyl]oxirane at different concentrations (mg/mL) against DPPH. The values correspond to the mean ± SEM ($n = 3$). (a) Methyl chavicol (50 to 750 mg/mL); (b) 2-[(4-methoxyphenyl)methyl]oxirane (5 to 75 mg/mL).

minutes (2-[(4-methoxyphenyl)methyl]oxirane), the reaction between antioxidant and DPPH ceased. Thus, it was possible to calculate the real amount of DPPH radicals that was reduced by MC, avoiding the selection of an inadequate time interval in which the reaction still occurs.

The antioxidant potential of MC, MPMO, and BHT against DPPH is presented in Table 1. IC_{50} values of the samples ranged from 0.01 ± 0.01 to 312.50 ± 2.28 mg/mL and were significantly different from each other ($P < 0.001$). Considering the concentration of 50 mg/mL, MPMO was more effective than MC in inhibiting DPPH, since it produced an activity percentage (%) of approximately 80% of inhibition.

3.3. Cooxidation of the β-Carotene/Linoleic Acid Method. Figure 2 shows the decay of the absorbances in relation to the time using the cooxidation of the β-carotene/linoleic acid method. MC was more effective in inhibiting lipid peroxidation, since this compound presented a lower decay when compared to MPMO. After 15 minutes, the absorbances of the compounds are different from those of the negative control ($P < 0.001$).

With the data in Table 2, one can observe that MC inhibited $73.08 \pm 4.79\%$ of the lipid peroxidation, while MPMO produced a reduction of $36.16 \pm 4.11\%$. These data also show that MC was more effective than BHT (positive control) in the inhibition of lipid peroxidation.

3.4. Thiobarbituric Acid Method. The concentration of malonaldehyde (MDA) decreased in the homogenate treated with BHT, MC, and MPMO when compared to that of the negative control (Table 3). On the 5th day (day 4) of the experiment, MC presented an antioxidant activity similar to that of BHT in inhibiting the formation of MDA ($P < 0.001$).

Table 1: IC_{50} values of the methyl chavicol and 2-[(4-methoxyphenyl)methyl]oxirane by the DPPH method.

Compound	IC_{50} (mg/mL)
BHT	0.01 ± 0.01
MC	$312.50 \pm 2.28^{***}$
MPMO	$8.29 \pm 0.80^{***}$

The values correspond to the mean ± SEM ($n = 3$). BHT: 3,5-di-*tert*-butyl-4-hydroxy toluene; MC: methyl chavicol; MPMO: 2-[(4-methoxyphenyl)methyl]oxirane. The means differed from those of the positive control (BHT) after analysis of variance followed by Tukey's HSD (honest significant difference) test for $^{***}P < 0.001$.

3.5. Inhibitory Effect of the Methyl Chavicol and 2-[(4-Methoxyphenyl)methyl]oxirane on the Pancreatic Lipase. The inhibitory activity of MC and MPMO on the pancreatic lipase was 58.12 and 26.93%, respectively. Orlistat, the positive control, was effective by 76.80% of inhibition (Figure 3).

3.6. Molecular Docking Study. In this investigation, the parameters were validated using the redocking method to reproduce a protein-ligand crystallographic complex with a root-mean-square deviation (RMSD) of less than 2 Å. The redocking of the crystallographic ligand, diundecylphosphatidylcholine (PLC) (1.3232 Å) and orlistat (1.84 Å) (Supplementary 8), at the pancreatic lipase binding site showed an expressive reconstruction of the crystallographic complexes, which was essential to conduct this study. From these data, the molecular docking on the pancreatic lipase (PDB 1LPA) (Supplementary 9) was performed to obtain the orientation of the ligands. The amino acid residues Ser153, Asp177, and His264, components of the catalytic triad, constituted the most important molecular interactions, and Ser153 was the main amino acid involved in the lipolysis. Van der Waals and hydrogen bond interactions are

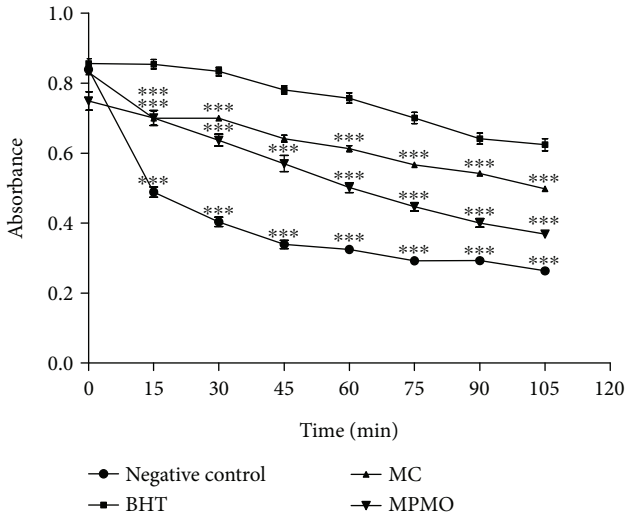


Figure 2: Decay of absorbance versus time by the cooxidation of the β-carotene/linoleic acid method. The values correspond to the mean ± SEM ($n = 3$). BHT: 3,5-di-*tert*-butyl-4-hydroxy toluene; MC: methyl chavicol; MPMO: 2-[(4-methoxyphenyl)methyl]oxirane. The means differed from those of the negative control after analysis of variance followed by Tukey's HSD (honest significant difference) test for $^{***}P < 0.001$.

Table 2: Inhibition of lipid peroxidation by the cooxidation of the β-carotene/linoleic acid method.

Compound	Inhibition of lipid peroxidation (%)
BHT	59.66 ± 0.52
MC	73.08 ± 4.79***
MPMO	36.16 ± 4.11***

The values correspond to the mean ± SEM ($n = 3$). BHT: 3,5-di-*tert*-butyl-4-hydroxy toluene; MC: methyl chavicol; MPMO: 2-[(4-methoxyphenyl)methyl]oxirane. The means differed from those of the positive control (BHT) after analysis of variance followed by Tukey's HSD (honest significant difference) test for $^{***}P < 0.001$.

associated with Ser153 and His264 residues, respectively, possible targets of inhibition for the antilipase agents (Supplementary 9).

The molecular interactions between MC or MPMO and pancreatic lipase are of hydrogen bonding type that exhibited Ser153 and His264 residues as target amino acids (Supplementary 9). The molecular docking study also revealed that the effect of MC and MPMO on the lipase produced a binding affinity value equal to −6.1 kcal·mol^{-1}, which was greater than that of orlistat (−6.5 kcal·mol^{-1}) and lower when compared to that of PLC (−5.6 kcal·mol^{-1}) (Table 4).

4. Discussion

MPMO synthesis consisted of an epoxidation in the olefinic group, since the epoxides are versatile and provide chirality to the molecules. Epoxides are also susceptible to reactions with a large number of nucleophiles, electrophiles, acids, and bases, with reducing agents and some oxidizing due to ring tension and polarity [38]. In this synthesis, the Prislaschajew reaction was carried out using the *meta*-chloroperbenzoic acid as an epoxidizing reagent. In addition, the structure of MC allows the functionalization of the olefin with peracids and occurs by a possible electrophilic biomolecular mechanism, where the peracid would be in a cyclic structure, stabilized by an intramolecular hydrogen bond to form a chelate (Scheme 2). The main evidence for this mechanism is the increase in the reaction rate due to the presence of electron-withdrawing substituents in the peracid. This fact increases the electrophilicity of O–O bond and/or the presence of electron donor groups that raise the nucleophilicity of C–C bond. The electrophilic mechanism is reinforced by the basicity of the solvent, breaking the intramolecular hydrogen bond, which decelerates the reaction. In this sense, the epoxidation of olefins with peracids is generally carried out in low polar aprotic solvents such as dichloromethane [38].

The results of the antioxidant activity showed that MC and MPMO presented an antioxidant potential in trials that differ in relation to the evaluated mechanism. In the DPPH free radical sequestration assay, the IC_{50} value of MC (312.50 ± 2.28 mg/mL) was about 38 times lower than that of MPMO (8.29 ± 0.80 mg/mL). The presence of the epoxide in MPMO can justify the higher antioxidant action when compared to MC, since this group increases its polarity and allows the electron donation to the DPPH radical. In addition, components of essential oils containing hydroxyl groups attached to the aromatic ring, unsaturations, and availability of electrons are associated with the antioxidant activity [39]. However, the essential oil of *Tagetes lucida*, which contains 95.7% of MC, produced IC_{50} of 37.9 μg/mL [40], while sweet basil essential oil (17.06% of MC) showed IC_{50} of 1.092 ± 0.066 mg/mL [41]. Probably, this difference is related to compounds that may promote synergistic action among them [41, 42].

Lipid peroxidation by the cooxidation of β-carotene/linoleic acid system and thiobarbituric acid assays are *in vitro* tests that reproduce physiological situations of oxidative stress, which can lead to cell death in extreme cases [43, 44]. As observed in the decay of the absorbance plot as a function of time (Figure 2), MC and MPMO delayed and reduced lipoperoxidation and, consequently, the oxidation of β-carotene. Thus, these findings show that MC is a molecule with antioxidant potential against lipid peroxidation, since it inhibited 73.08% of the oxidative process, while BHT (positive control) presented 59.66%, and MPMO was able to inhibit 36.16%. In addition, the results of the thiobarbituric acid assay corroborate the data on cooxidation of β-carotene/linoleic acid system, since MC was more active in inhibiting the formation of malonaldehyde in the homogenate. Lipoperoxidation is a process that involves the initiation, propagation, and termination steps, and antioxidants can block the first step (initiation) by neutralizing reactive oxygen species and/or inhibiting the propagation by suppressing the peroxyl radicals [44, 45]. Probably, MC reduced the generation of lipoperoxides by decreasing the formation of chelate complexes with reduced metals. Because it is a compound with lower polarity, MC has higher affinity

Table 3: Concentration of malonaldehyde (MDA) obtained by the thiobarbituric acid method.

Sample	Concentration (mg)	MDA content (μM)				
		Day 0	Day 1	Day 2	Day 3	Day 4
Control	Saline	0.42 ± 0.02	0.59 ± 0.04	0.67 ± 0.06	0.64 ± 0.03	0.91 ± 0.08
BHT	7.5	$0.08 \pm 0.02^{***}$	$0.16 \pm 0.02^{***}$	$0.12 \pm 0.03^{***}$	$0.16 \pm 0.00^{***}$	$0.22 \pm 0.05^{***}$
	15	$0.10 \pm 0.02^{***}$	$0.11 \pm 0.03^{***}$	$0.08 \pm 0.01^{***}$	$0.17 \pm 0.06^{***}$	$0.16 \pm 0.01^{***}$
	30	$0.20 \pm 0.01^{***}$	$0.20 \pm 0.03^{***}$	$0.23 \pm 0.06^{***}$	$0.12 \pm 0.08^{***}$	$0.22 \pm 0.08^{***}$
MC	7.5	0.41 ± 0.01	$0.18 \pm 0.02^{***}$	$0.22 \pm 0.06^{***}$	$0.22 \pm 0.06^{***}$	$0.16 \pm 0.02^{***}$
	15	0.39 ± 0.06	$0.29 \pm 0.04^{***}$	$0.53 \pm 0.06^{***}$	$0.32 \pm 0.07^{***}$	$0.21 \pm 0.08^{***}$
	30	$0.35 \pm 0.02^{***}$	$0.20 \pm 0.01^{***}$	$0.40 \pm 0.06^{***}$	$0.38 \pm 0.03^{***}$	$0.22 \pm 0.02^{***}$
MPMO	7.5	$0.24 \pm 0.02^{***}$	$0.39 \pm 0.07^{***}$	$0.46 \pm 0.01^{***}$	$0.35 \pm 0.03^{***}$	$0.54 \pm 0.04^{***}$
	15	$0.20 \pm 0.01^{***}$	$0.25 \pm 0.00^{***}$	$0.37 \pm 0.05^{***}$	$0.39 \pm 0.01^{***}$	$0.38 \pm 0.01^{***}$
	30	$0.18 \pm 0.06^{***}$	$0.26 \pm 0.02^{***}$	$0.32 \pm 0.00^{***}$	$0.37 \pm 0.01^{***}$	$0.34 \pm 0.05^{***}$

The values correspond to the mean ± SEM ($n = 3$). BHT: 3,5-di-*tert*-butyl-4-hydroxy toluene; MC: methyl chavicol; MPMO: 2-[(4-methoxyphenyl)methyl]oxirane. The means differed from those of the negative control (saline) after analysis of variance followed by Tukey's HSD (honest significant difference) test for $^{***}P < 0.001$.

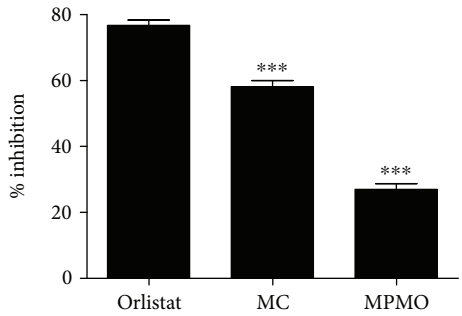


Figure 3: Inhibitory effect of the methyl chavicol and 2-[(4-methoxyphenyl)methyl]oxirane on the pancreatic lipase. The values correspond to the mean ± SEM ($n = 3$). MC: methyl chavicol (10 mg/mL); MPMO: 2-[(4-methoxyphenyl)methyl]oxirane (10 mg/mL). The means differed from those of the positive control (orlistat) after analysis of variance followed by Tukey's HSD (honest significant difference) test for $^{***}P < 0.001$.

Table 4: Binding affinity between ligands and pancreatic lipase.

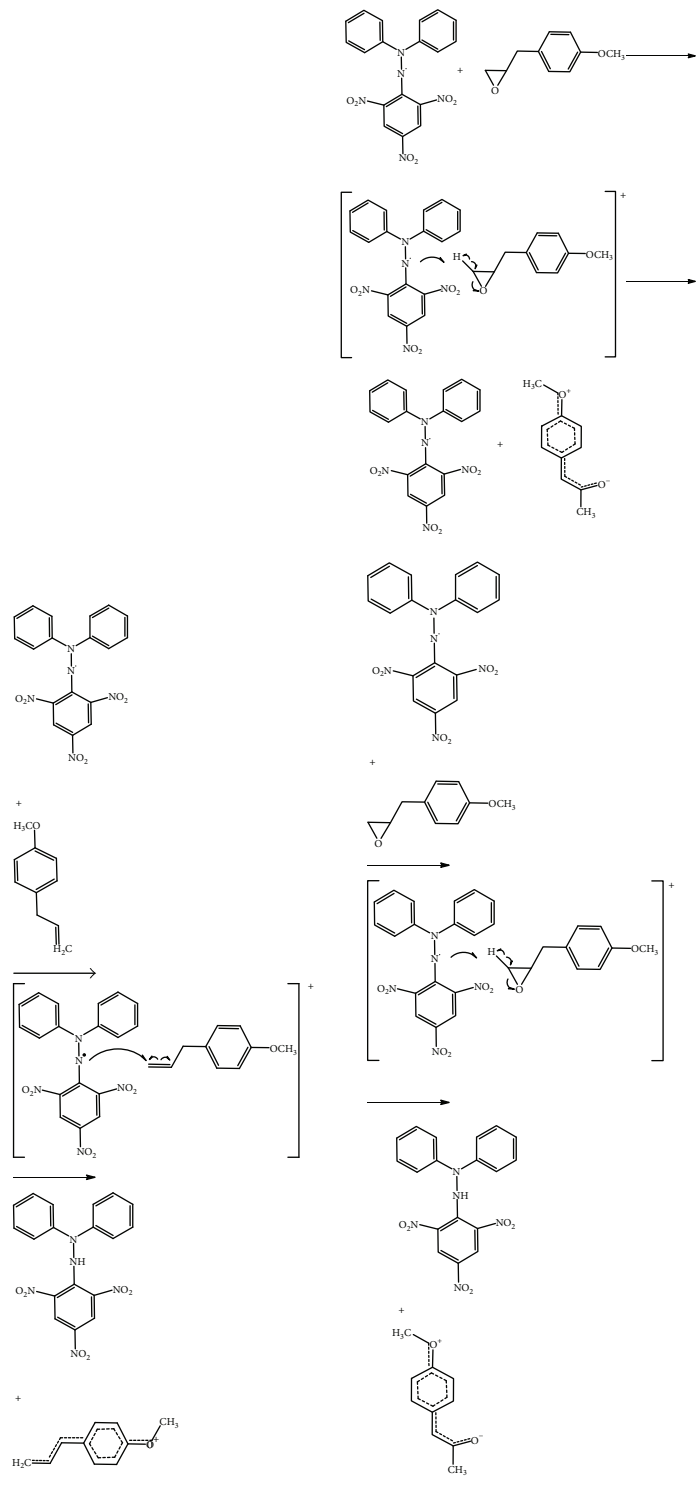
Compound	Binding affinity (kcal·mol^{-1})
PLC	−5.6
Orlistat	−6.5
MC	−6.1
MPMO	−6.1

PLC: diundecylphosphatidylcholine; MC: methyl chavicol; MPMO: 2-[(4-methoxyphenyl)methyl]oxirane.

for lipid media than has MPMO. In addition, unlike the epoxide group in the MPMO structure, the presence of the olefin in MC reduces the polarity and allows a greater lipid peroxidation inhibition.

The evaluation of the antilipase activity of MC is based on the kinetics of the lipolytic hydrolysis reaction of the *p*-nitrophenol palmitate in palmitic acid and *p*-nitrophenol. The release of these substances results in a yellow-colored chromogen that is intensified at high pH (optimum pH equals 8) [46]. A surfactant, such as Triton X-100, increases lipophilicity and stabilizes the reaction medium as well as enhances the permeability. Salts such as NaCl and $CaCl_2$ decrease the solvation layer of the enzyme, which implies a better dissolution, increases the ionic strength of the medium, and enables the formation of the ligand-protein complex [29]. Our results show that MC was more effective than MPMO, since MC was able to inhibit the *in vitro* pancreatic lipase by 58.12% (Figure 3), and these data were corroborated by the molecular docking study (Supplementary 9). Although Ser153 is the most important amino acid involved in the lipolysis, the pancreatic lipase inhibition occurs at the catalytic triad containing Ser153, Asp177, and His264 residues [47]. Both MC and MPMO interacted with Ser153 through a hydrogen bond, while the crystallographic binder (PLC) interacted with Ser153 by means of van der Waals force and His264 through a hydrogen bond. The formation of an electrostatic interaction between His264 and carbonyl oxygen differentiated the orlistat action of the other ligands at the catalytic site. It is important to note that a hydrogen bond interaction is typically more stable than a van der Waals force [48]. These findings also showed that MC and MPMO were less active than orlistat but were more active than PLC, since they produced a lower-affinity energy value (Table 4). Furthermore, it was verified that the oxygen of the epoxide found in MPMO was able to maintain a hydrogen bond at the Ser153 residue, but there was no interaction with the other residues responsible for its lipolytic action.

Natural compounds, such as alkaloids, carotenoids, glycosides, polyphenols, polysaccharides, saponins, and terpenoids, are described as pancreatic lipase inhibitors [48]. In particular, terpenes, such as carnosic acid, carnosol, roylenoic acid, 7-methoxyrosmanol, and oleanolic acid, were



Scheme 2: Proposal of the antioxidant mechanism of the methyl chavicol and 2-[(4-methoxyphenyl)methyl]oxirane based on the reaction with DPPH. (a) Stages of reactions between methyl chavicol and DPPH. (b) Stages of reactions between 2-[(4-methoxyphenyl)methyl]oxirane and DPPH.

reported to inhibit pancreatic lipase [49, 50]. Considering these aspects, MC and its synthetic analogue MPMO may constitute a new class of antilipase agents belonging to the phenylpropanoid derivatives.

Although methyl chavicol is found in essential oils of medicinal plants widely used by the population [43], the carcinogenic and teratogenic effects should be considered in possible therapeutic applications. Estragole and its

metabolite 1′-hydroxyestragole, for example, induced hepatic tumors in mice either after dietary chronic exposure or after intraperitoneal or subcutaneous injections [12–14]. In addition, the electrophilic epoxides of estragole and 1′-hydroxyestragole are directly mutagenic in *S. typhimurium*. Both estragole and its 1′-hydroxy metabolite produced unscheduled DNA synthesis in rat hepatocytes *in vitro* and estragole also *in vivo*. The formation of hepatic DNA adducts has also been demonstrated in mice. In this sense, the toxicity of methyl chavicol and its analogue with generation of toxic metabolites [12–15] may prevent their therapeutic use as antioxidant and antilipase agents.

5. Conclusion

MC and MPMO have an antioxidant activity and are capable of inhibiting the pancreatic lipase enzyme using the *in vitro* and in silico assays. The results suggest that these compounds may be promising for the development of new therapeutic options for the treatment of diseases associated with oxidative processes and metabolic alterations.

Disclosure

The results of this article were presented at the 11th International Congress of Pharmaceutical Sciences (CIFARP) focused meeting on "Pharmaceutical sciences in an emerging economy: challenges for a sustainable world" (Ribeirão Preto, Brazil, November 15–18, 2017).

Conflicts of Interest

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

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Supplementary Materials

Supplementary 1: ^{1}HNMR spectrum of the methyl chavicol. Supplementary 2: ^{13}CNMR spectrum of the methyl chavicol. Supplementary 3: mass spectrum of the methyl chavicol. Supplementary 4: ^{1}HNMR spectrum of the 2-[(4-methoxyphenyl) methyl]oxirane. Supplementary 5: ^{13}CNMR spectrum of the 2-[(4-methoxyphenyl)methyl]oxirane. Supplementary 6: mass spectrum of the 2-[(4-methoxyphenyl)methyl]oxirane. Supplementary 7: physicochemical properties and spectral data of the 2-[(4-methoxyphenyl)-methyl]oxirane. Supplementary 8: redocking of the crystallographic ligands PLC and orlistat with RMSD 1.3232 Å and 1.84 Å, respectively. *(Supplementary Materials)*

References

[1] V. Sosa, T. Moliné, R. Somoza, R. Paciucci, H. Kondoh, and M. E. LLeonart, "Oxidative stress and cancer: an overview," *Ageing Research Reviews*, vol. 12, no. 1, pp. 376–390, 2013.

[2] M. Valko, D. Leibfritz, J. Moncol, M. T. D. Cronin, M. Mazur, and J. Telser, "Free radicals and antioxidants in normal physiological functions and human disease," *The International Journal of Biochemistry & Cell Biology*, vol. 39, no. 1, pp. 44–84, 2007.

[3] P. X. Picon, C. M. Zanatta, F. Gerchman, T. Zelmanovitz, J. L. Gross, and L. H. Canani, "Análise dos critérios de definição da Sídrome Metabólica em Pacientes com Diabetes Metilo tipo 2," *Arquivos Brasileiros Endocrinologia & Metabologia*, vol. 50, no. 2, pp. 264–270, 2006.

[4] M. P. Mattson, "Dietary factors, hormesis and health," *Ageing Research Reviews*, vol. 7, no. 1, pp. 43–48, 2008.

[5] L. Li, G. Ishdorj, and S. B. Gibson, "Reactive oxygen species regulation of autophagy in cancer: implications for cancer treatment," *Free Radical Biology & Medicine*, vol. 53, no. 7, pp. 1399–1410, 2012.

[6] L. M. Magalhães, M. A. Segundo, S. Reis, and J. L. F. C. Lima, "Methodological aspects about *in vitro* evaluation of antioxidant properties," *Analytica Chimica Acta*, vol. 613, no. 1, pp. 1–19, 2008.

[7] J. P. Paula, P. V. Farago, J. L. C. Ribas et al., "*In vivo* evaluation of the mutagenic potencial of estragole and eugenol chemotypes of *Ocimum selloi* Benth. essential oil," *Latin American Journal of Pharmacy*, vol. 26, no. 6, pp. 846–851, 2007.

[8] J. P. de Paula, M. R. Gomes-Carneiro, and F. J. R. Paumgartten, "Chemical composition, toxicity and mosquito repellency of *Ocimum selloi* oil," *Journal of Ethnopharmacology*, vol. 88, no. 2-3, pp. 253–260, 2003.

[9] K. S. Silva-Alves, F. W. Ferreira-da-Silva, D. Peixoto-Neves et al., "Estragole blocks neuronal excitability by direct inhibition of Na^{+} channels," *Brazilian Journal of Medical and Biological Research*, vol. 46, no. 12, pp. 1056–1063, 2013.

[10] F. M. de Souza Silva-Comar, L. A. M. Wiirzler, S. E. Silva-Filho et al., "Effect of estragole on leukocyte behavior and phagocytic activity of macrophages," *Evidence-based Complementary and Alternative Medicine*, vol. 2014, Article ID 784689, 7 pages, 2014.

[11] S. Pattnaik, V. R. Subramanyam, M. Bapaji, and C. R. Kole, "Antibacterial and antifungal activity of aromatic constituents of essential oils," *Microbios*, vol. 89, no. 358, pp. 39–46, 1997.

[12] N. R. Drinkwater, E. C. Miller, J. A. Miller, and H. C. Pitot, "Hepatocarcinogenicity of estragole (1-allyl-4-methoxybenzene) and 1′-hydroxyestragole in the mouse and mutagenicity of 1′-acetoxyestragole in bacteria," *Journal of the National Cancer Institute*, vol. 57, no. 6, pp. 1323–1331, 1976.

[13] E. C. Miller, A. B. Swanson, D. H. Phillips, T. L. Fletcher, A. Liem, and J. A. Miller, "Structure-activity studies of the carcinogenicities in the mouse and rat of some naturally occurring and synthetic alkenylbenzene derivatives related to safrole and estragole," *Cancer Research*, vol. 43, no. 3, pp. 1124–1134, 1983.

[14] R. W. Wiseman, T. R. Fennell, J. A. Miller, and E. C. Miller, "Further characterization of the DNA adducts formed by electrophilic esters of the hepatocarcinogens 1′-hydroxysafrole and 1′-hydroxyestragole in vitro and in mouse liver

in vivo, including new adducts at C-8 and N-7 of guanine residues," *Cancer Research*, vol. 45, no. 7, pp. 3096–3105, 1985.

[15] S. M. F. Jeurissen, A. Punt, M. G. Boersma et al., "Human cytochrome P450 enzyme specificity for the bioactivation of estragole and related alkenylbenzenes," *Chemical Research in Toxicology*, vol. 20, no. 5, pp. 798–806, 2007.

[16] A. Anthony, J. Caldwell, A. J. Hutt, and R. L. Smith, "Metabolism of estragole in rat and mouse and influence of dose size on excretion of the proximate carcinogen 1′-hydroxyestragole," *Food and Chemical Toxicology*, vol. 25, no. 11, pp. 799–806, 1987.

[17] T. M. Guenthner and G. Luo, "Investigation of the role of the 2′,3′-epoxidation pathway in the bioactivation and genotoxicity of dietary allylbenzene analogs," *Toxicology*, vol. 160, no. 1-3, pp. 47–58, 2001.

[18] G. Luo, M. K. Qato, and T. M. Guenthner, "Hydrolysis of the 2′,3′-allylic epoxides of allylbenzene, estragole, eugenol, and safrole by both microsomal and cytosolic epoxide hydrolases," *Drug Metabolism and Disposition*, vol. 20, no. 3, pp. 440–445, 1992.

[19] D. H. Phillips, J. A. Miller, E. C. Miller, and B. Adams, "Structures of the DNA adducts formed in mouse liver after administration of the proximate hepatocarcinogen 1′-hydroxyestragole," *Cancer Research*, vol. 41, no. 1, pp. 176–186, 1981.

[20] S. A. Sangster, J. Caldwell, A. J. Hutt, A. Anthony, and R. L. Smith, "The metabolic disposition of [methoxy-^{14}C]-labeled *trans*-anethole, estragole and *p*-propylanisole in human volunteers," *Xenobiotica*, vol. 17, no. 10, pp. 1223–1232, 1987.

[21] E. Solheim and R. R. Scheline, "Metabolism of alkenebenzene derivatives in the rat I. *p*-Methoxyallylbenzene (estragole) and *p*-methoxypropenylbenzene (anethole)," *Xenobiotica*, vol. 3, no. 8, pp. 493–510, 1973.

[22] D. H. Phillips, M. V. Reddy, and K. Randerath, "^{32}P-Post-labelling analysis of DNA adducts formed in the livers of animals treated with safrole, estragole and other naturally-occurring alkenylbenzenes. II. Newborn male B6C3F1 mice," *Carcinogenesis*, vol. 5, no. 12, pp. 1623–1628, 1984.

[23] K. Randerath, R. E. Haglund, D. H. Phillips, and M. V. Reddy, "^{32}P-Post-labelling analysis of DNA adducts formed in the livers of animals treated with safrole, estragole and other naturally-occurring alkenylbenzenes. I. Adult female CD-1 mice," *Carcinogenesis*, vol. 5, no. 12, pp. 1613–1622, 1984.

[24] L. L. Mensor, F. S. Menezes, G. G. Leitão et al., "Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method," *Phytotherapy Research*, vol. 15, no. 2, pp. 127–130, 2001.

[25] K. Mishra, H. Ojha, and N. K. Chaudhury, "Estimation of antiradical properties of antioxidants using DPPH assay: a critical review and results," *Food Chemistry*, vol. 130, no. 4, pp. 1036–1043, 2012.

[26] I. I. Koleva, T. A. van Beek, J. P. H. Linssen, A. de Groot, and L. N. Evstatieva, "Screening of plant extracts for antioxidant activity: a comparative study on three testing methods," *Phytochemical Analysis*, vol. 13, no. 1, pp. 8–17, 2002.

[27] A. Zeb and F. Ullah, "A simple spectrophotometric method for the determination of thiobarbituric acid reactive substances in fried fast foods," *Journal of Analytical Methods in Chemistry*, vol. 2016, Article ID 9412767, 5 pages, 2016.

[28] J. A. Buege and S. D. Aust, "[30] Microsomal lipid peroxidation," *Methods in Enzymology*, vol. 52, pp. 302–310, 1978.

[29] S. P. de Souza, L. L. S. Pereira, A. A. Souza, and C. D. dos Santos, "Inhibition of pancreatic lipase by extracts of *Baccharis trimera* (Less.) DC., Asteraceae: evaluation of antinutrients and effect on glycosidases," *Revista Brasileira de Farmacognosia*, vol. 21, no. 3, pp. 450–455, 2011.

[30] S. L. Mayo, B. D. Olafson, and W. A. Goddard, "DREIDING: a generic force field for molecular simulations," *The Journal of Physical Chemistry*, vol. 94, no. 26, pp. 8897–8909, 1990.

[31] J. J. P. Stewart, "Optimization of parameters for semiempirical methods VI: more modifications to the NDDO approximations and re-optimization of parameters," *Journal of Molecular Modeling*, vol. 19, no. 1, pp. 1–32, 2013.

[32] H. Van Tilbeurgh, M. P. Egloff, C. Martinez, N. Rugani, R. Verger, and C. Cambillau, "Interfacial activation of the lipase-procolipase complex by mixed micelles revealed by X-ray crystallography," *Nature*, vol. 362, no. 6423, pp. 814–820, 1993.

[33] E. H. B. Maia, V. A. Campos, B. dos Reis Santos et al., "Octopus: a platform for the virtual high-throughput screening of a pool of compounds against a set of molecular targets," *Journal of Molecular Modeling*, vol. 23, no. 1, p. 26, 2017.

[34] O. Trott and A. J. Olson, "AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading," *Journal of Computational Chemistry*, vol. 31, no. 2, pp. 455–461, 2010.

[35] M. E. de Oliveira, G. Cenzi, R. R. Nunes et al., "Antimalarial activity of 4-metoxychalcones: docking studies as falcipain/plasmepsin inhibitors, ADMET and lipophilic efficiency analysis to identify a putative oral lead candidate," *Molecules*, vol. 18, no. 12, pp. 15276–15287, 2013.

[36] G. M. Morris, R. Huey, W. Lindstrom et al., "AutoDock4 and AutoDockTools4: automated docking with selective receptor flexibility," *Journal of Computational Chemistry*, vol. 30, no. 16, pp. 2785–2791, 2009.

[37] M. C. Santos Junior, S. A. Assis, A. Góes-Neto et al., "Structure-based drug design studies of UDP-*N*-acetylglucosamine pyrophosphosrylase, a key enzyme for the control of witches' broom disease," *Chemistry Central Journal*, vol. 7, pp. 1–7, 2013.

[38] M. L. A. Von Holleben and C. M. Schuch, "Ativação do peróxido de hidrogênio para a epoxidação de olefinas não funcionalizadas," *Química Nova*, vol. 20, no. 1, pp. 58–71, 1996.

[39] D. Amic, D. Davidovic-Amic, D. Beslo, V. Rastija, B. Lucic, and N. Trinajstic, "SAR and QSAR of the antioxidant activity of flavonoids," *Current Medicinal Chemistry*, vol. 14, no. 7, pp. 827–845, 2007.

[40] J. Olivero-Verbel, T. González-Cervera, J. Güette-Fernandez, B. Jaramillo-Colorado, and E. Stashenko, "Chemical composition and antioxidant activity of essential oils isolated from Colombian plants," *Brazilian Journal of Pharmacognosy*, vol. 20, no. 4, pp. 568–574, 2010.

[41] H. Li, Y. Ge, Z. Luo et al., "Evaluation of the chemical composition, antioxidant and anti-inflammatory activities of distillate and residue fractions of sweet basil essential oil," *Journal of Food Science and Technology*, vol. 54, no. 7, pp. 1882–1890, 2017.

[42] H. J. Damien Dorman, S. G. Deans, R. C. Noble, and P. Surai, "Evaluation *in vitro* of plant essential oils as natural antioxidants," *Journal of Essential Oil Research*, vol. 7, no. 6, pp. 645–651, 1995.

[43] A. M. Pisoschi and A. Pop, "The role of antioxidants in the chemistry of oxidative stress: a review," *European Journal of Medicinal Chemistry*, vol. 97, pp. 55–74, 2015.

[44] E. S. Lima and D. S. P. Abdalla, "Peroxidação lipídica: mecanismos e avaliação em amostras biológicas," *Revista Brasileira de Ciências Farmacêuticas*, vol. 37, no. 3, pp. 293–303, 2010.

[45] J. M. Duarte-Almeida, R. J. dos Santos, M. I. Genovese, and F. M. Lajolo, "Avaliação da atividade antioxidante utilizando sistema β-caroteno/ácido linoleico e método de seqüestro de radicais DPPH," *Ciência e Tecnologia de Alimentos*, vol. 26, no. 2, pp. 446–452, 2006.

[46] Y. Teng and Y. Xu, "A modified *para*-nitrophenyl palmitate assay for lipase synthetic activity determination in organic solvent," *Analytical Biochemistry*, vol. 363, no. 2, pp. 297–299, 2007.

[47] M. E. Lowe, "The catalytic site residues and interfacial binding of human pancreatic lipase," *The Journal of Biological Chemistry*, vol. 267, no. 24, pp. 17069–17073, 1992.

[48] G. K. Veeramachaneni, K. K. Raj, L. M. Chalasani, S. K. Annamraju, J. S. Bondili, and V. R. Talluri, "Shape based virtual screening and molecular docking towards designing novel pancreatic lipase inhibitors," *Bioinformation*, vol. 11, no. 12, pp. 535–542, 2015.

[49] N. A. Lunagariya, N. K. Patel, S. C. Jagtap, and K. K. Bhutani, "Inhibitors of pancreatic lipase: state of the art and clinical perspectives," *EXCLI Journal*, vol. 13, pp. 897–921, 2014.

[50] K. Ninomiya, H. Matsuda, H. Shimoda et al., "Carnosic acid, a new class of lipid absorption inhibitor from sage," *Bioorganic & Medicinal Chemistry Letters*, vol. 14, no. 8, pp. 1943–1946, 2004.

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Research Article

Clarified Açaí (*Euterpe oleracea*) Juice as an Anticonvulsant Agent: *In Vitro* Mechanistic Study of GABAergic Targets

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Seizures affect about 50 million people around the world. Approximately 30% of seizures are refractory to the current pharmacological arsenal, so, the pursuit of new therapeutic alternatives is essential. Clarified *Euterpe oleracea* (EO) juice showed anticonvulsant properties similar to diazepam in an *in vivo* model with pentylenetetrazol, a $GABA_A$ receptor blocker. This study investigated the effects of EO on the main GABAergic targets for anticonvulsant drugs, analyzing the effect on the GABA receptor's benzodiazepine and picrotoxinin binding sites and the GABA uptake. Primary cultures of cortical neurons and astrocytes were treated with EO (0–25%) for up to 90 min. [^{3}H]Flunitrazepam and [^{3}H]TBOB binding, [^{3}H]GABA uptake, cell viability, and morphology were assayed. Nonlethal concentrations of EO increased agonist binding and decreased antagonist binding in cortical neurons. Low concentrations significantly inhibited GABA uptake, especially in astrocytes, suggesting an accumulation of endogenous GABA in the synaptic cleft. The results demonstrate, for the first time, that EO can improve GABAergic neurotransmission via interactions with $GABA_A$ receptor and modulation of GABA uptake. Understanding these molecular mechanisms will help in the treatment of seizures and epilepsy, especially in developing countries where geographic isolation and low purchasing power are the main barriers to access to adequate treatment.

1. Introduction

Seizures are deleterious consequences of serious insults to the brain (e.g., trauma and stroke) and primary manifestations in epilepsy, affecting more than 50 million people worldwide [1]. Approximately 30% of seizures are refractory to the current pharmacological arsenal. Nearly 80% of all epileptic patients live in low-income or developing countries, and approximately 75% of these patients do not get adequate treatment [1]. Geographic isolation and low purchasing power are the main barriers to access the treatment in these countries, so, an easily available fruit juice may have an important impact.

Recently, the potent anticonvulsant properties of açaí (*Euterpe oleracea* Martius, family Arecaceae) were demonstrated in an *in vivo* pentylenetetrazol (PTZ) mouse model [2]. Açaí is the fruit of a common palm found in the eastern Amazonian floodplains, and its juice is highly consumed in

northern Brazil (up to 1 l/day per person) [3]. It is also available at the international market as a growing economic value (e.g., in 2011, açaí generated an estimated monetary movement of US$ 700,000 in Brazil) [4].

In the previous study, four doses of clarified açaí juice (10 μl/g body weight, equivalent to approximately 700 ml/day for a person weighing 70 kg) were sufficient to significantly protect against PTZ-induced seizures and seizure-related oxidative stress in mice [2]. Understanding the molecular mechanisms underlying this effect will help in the treatment of the disease. Considering that PTZ blocks the chloride channel coupled to the $GABA_A$ receptor complex, the present study aimed to analyze the possible modulation of GABAergic homeostasis within synaptic clefts *in vitro*.

2. Material and Methods

2.1. Culture of Neurons and Astrocytes. Animals were handled in compliance with protocols approved by the Autonomous Government of Catalonia, Spain, following European Union guidelines. All efforts were carried out to reduce the number of animals and minimize their suffering.

Primary cultures of neocortical neurons were obtained from 16-day-old NMRI mouse embryos (Charles River, Iffa Credo, Saint-Germain-sur-l'Arbresle, France) [5, 6]. The cell suspension for astrocyte culture was obtained in the same way as for neurons. Cells were grown for 2 weeks in DMEM : F12 containing 10% fetal bovine serum (FBS) until reaching confluence. Cytosine arabinoside (AraC) 10 μM was added to the media the last 2 days to prevent proliferation of other glial cells. Cultures were harvested with trypsin-EDTA, gently disaggregated, and seeded in 24-well plates at a density of 450,000 cells/ml with DMEM : F12 plus 10% FBS for 15 days. Thereafter, 200 μM dibutyryl cAMP was added to fully differentiate the astrocytes.

2.2. Clarified Euterpe oleracea (EO) Juice. Amazon Dreams (Belém, Pará, Brazil) kindly provided the commercial clarified *Euterpe oleracea* Martius, family Arecaceae, juice used in this work. The patented process to produce the juice was licensed by both Amazon Dreams and Universidade Federal do Pará (PI 8 1003060-3). It includes the microfiltration and centrifugation of a juice prepared with fresh fruit [2]. In order to quantify the anthocyanins and major flavonoids present in clarified juice, two validated UHPLC-DAD methods were used [7, 8], with the standard compounds (orientin, homoorientin, taxifolin, cyanidin 3-glucoside, and cyanidin 3-rutinoside) purchased from Extrasynthèse.

2.3. Treatments. After 7–12 days *in vitro* (div), the culture medium was removed and cells were rinsed with Hank's buffer. Cells were treated with 0–25% EO in Hank's buffer (250 μl final volume) for binding and uptake assays. Osmolality was maintained at 257–332 mOsmol/kg (data not shown) as recommended [9]. Cell viability and morphology were evaluated after exposure to 0–50% EO.

2.4. [^{3}H]Flunitrazepam Binding. After treatment with EO for 30 min, binding to the benzodiazepine site of the $GABA_A$ receptor in neuronal cultures was assayed using 1.83 nM [^{3}H]flunitrazepam according to Sunol et al. [6] and Garcia et al. [5]. [^{3}H]Flunitrazepam (specific radioactivity 82.5 Ci/mmol) was purchased from Amersham, Life Sciences. Data were expressed as the percentage of basal specific binding.

2.5. [^{3}H]-t-Butylbicycloorthobenzoate ([^{3}H]TBOB) Binding. Cultured cortical neurons were treated with EO for 60 min and binding to the picrotoxinin site at the $GABA_A$ receptor channel assayed using 1.84 nM [^{3}H]TBOB in Hank's buffer. [^{3}H]TBOB (16.2 Ci/mmol) was purchased from Amersham, Life Sciences. The method in van Rijn et al. [10] was adapted to evaluate TBOB binding in intact cells (unpublished results). Nonspecific binding was determined in the presence of 100 μM picrotoxinin. After 30 min of incubation at 25°C, cold buffer was added and rapidly removed by suction. The cells were rinsed two times with cold buffer and disaggregated with 0.2 N NaOH overnight at 4°C. The radioactivity of the samples was quantified by liquid scintillation spectroscopy using the OptiPhase cocktail (Wallac, UK). Data were expressed as the percentage of basal specific binding.

2.6. [^{3}H]GABA Uptake. After 55 min of treatment with EO, [^{3}H]GABA uptake was assayed in both mature cultures of neurons and astrocytes using 1.46 nM [^{3}H]GABA according to Vale et al. [11]. [^{3}H]GABA (90 Ci/mmol) was purchased from Amersham, Life Sciences. Data were expressed as the percentage relative to the control group.

2.7. Cell Viability and Morphology. After 90 min of treatment with EO, cell morphology and cytoskeletal performance was determined by tau immunocytochemistry (primary antibody, Sigma T-6402, 1 : 1000) as described elsewhere [12]. The cell viability of neuronal cells was evaluated using the 4,5-dimethylthiazol-3,5-diphenyltetrazolium (MTT) method as described previously [13]. Cell viability was reported as the percentage of reduced MTT compared to the control group.

2.8. Data Analysis. The results are presented as mean ± SEM of at least three independent experiments performed in triplicate. Student's *t*-test and one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test were applied. Significance was set at $P < 0.05$.

3. Results

EO showed five major phenolic compounds, all expressed per 100 ml of juice: 38 mg of orientin, 25 mg homoorientin, 31 mg of taxifolin deoxyhexose, 18 mg of cyanidin 3-glucoside, and 45 mg of cyanidin 3-rutinoside.

Treatment with EO significantly decreased [^{3}H]TBOB binding of the $GABA_A$ receptor in cortical neurons treated with ≥25% EO (Figure 1). Because of a high number of groups and the use of demanding statistical tests (such as ANOVA followed by Dunnett) could ignore slight differences between groups, an additional analysis with *t*-test was also carried out between control and EO-treated groups. In this analysis, 5% EO was sufficient to significantly reduce [^{3}H]TBOB binding (Figure 1, inset).

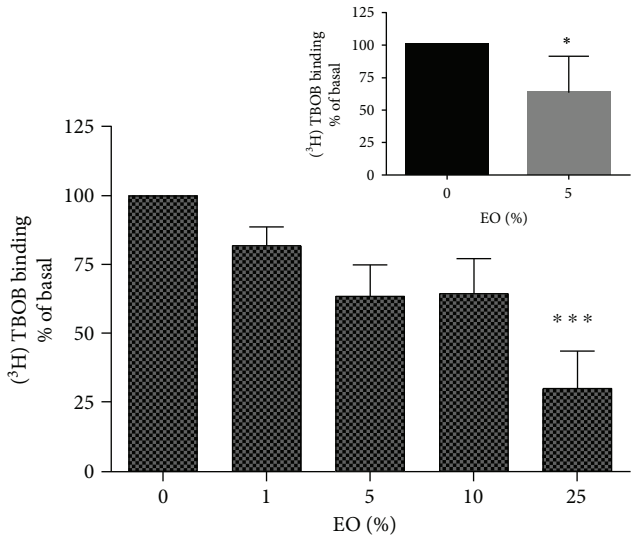

Figure 1: [^{3}H]TBOB binding in cultures of cortical neurons treated with clarified *Euterpe oleracea* (EO) juice. All data were evaluated by one-way ANOVA followed by the Dunnett' post hoc test, except data in inset that were evaluated using *t*-test. $^*P < 0.05$ and $^{***}P < 0.001$ versus control.

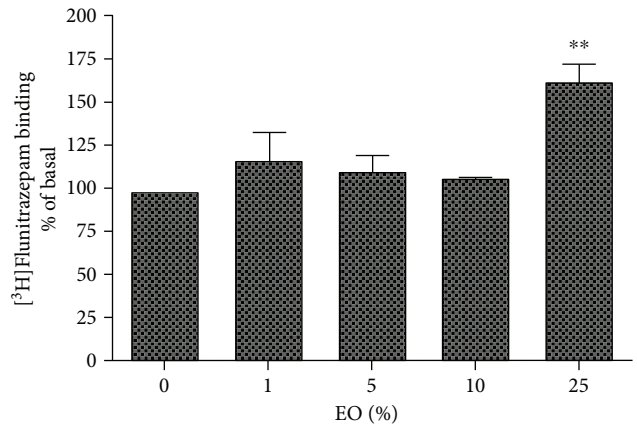

Figure 2: [^{3}H]Flunitrazepam binding in cultures of cortical neurons treated with clarified *Euterpe oleracea* (EO) juice. Data were evaluated by one-way ANOVA followed by the Dunnett's post hoc test. $^{**}P < 0.01$ versus control.

A significant increase in [^{3}H]flunitrazepam binding of the $GABA_A$ receptor (>50%) was observed after treatment with 25% EO (Figure 2).

Treatment with 25% EO significantly inhibited the [^{3}H]GABA uptake in cortical neurons (Figure 3(a)). Interestingly, cultures of cortical astrocytes were more sensible to the effect of EO on [^{3}H]GABA uptake, showing significant dose-response inhibition with ≥5% EO (Figure 3(b)).

Exposure to EO (0–50%) did not alter the cell morphology (Figure 4) or reduce cell viability (Figure 5).

4. Discussion

This work demonstrated, for the first time, that EO is able to interact with $GABA_A$ receptor and affect GABA uptake. Noncytotoxic concentrations of EO increased flunitrazepam binding and decreased TBOB binding in cortical neurons. Lower concentrations of EO significantly inhibited GABA uptake, especially in astrocytes. These events could possibly lead to the accumulation of endogenous GABA in the synaptic cleft and enhanced inhibitory neurotransmission in the brain.

Here, we used primary cultures of cortical neurons and astrocytes. After 7-8 days *in vitro*, neocortical neurons are mature and comprise mainly of GABAergic neurons [11], making them an excellent model for mechanistic studies. In these cells, $GABA_A$ receptor is a major pharmacological target for anticonvulsant drugs, such as benzodiazepines.

Although the allosteric modulation of $GABA_A$ receptor via multiple drug-binding sites is very complex (reviewed by [14]), the main targets for flunitrazepam and TBOB on the channel are the anticonvulsant benzodiazepine site and the convulsant picrotoxinin site, respectively. Thus, analysis of the interaction between the GABA receptor and possible therapeutic candidates using radioligand-binding assays is well recognized for elucidating the molecular mechanisms underlying the effect of anticonvulsant/proconvulsant agents [5, 15, 16]. Positive allosteric modulators or agonists of $GABA_A$ receptor exhibiting anticonvulsant actions can increase the binding of [^{3}H]flunitrazepam and/or modify the binding of [^{3}H]TBPS, a TBOB analog [5]. For example, the anticonvulsant drug felbamate inhibits [^{3}H]TBOB binding and increases chloride current (an indicative of a possible opening of the channel) [15]. Our results demonstrate that exposure to EO positively modulates the benzodiazepine site in addition to the possibly more potent negative modulation of the picrotoxinin site (Figures 1 and 2). Both actions facilitate the inhibitory role of GABA in the brain, making the initiation and propagation of exacerbated excitatory activity, as occurs in a seizure, more difficult.

Other important molecular targets for anticonvulsant drugs are the GABA transporters (GATs), which are inhibited by the anticonvulsant tiagabine [16, 17]. EO significantly inhibited GABA uptake in both cortical neurons and astrocytes (Figure 3). EO was particularly potent in astrocytes, causing significant inhibition at less than half the concentration than in neurons (Figure 3). Hypothetically, this finding may point to a more potent effect of EO toward GAT3, which is mainly found in astrocytes, than GAT1, which is mainly found in neurons. Interestingly, the inhibition of astrocytic GAT could have a superior anticonvulsant effect because GABA is nonreutilized and effectively eliminated in astrocytes, leading to clearance of this neurotransmitter from presynaptic neurons [17]. Inhibition of GABA reuptake has been shown to have significant therapeutic efficacy in other models [18], indicating a possible beneficial effect of EO in comorbidities such as depression and anxiety. By blocking GAT, endogenous GABA accumulates in the synaptic cleft, increasing inhibitory neurotransmission. This high levels of GABA caused by incubation with EO could be responsible, partially at least, for the effects detected in the receptor, since GABA is able of increasing benzodiazepine binding

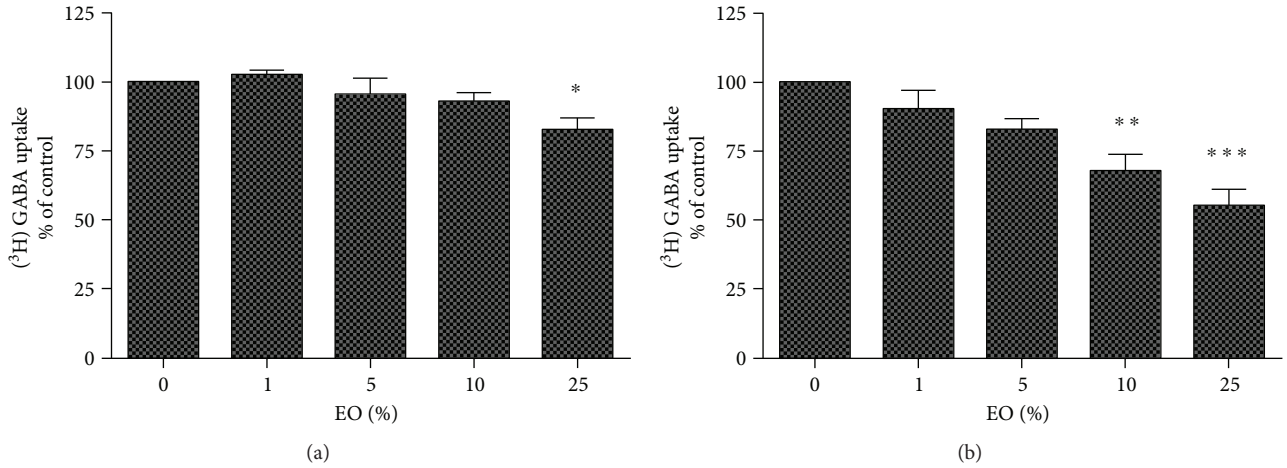

Figure 3: [^{3}H]GABA uptake in cultures of cortical neurons (a) and astrocytes (b) treated with clarified *Euterpe oleracea* (EO) juice. Data were evaluated by one-way ANOVA followed by the Dunnett's post hoc test. $^*P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ versus control.

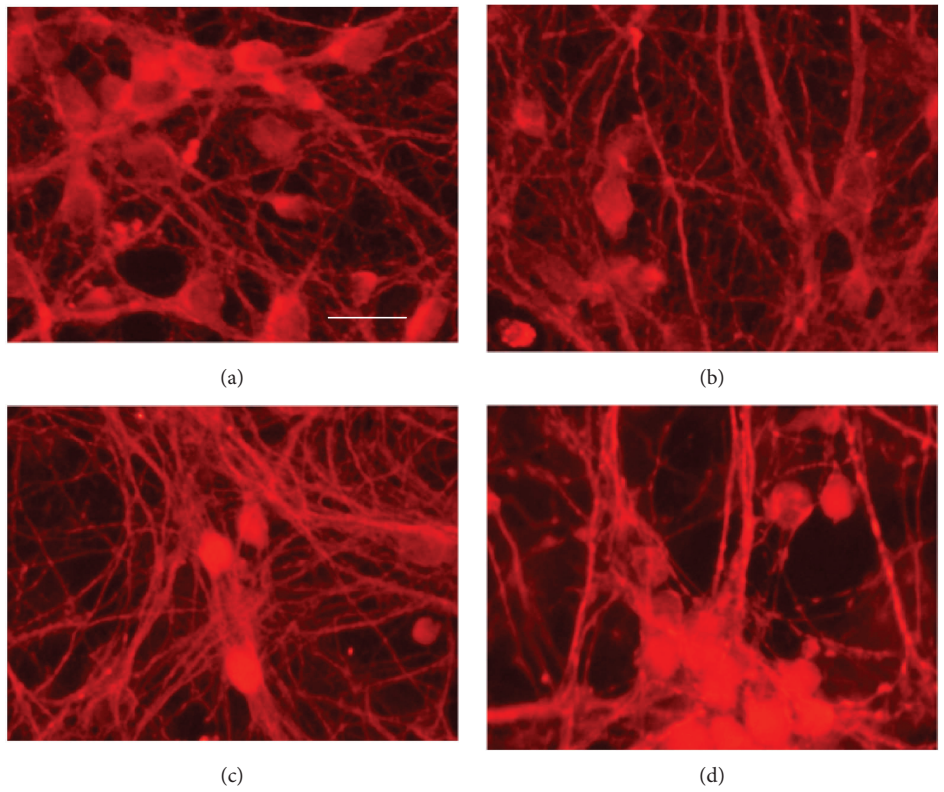

Figure 4: Representative micrographs of cortical neurons exposed to clarified *Euterpe oleracea* (EO) juice for 90 minutes. (a) Control; (b) 10% EO; (c) 25% EO; and (d) 50% EO. Neurons were labelled with anti-tau, allowing a comparison of neuronal arborization among treatments. Scale bar = 20 μm.

and decreasing TBOB binding. Additionally, the possible presence of GABA in the composition of EO must not be discarded, since this neurotransmitter is a constituent found in many plants [19]. However, to date, GABA has not been described as a main component of EO and it seems to be unprovable that the anticonvulsant effect of EO can be totally attributed to this compound due to the absence of sedative effects of EO [2].

Moreover, our data showed that doses of EO that affect the GABA receptor and uptake did not alter cellular viability or morphology (Figures 4 and 5), confirming that they are not the consequence of a reduced number of cells and suggesting that EO acts on diverse molecular targets in the GABAergic system. The latter hypothesis is in agreement with the notable potency of the anticonvulsant effect of EO observed *in vivo* [2]. In a similar model of PTZ-induced

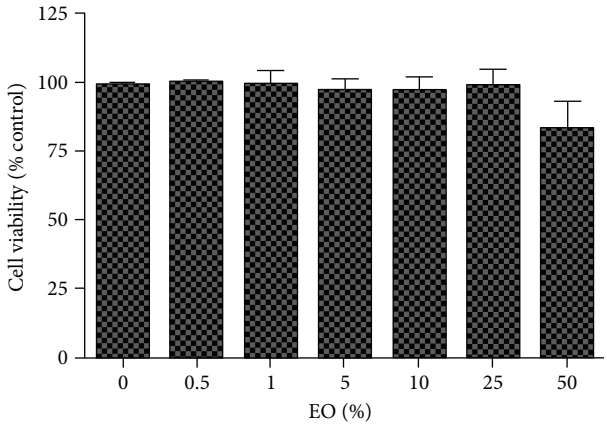

Figure 5: Cell viability of cortical neurons exposed to clarified *Euterpe oleracea* (EO) juice for 90 minutes. No significant difference was found between groups using one-way ANOVA.

seizures, diazepam (3 mg/kg) reduced the duration of clonic-tonic convulsions in a similar way as EO (9.2 ± 1.5 and 8.0 ± 1.12 seconds for diazepam and EO, resp.; $P > 0.05$, unpaired t-test). Also, EO caused a higher delay of the onset (405.1 ± 99.71 seconds) than that of diazepam (187 ± 7.2 seconds; $P < 0.001$, unpaired t-test) [2, 20]. Although our initial hypothesis was that the anticonvulsant effect of EO is due to its extraordinary antioxidant properties because a 1 : 100 EO dilution had greater scavenger action than 800 μM Trolox [2], this study is the first to show that EO also significantly influences on the GABAergic system.

Commercial EO was used to guarantee that the samples were indicative of human consumption. All macronutrients (i.e., lipid, fiber, and protein) are eliminated in the clarification process, reducing possible interference by these compounds. So, phenolic compounds, particularly anthocyanins, are the main compounds in this juice. Preliminary analysis of the samples indicated 1662.15 mg gallic acid equivalents/l of phenolic compounds, including 761 mg cyanide equivalents/l of anthocyanins. Major flavonoids present in clarified açaí were (per 100 mL) cyanidin 3-rutinoside (45 mg), orientin (38 mg), taxifolin deoxyhexose (31 mg), homoorientin (25 mg), and cyanidin 3-glucoside (18 mg). Many flavonoids are able to interact with the benzodiazepine site of GABA receptors and modulate the chloride flux [16, 19]. Interestingly, some of these flavonoids exhibit anticonvulsant activity in the absence of sedative effects [16]. Although additional studies are necessary, this finding may explain the effect of EO, found by Souza-Monteiro et al. and this work, which modulates the GABA receptor without sedative effects [2].

Our results demonstrate that EO can improve GABAergic neurotransmission via interactions with $GABA_A$ receptor and modulation of GABA uptake. These events could possibly lead to the accumulation of endogenous GABA in the synaptic cleft and enhanced inhibitory neurotransmission in the brain.

Knowledge of the molecular mechanisms underlying the anticonvulsant effect of açaí is of particular importance for use in folk medicine by isolated populations. Many of these populations live in the Amazon, where compliance with chronic pharmacological treatment with current anticonvulsant drugs is reduced due to socioeconomic factors (i.e., poverty and isolation) that make it difficult to access health services. Thus, a common palm widely distributed in the Amazon could be an extremely useful tool for treating seizures, especially in these populations.

Disclosure

The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Conflicts of Interest

All authors declare that no conflicts of interest exist. The donations from Amazon Dreams do not imply any competing interests.

Authors' Contributions

Cristina Suñol and Maria Elena Crespo-López contributed equally to this work.

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References

[1] WHO-World Health Organization, "Epilepsy, Fact sheet N°999," 2017, http://www.who.int/mediacentre/factsheets/fs999/en/.

[2] J. R. Souza-Monteiro, M. Hamoy, D. Santana-Coelho et al., "Anticonvulsant properties of *Euterpe Oleracea* in mice," *Neurochemistry International*, vol. 90, pp. 20–27, 2015.

[3] C. M. G. Bichara and H. Rogez, "Açai (*Euterpe oleracea* Mart.)," in *Postharvest Biology and Technology of Tropical and Subtropical Fruits, Açai to Citrus*, E. Yahia, Ed., Woodhead Publishing, Cambridge, 2015.

[4] K. K. Yamaguchi, L. F. Pereira, C. V. Lamarao, E. S. Lima, and V. F. da Veiga-Junior, "Amazon acai: chemistry and biological activities: a review," *Food Chemistry*, vol. 179, pp. 137–151, 2015.

[5] D. A. Garcia, J. Bujons, C. Vale, and C. Sunol, "Allosteric positive interaction of thymol with the $GABA_A$ receptor in primary cultures of mouse cortical neurons," *Neuropharmacology*, vol. 50, no. 1, pp. 25–35, 2006.

[6] C. Sunol, D. A. Garcia, J. Bujons et al., "Activity of B-nor analogues of neurosteroids on the $GABA_A$ receptor in primary neuronal cultures," *Journal of Medicinal Chemistry*, vol. 49, no. 11, pp. 3225–3234, 2006.

[7] A. L. Dias, E. Rozet, G. Chataigne et al., "A rapid validated UHPLC–PDA method for anthocyanins quantification from *Euterpe oleracea* fruits," *Journal of Chromatography, B*, vol. 907, pp. 108–116, 2012.

[8] A. L. Dias, E. Rozet, Y. Larondelle, P. Hubert, H. Rogez, and J. Quetin-Leclercq, "Development and validation of an UHPLC-LTQ-Orbitrap MS method for non-anthocyanin flavonoids quantification in *Euterpe oleracea* juice," *Analytical and Bioanalytical Chemistry*, vol. 405, no. 28, pp. 9235–9249, 2013.

[9] R. I. Freshney, *Culture of Animal Cells, pp. a Manual of Basic Technique and Specialized Applications*, Wiley-Blackwell, Hoboken, NJ, USA, 2015.

[10] C. M. van Rijn, E. Willems-van Bree, R. Dirksen, and J. F. Rodrigues de Miranda, "The $GABA_A$ receptor complex in relation to epilepsy. Reversal of [^{3}H]TBOB inhibition: a prediction of proconvulsive properties?," *Epilepsy Research*, vol. 12, no. 2, pp. 163–170, 1992.

[11] C. Vale, M. T. Vilaro, E. Rodriguez-Farre, and C. Sunol, "Effects of the conformationally restricted GABA analogues, *cis*- and *trans*-4-aminocrotonic acid, on GABA neurotransmission in primary neuronal cultures," *Journal of Neuroscience Research*, vol. 57, no. 1, pp. 95–105, 1999.

[12] N. M. Carretero, M. P. Lichtenstein, E. Perez, L. Cabana, C. Sunol, and N. Casan-Pastor, "IrOx–carbon nanotube hybrids: a nanostructured material for electrodes with increased charge capacity in neural systems," *Acta Biomaterialia*, vol. 10, no. 10, pp. 4548–4558, 2014.

[13] T. Mosmann, "Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays," *Journal of Immunological Methods*, vol. 65, no. 1-2, pp. 55–63, 1983.

[14] W. Sieghart, "Chapter three - allosteric modulation of $GABA_A$ receptors via multiple drug-binding sites," *Advances in Pharmacology*, vol. 72, pp. 53–96, 2015.

[15] A. Kume, L. J. Greenfield Jr, R. L. Macdonald, and R. L. Albin, "Felbamate inhibits [3H]t-butylbicycloorthobenzoate (TBOB) binding and enhances Cl-current at the gamma-aminobutyric AcidA (GABAA) receptor," *The Journal of Pharmacology and Experimental Therapeutics*, vol. 277, no. 3, pp. 1784–1792, 1996.

[16] N. J. Sucher and M. C. Carles, "A pharmacological basis of herbal medicines for epilepsy," *Epilepsy & Behavior*, vol. 52, Part B, pp. 308–318, 2015.

[17] A. Schousboe, K. K. Madsen, M. L. Barker-Haliski, and H. S. White, "The GABA synapse as a target for antiepileptic drugs: a historical overview focused on GABA transporters," *Neurochemical Research*, vol. 39, no. 10, pp. 1980–1987, 2014.

[18] K. Salat, A. Podkowa, P. Kowalczyk et al., "Anticonvulsant active inhibitor of GABA transporter subtype 1, tiagabine, with activity in mouse models of anxiety, pain and depression," *Pharmacological Reports*, vol. 67, no. 3, pp. 465–472, 2015.

[19] G. A. Johnston, "Flavonoid nutraceuticals and ionotropic receptors for the inhibitory neurotransmitter GABA," *Neurochemistry International*, vol. 89, pp. 120–125, 2015.

[20] N. Karim, S. Irshad, I. Khan et al., "$GABA_A$ receptor modulation and neuropharmacological activities of viscosine isolated from *Dodonaea viscosa* (Linn)," *Pharmacology, Biochemistry, and Behavior*, vol. 136, pp. 64–72, 2015.

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Research Article

Antioxidant and Hepatoprotective Activities of Polysaccharides from Spent Mushroom Substrates (*Laetiporus sulphureus*) in Acute Alcohol-Induced Mice

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In order to contribute to the exploitation and utilization of spent mushroom substrates (SMS) of *Laetiporus sulphureus*, hot-water-extractable polysaccharides (H-SMPS) and enzymatic-extractable polysaccharides (E-SMPS) were successfully isolated from SMS of *L. sulphureus*. Both H-SMPS and E-SMPS were found to have high reducing power and potential scavenging activities against hydroxyl, DPPH, and superoxide anion radicals. *In vivo* assays showed that the administration of H-SMPS and E-SMPS has potential hepatoprotective effects against alcohol-induced alcoholic liver disease (ALD), possibly brought about by improving liver function, increasing antioxidant status, and reducing lipid peroxidation. Furthermore, monosaccharide composition analysis showed that fucose may play a vital role in guaranteeing stronger hepatoprotection. These results may provide references for the exploitation of the SMS of *L. sulphureus* as a source of H-SMPS and E-SMPS, which in turn can be used as functional foods or natural drugs for the prevention of ALD and other liver diseases.

1. Introduction

With the prevalence of poor diet structures in China, increasing alcohol consumption has been demonstrated to be involved in the increasing incidence of alcoholic liver disease (ALD), which is a progressive disease that could subsequently induce hepatitis, hepatic fibrosis, cirrhosis, and liver cancer [1–3]. Furthermore, ALD could also cause other clinical diseases including hypertension, hyperlipidemia, and atherosclerosis [4, 5]. Alcohol metabolism mainly occurs in the liver, and many studies have indicated that many *in vivo* enzymes, such as alcohol dehydrogenase (ADH), aldehyde dehydrogenase (ALDH), and cytochrome P4502E1 (CYP2E1), are involved in alcohol metabolism [6, 7]. The toxicity of acute alcohol consumption in the liver is possibly due to the inhibition of ADH and ALDH activities, as well as the increase in CYP2E1 activity, which is accompanied by the excessive formation of reactive oxygen species (ROS) [5, 8]. Increasing amounts of evidence indicate that ROS could oxidize biological membranes, proteins, nucleic acids, and other biological macromolecules, thereby damaging cellular integrity and functionality [9]. At present, the mechanisms underlying acute alcohol metabolism and its metabolic consequences are still poorly understood. The traditional therapies for ALD are mainly abstinence and the administration of nutritional supplements and corticosteroid substances; however, their therapeutic effects have been proven to be poor [10]. Recently, natural substances from edible and medicinal mushrooms have attracted attention as potential drugs in preventing and treating alcohol-induced liver injury [11]. Hence, it seems necessary and significant to explore natural and nontoxic therapeutic strategies for the treatment of ALD.

It has been shown that more than five million tons of spent mushroom substrates (SMS), the cultivation matrix that remains after mushroom cultivations, are produced in China annually with low utilization, causing environmental pollution and resource waste [12]. SMS contains residual mycelia and fruiting bodies of mushrooms; trace elements

including Fe, Ca, Zn, and Mg; active enzymes such as cellulose, hemicellulose, and ligninase; and biomacromolecules including polysaccharides, proteins, and lipids [12, 13]. Polysaccharides extracted from SMS have received attention owing to their abundant pharmacological activities including antioxidation, hepatoprotection, and antihyperlipidemia [14, 15]. Additionally, these polysaccharides possess less toxic side effects and are more accessible locally and more environment friendly, compared to synthetic substances [14].

Laetiporus sulphureus, an edible and medicinal fungus belonging to *Basidiomycetes*, is widely distributed in Asia, Europe, and North America [16]. Documents in literature have indicated that intracellular polysaccharides and exopolysaccharides from *L. sulphureus* show antioxidant, anti-inflammatory, and antitumor activities [17–19]. However, studies on the hepatoprotective activities of polysaccharides isolated from *L. sulphureus* SMS are scarce. In this work, the hepatoprotective effects and antioxidant activities of hot-water-extractable SMPS (H-SMPS) and enzymatic-extractable SMPS (E-SMPS) from *L. sulphureus* SMS in acute alcohol-induced ALD mice were investigated.

2. Materials and Methods

2.1. Materials and Chemicals. SMS of *L. sulphureus* was obtained from Tai'an Agricultural Sciences Academy (Tai'an, China). Kunming strain mice (male) were purchased from Taibang Biologic Products Co. Ltd., Tai'an, China. Monosaccharide standard samples were provided by Sigma Chemicals Co. Ltd., St. Louis, USA The diagnostic kits for analyzing the activities of ADH, ALDH, CYP2E1, superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT), as well as the levels of malondialdehyde (MDA), lipid peroxidation (LPO), total cholesterol (TC), and triglyceride (TG), were purchased from Jiangsu Meibiao Biological Technology Co. Ltd., Jiangsu, China. All other reagents used in this experiment were provided by local chemical suppliers.

2.2. Preparation of H-SMPS and E-SMPS. The SMS of *L. sulphureus* was dried at 55°C and ground into powder. H-SMPS and E-SMPS were extracted by incubating the SMS in deionized water (90°C, 8 h) and snailase solutions (4% *w*/*v*, 37°C, 6 h), respectively. After centrifugation (3000 rpm, 10 min), the supernatant was collected, concentrated, and precipitated by incubation with three volumes of 95% *v*/*v* ethanol at 4°C overnight. The precipitate was deproteinated by the Sevage method [20] and dialyzed against deionized water.

2.3. Animal Experiments. Animal procedures were performed in accordance with the institutional ethical guidelines for animals' welfare of Shandong Agricultural University Committee. Seventy male Kunming strain mice (20 ± 2 g) were housed in an animal room under standardized environmental conditions (temperature of 23 ± 2°C, a relative humidity of 50 ± 5%, and a 12/12 h light/dark cycle) for 5 days with free access to food and water.

After domestication, the mice were randomly divided into seven groups of ten mice each. These groups were the normal control (NC) group, model control (MC) group, positive control (PC) group (received bifendatatum at 150 mg/kg), H-SMPS high-dose group (400 mg/kg), H-SMPS low-dose group (100 mg/kg), E-SMPS high-dose group (400 mg/kg), and E-SMPS low-dose group (100 mg/kg). The mice in the NC and MC groups received isometric normal saline until the 25th day as a blank. On the 26th day, all mice except those in the NC group received alcohol (50% *v*/*v*) at a dose of 12 mL/kg by gavages. All mice were sacrificed quickly by euthanasia overnight after inducing toxicity with alcohol. The animal experiment design thought was in accordance with previous literature [6, 21, 22].

Blood samples were collected and centrifuged (10,000 rpm, 10 min, 4°C) to obtain the sera. Alanine aminotransferase (ALT) activity and aspartate aminotransferase (AST) activity in the sera were measured using an automatic biochemical analyzer (BS-380, Shenzhen, China).

The liver was surgically removed, weighed, and homogenized (10% *w*/*v*) in phosphate buffer solution (0.2 M, pH 7.4). After centrifugation (3000 rpm, 10 min, 4°C), the supernatant was collected for further biochemical assay. The hepatic activities of ADH, ALDH, CYP2E1, SOD, GSH-Px, and CAT, as well as the levels of MDA, LPO, TC, and TG, were determined using commercial reagent kits following the manufacturer's instructions. The liver samples used for histopathological observations were soaked in formalin (10% *w*/*v*). Thin sections (4-5 μm thickness) were acquired using a microtome and stained with hematoxylin and eosin. Hepatic histopathological changes were observed and photographed using a microscope under ×400 magnification.

2.4. In Vitro Antioxidant Analysis. The reducing powers of H-SMPS and E-SMPS were determined using the methods reported by Oyaizu [23]. The reaction system, which contained 1 mL of either of the polysaccharides (0–800 μg/mL), 2.5 mL of phosphate buffer (0.2 M, pH 6.6), and 1.0 mL of potassium ferricyanide (1% *w*/*v*), was kept warm in a water bath at 50°C for 20 min. The reaction was terminated by adding 2 mL of trichloroacetic acid (10% *w*/*v*) and 1.2 mL of ferric chloride (0.1% *w*/*v*). The absorbance was measured at 700 nm using deionized water as a blank control and butylated hydroxytoluene (BHT) as a positive control.

The scavenging activities of H-SMPS and E-SMPS against hydroxyl radicals were measured according to a reported method [24]. The reaction mixture, composed of 1 mL of ferrous sulfate (9 mM), 1 mL of salicylic acid (9 mM), 1 mL of either of the polysaccharides (0–800 μg/mL), and 1 mL of hydrogen peroxide (8.8 mM), was incubated for 30 min at 37°C. After centrifugation (12,000 rpm, 6 min), the absorbance was measured at 510 nm using deionized water as a blank control and BHT as a positive control. The scavenging rate was calculated using the following formula:

$$\text{Scavenging rate}\,(\%) = \left[\frac{A_0 - A_1}{A_0}\right] \times 100, \quad (1)$$

where A_0 is the absorbance of the blank and A_1 is the absorbance of the polysaccharides or BHT.

The DPPH radical scavenging activities of H-SMPS and E-SMPS were measured using the method reported by Cheng

et al. [25]. The reaction mixture, which contained either of the polysaccharides (0.2 mL, 0–800 μg/mL) and DPPH solution (0.6 mL, 0.004% *w*/*v* in methanol), was stored in the dark for 30 min without moving. The absorbance was measured at 517 nm using deionized water as a blank control and BHT as a positive control. The scavenging rate was evaluated as follows:

$$\text{Scavenging rate}\,(\%) = \left[\frac{A_0 - A_1}{A_1}\right] \times 100, \quad (2)$$

where A_0 is the absorbance of the blank control and A_1 is the absorbance of the polysaccharides or BHT.

Superoxide anion radical scavenging activities were determined using the method reported by Wei et al. [26]. The reaction system, composed of 1 mL of either of the polysaccharides (0–800 μg/mL) and 2 mL of Tris-HCl buffer (50 mM, pH 8.2), was incubated for 20 min at 25°C. After incubation, 0.4 mL of 1,2,3-phentriol (5 mM) was added to terminate the reaction. The absorbance was measured at 325 nm using deionized water as a blank control and BHT as a positive control. The scavenging rate was calculated using the following equation:

$$\text{Scavenging rate}\,(\%) = \left[\frac{A_0 - A_1}{A_0}\right] \times 100, \quad (3)$$

where A_0 is the absorbance of the blank and A_1 is the absorbance of polysaccharides or BHT.

The IC_{50} values (μg/mL) were defined as the effective concentrations of the sample at which the radicals were inhibited by 50%.

2.5. Monosaccharide Composition Determination. Monosaccharide compositions of H-SMPS and E-SMPS were analyzed by gas chromatography (GC-2010, Shimadzu, Japan) equipped with a flame ionization detector (FID) according to a reported method [27]. Briefly, 0.1 g of the sample was hydrolyzed with 1.8 mL of trifluoroacetic acid (2 M) at 100°C for 4 h. The resulting hydrolysate was neutralized with 0.6 mL of ammonium hydroxide (12 M), restored with 0.4 mL of sodium borohydride in ammonium hydroxide (2% *w*/*v*), and acetylated with 0.6 mL of methylimidazole and 4 mL of acetic anhydride. After centrifugation (3000 rpm, 10 min), 1 μL of the supernatant was injected into a capillary column of Rtx-1 (30 mm × 0.25 mm × 0.25 μm). Monosaccharide components were evaluated based on the standard curves of rhamnose, arabinose, xylose, mannose, galactose, glucose, ribose, and fucose.

2.6. Statistical Analysis. The software SAS was used to perform statistical evaluation. Data are expressed as mean ± SD (standard deviations). One-way ANOVA was performed to analyze the data. Significant differences between experimental groups were determined using Tukey's tests. $P < 0.05$ was considered as the threshold for statistical significance.

3. Results

3.1. Effects of H-SMPS and E-SMPS on AST and ALT Activities in Sera. The mice in the MC group showed abnormally higher serum AST and ALT activities than the mice in the NC group ($P < 0.05$, Figure 1), indicating that alcohol-induced liver injury was successfully established in mice. Interestingly, the elevation of AST and ALT activities could be attenuated by supplementation of H-SMPS and E-SMPS, especially at high doses. Compared to mice in the MC group, the serum AST and ALT activities of mice in the H-SMPS high-dose group decreased by 28.29% and 28.0%, respectively, while those of mice in the E-SMPS high-dose group decreased by 36.6% and 50.0% of mice, respectively. In addition, bifendatatum was also observed to decrease the serum enzyme activities. These results demonstrate that both H-SMPS and E-SMPS have the potential to suppress acute alcohol-induced elevation of AST and ALT activities in order to maintain liver function.

3.2. Effects of H-SMPS and E-SMPS on Hepatic Lipid Properties. As shown in Figure 2, the mice in the MC group exhibited higher hepatic TG and TC levels than the mice in the NC group ($P < 0.05$), indicating that hepatic lipid metabolic disturbance was induced by acute alcohol toxicity. The administration of H-SMPS and E-SMPS attenuated the elevation of TG and TC levels, especially with E-SMPS administered at a high dose. Briefly, the hepatic TG and TC levels in mice treated with a high dose of E-SMPS reached 1.25 ± 0.14 and 3.7 ± 0.18 mmol/mg prot, respectively, which were lower than those observed in the H-SMPS group at the same dose (TG: 1.54 ± 0.11 mmol/mg prot and TC: 4.8 ± 0.16 mmol/mg prot), and were almost equal to those in the NC group (TG: 1.12 ± 0.13 mmol/mg prot and TC: 3.5 ± 0.16 mmol/mg prot). The present results indicate that both H-SMPS and E-SMPS could potentially restore lipid metabolism to its state before acute alcohol-induced toxicity.

3.3. Effects of H-SMPS and E-SMPS on Hepatic ADH and ALDH Activities and CYP2E1 Level. The hepatic ADH and ALDH activities and CYP2E1 levels are shown in Figure 3. Compared with mice in the NC group, the mice in the MC group had decreased ADH and ALDH activities and increased CYP2E1 levels ($P < 0.05$), indicating that alcohol metabolism was partly suppressed in the alcohol dehydrogenase oxidation system and accelerated in the microsome alcohol oxidation system in response to acute alcohol-induced toxicity. Interestingly, the remarkable elevation in ADH and ALDH activities and reduction in CYP2E1 levels could be observed when H-SMPS and E-SMPS are administered at the tested dosages ($P < 0.05$). The ADH and ALDH activities of mice treated with a high dose of E-SMPS reached maximum values of 58.9 ± 3.4 and 18.5 ± 1.9 U/mg prot, respectively, which were higher than those of mice in the H-SMPS group (ADH: 55.3 ± 2.8 U/mg prot and ALDH: 15.9 ± 2.1 U/mg prot) at the same dose. Additionally, compared with the CYP2E1 levels in the H-SMPS high-dose group (64.30 ± 2.72 ng/mL), the CYP2E1 levels in the E-SMPS high-dose group were lower (56.73 ± 2.98 ng/mL).

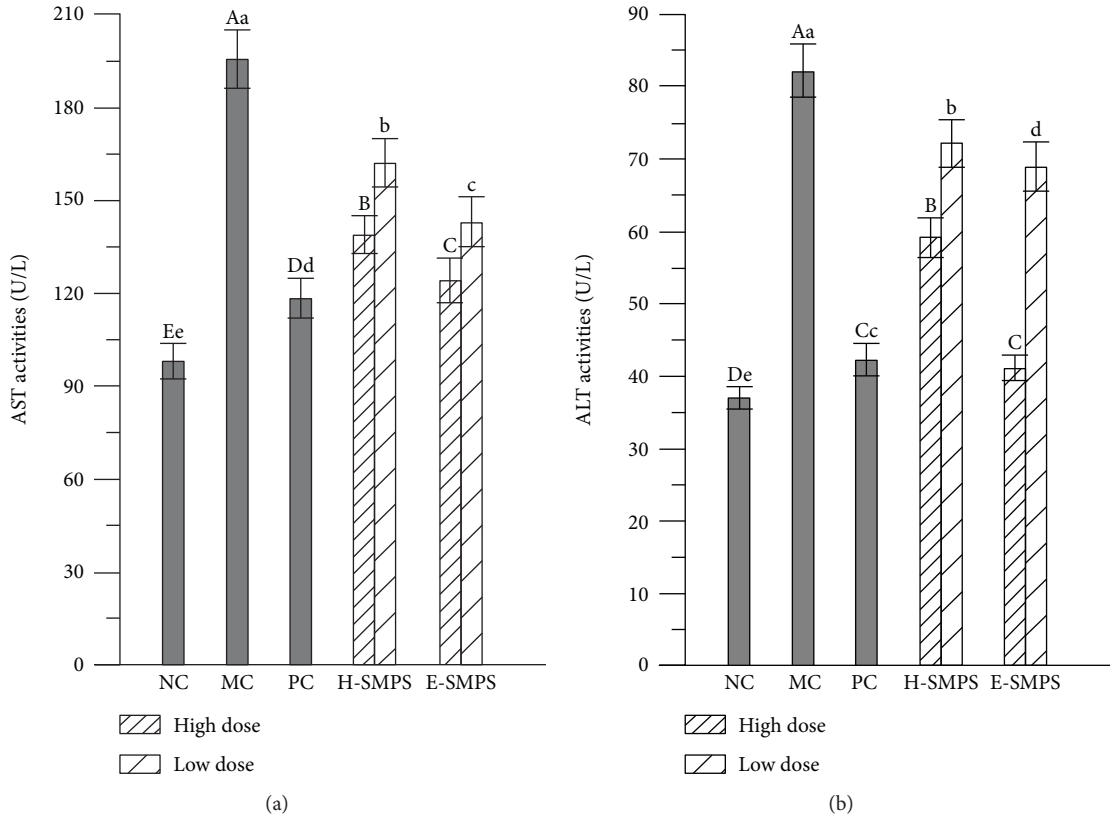

Figure 1: Effects of H-SMPS and E-SMPS on the serum activities of AST (a) and ALT (b). The values were reported as means ± SD. Bars with different letters were significantly different ($P < 0.05$).

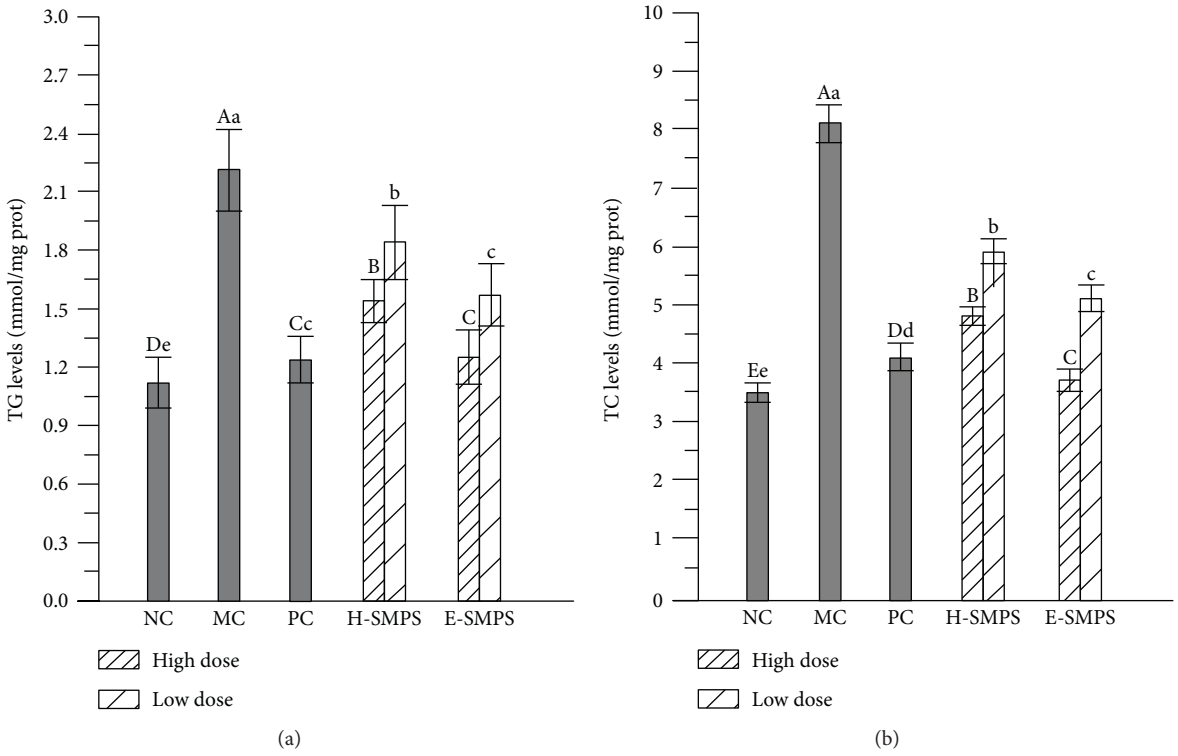

Figure 2: Effects of H-SMPS and E-SMPS on hepatic lipid profiles of TG (a) and TC (b). The values were reported as means ± SD. Bars with different letters were significantly different ($P < 0.05$).

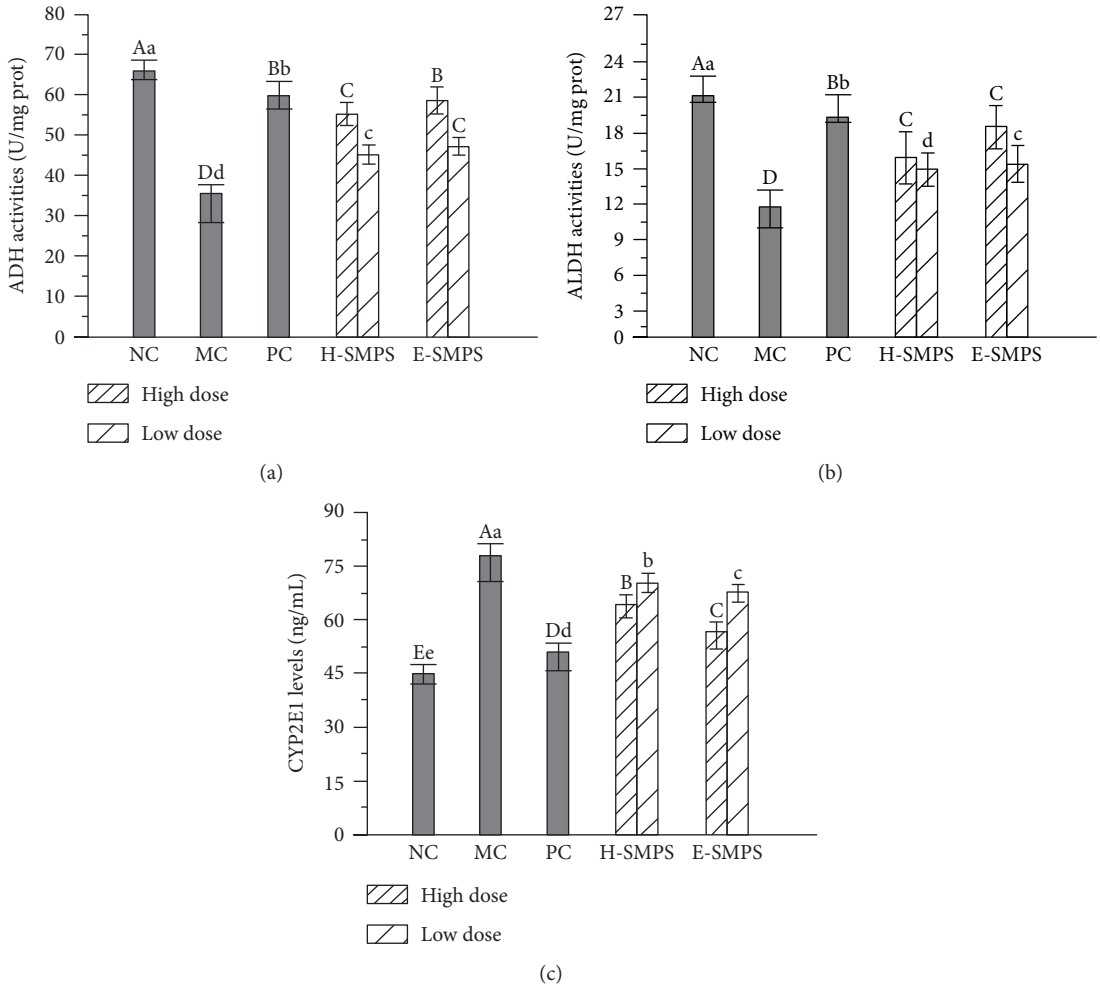

Figure 3: Effects of H-SMPS and E-SMPS on alcohol-metabolizing enzyme activities of ADH (a) and ALDH (b) as well as levels of CYP2E1 (c). The values were reported as means ± SD. Bars with different letters were significantly different ($P < 0.05$).

Meanwhile, bifendatatum-treated mice also exhibited an increase in the activities of ADH and ALDH, as well as an attenuation in the CYP2E1 levels when compared with the MC group mice ($P < 0.05$).

3.4. Effects of H-SMPS and E-SMPS on Hepatic Antioxidative Enzymes. Compared with the mice in the NC group, the mice in the MC group were observed to have an abnormal reduction in hepatic SOD, GSH-Px, and CAT activities ($P < 0.05$, Figures 4(a)–4(c)), indicating that the hepatic antioxidative defense had been damaged by the administration of alcohol. The changes in these parameters were ameliorated by treatment with H-SMPS, E-SMPS, and bifendatatum at all doses (with all $P < 0.05$). The hepatic SOD, GSH-Px, and CAT activities in the H-SMPS group were elevated by 30.6%, 36.8%, and 44.6%, respectively, compared with those in the MC group. In the E-SMPS high-dose group, hepatic SOD, GSH-Px, and CAT activities were elevated by 40.8%, 58.4%, and 54.5%, respectively, compared with those in the MC group. Based on the present results, H-SMPS, E-SMPS, and bifendatatum can potentially restore the damaged antioxidative defense.

3.5. Effects of H-SMPS and E-SMPS on Hepatic Lipid Peroxidation. In the MC group, the hepatic lipid peroxidation of MDA and LPO was remarkably increased by 104.8% and 159.4%, respectively, as compared to the NC group ($P < 0.05$, Figures 4(d)–4(e)). This indicates that oxidative stress had occurred in the liver as a result of the administration of alcohol. However, treatment with H-SMPS and E-SMPS significantly mitigated the abnormal increases in MDA and LPO levels ($P < 0.05$). A high dose of H-SMPS inhibited MDA and LPO levels by 27.9% and 41.0%, respectively, while E-SMPS inhibited MDA and LPO levels by 40.7% and 50.6%, respectively, when compared with levels in the MC group. These results demonstrate that both E-SMPS and H-SMPS could protect the liver against oxidative stress by inhibiting lipid peroxidation. Furthermore, the MDA and LPO levels in the PC group were almost equal to those in the NC group.

3.6. Effects of H-SMPS and E-SMPS on Hepatic Histological Changes. Images of hepatic histological sections are displayed in Figure 5. No obvious histological changes were observed in the livers of mice in the NC group. In contrast, hepatic

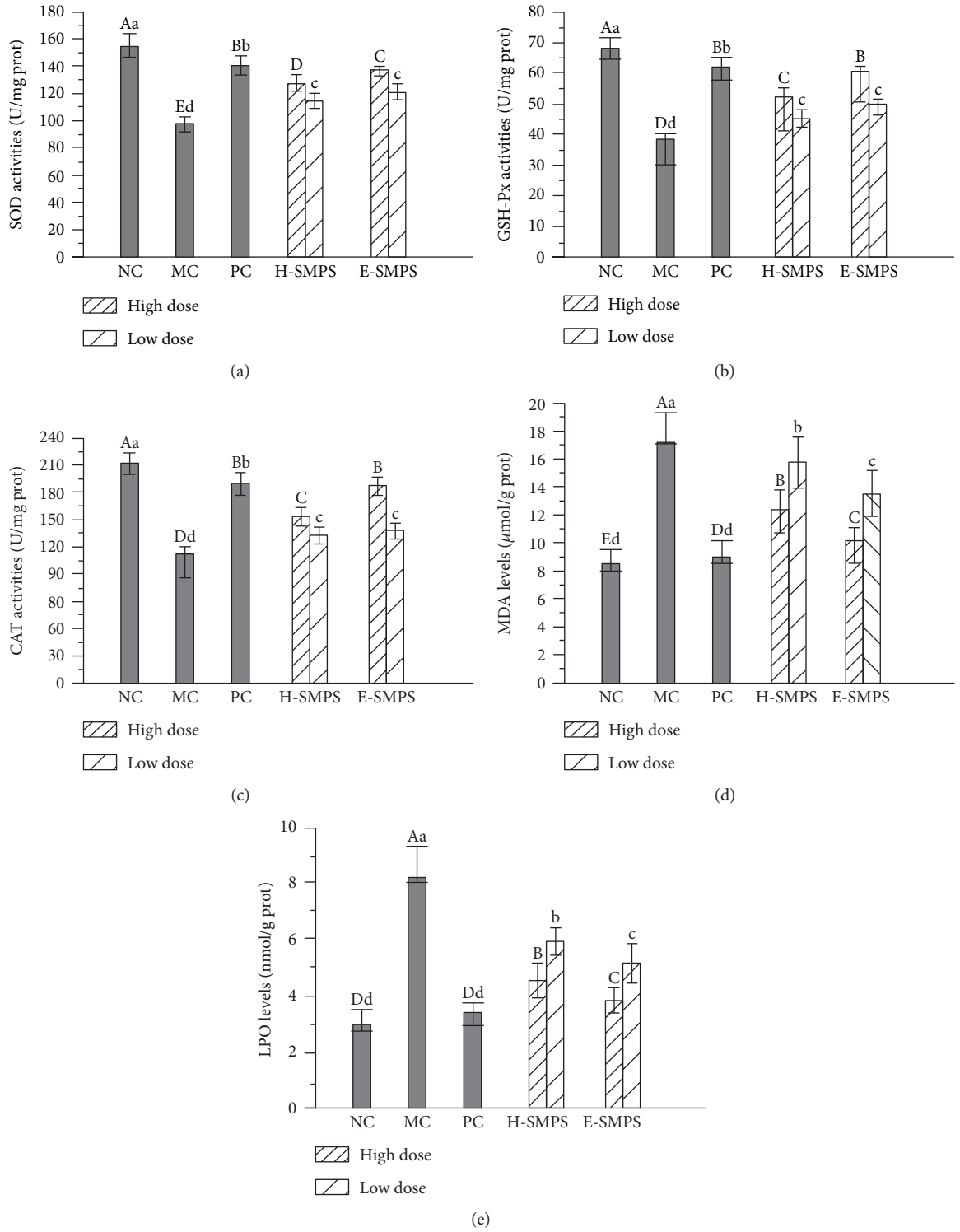

Figure 4: Effects of H-SMPS and E-SMPS on antioxidant enzymes of SOD (a), GSH-Px (b), and CAT (c), as well as lipid peroxides of MDA (d) and LPO (e). The values were reported as means ± SD. Bars with different letters were significantly different ($P < 0.05$).

sections of mice in the MC group showed nucleus contraction, loss of cellular boundaries in the cytoplasm of hepatocytes, necrotic hepatocytes around the central vein, and massive fatty tissues. Interestingly, the hepatic sections of the mice treated with H-SMPS and E-SMPS showed obvious improvement compared to the mice in the MC group. In particular, the mice treated with a high dose of E-SMPS had hepatic sections similar to those from the NC group, indicating that H-SMPS and E-SMPS could protect liver tissue from acute alcohol-induced hepatic injury.

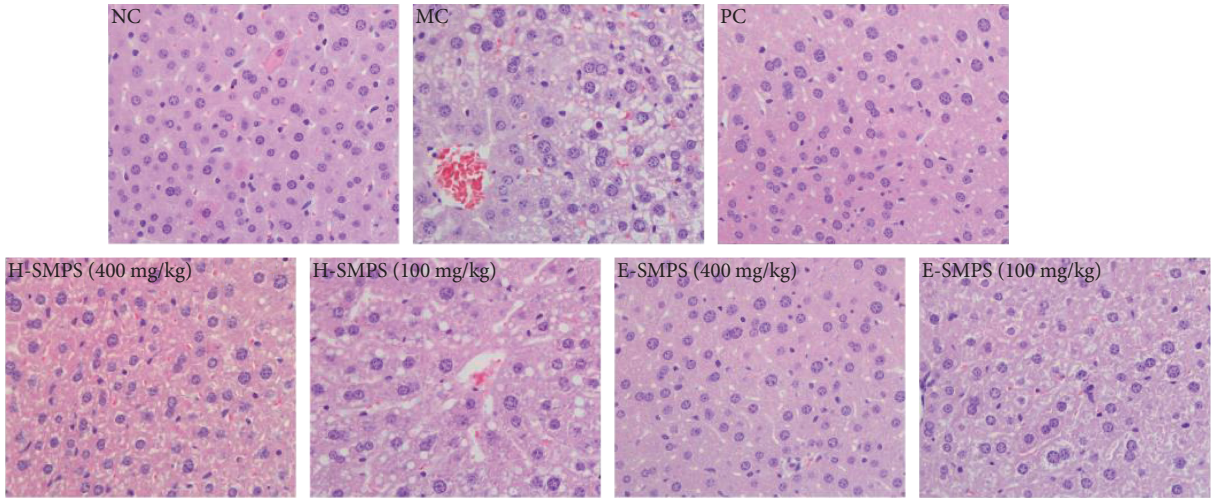

Figure 5: Effects of H-SMPS and E-SMPS on liver damages in acute alcohol-induced mice.

3.7. Antioxidant Activities In Vitro. The reducing power of H-SMPS and E-SMPS increased as the polysaccharide concentration increased from 0 to 800 μg/mL (Figure 6(a)). At a concentration of 800 μg/mL, the reducing power of E-SMPS (1.67 ± 0.08) was higher than those of H-SMPS (1.39 ± 0.06) and BHT (1.11 ± 0.09).

The scavenging activities of E-SMPS and H-SMPS against hydroxyl radicals were shown to be dose dependent (Figure 6(b)). At a concentration of 800 μg/mL, the hydroxyl radical scavenging activities of H-SMPS, E-SMPS, and BHT reached $74.10 \pm 4.11\%$, $86.6 \pm 4.52\%$, and $66.22 \pm 3.77\%$, respectively. Moreover, the IC_{50} values of H-SMPS, E-SMPS, and BHT were found to be 500.31 ± 2.70, 375.75 ± 2.58, and 564.64 ± 2.75 μg/mL, respectively, indicating that E-SMPS has stronger scavenging activities against hydroxyl radicals than H-SMPS does.

The color of DPPH solutions was reduced with the enhancement of antioxidant activities. H-SMPS and E-SMPS showed observable scavenging activities against DPPH radicals following an increase in the concentrations of the polysaccharides (Figure 6(c)). At a concentration of 800 μg/mL, the DPPH scavenging activity of E-SMPS reached $86.6 \pm 4.73\%$, which was higher than those of H-SMPS ($73.88 \pm 4.25\%$) and BHT ($61.27 \pm 3.41\%$). The IC_{50} values of H-SMPS (479.90 ± 2.68 μg/mL), E-SMPS (378.12 ± 2.58 μg/mL), and BHT (604.12 ± 2.78 μg/mL) were consistent with the above results.

The superoxide radical scavenging activities of H-SMPS, E-SMPS, and BHT are shown in Figure 6(d). A positive correlation between scavenging activity and concentration is seen. The scavenging activities of H-SMPS, E-SMPS, and BHT against superoxide radicals were $59.51 \pm 2.84\%$, $67.22 \pm 3.11\%$, and $48.40 \pm 2.91\%$, respectively. Furthermore, the IC_{50} values of H-SMPS, E-SMPS, and BHT were 674.35 ± 2.83, 553.09 ± 2.72, and 868.62 ± 2.94 μg/mL, respectively, indicating that E-SMPS displays stronger superoxide radical scavenging activity than H-SMPS and BHT.

3.8. Monosaccharide Composition Analysis. The monosaccharide compositions of H-SMPS and E-SMPS were determined by comparing their retention times with those of reference monosaccharides (Figure 7(a)). As seen in Figures 7(b) and 7(c), H-SMPS is composed of arabinose, xylose, mannose, galactose, and glucose, with mass percentages of 4.45%, 10.08%, 6.78%, 17.22%, and 61.37% (Figure 7(b)), respectively. However, E-SMPS was found to be composed of fucose, arabinose, xylose, mannose, galactose, and glucose, with mass percentages of 6.88%, 15.04%, 22.71%, 8.81%, 18.43%, and 28.13% (Figure 7(c)), respectively.

4. Discussion

In recent years, the use of polysaccharides from fungus as natural medicines has gained increasing attention owing to the lack of negative consequences in using them as treatments against many pathological diseases, as compared to chemical synthetic drugs. Several publications have established many polysaccharide extraction methods including hot water, alkaline, acidic, enzymatic, ultrasonic, and microwave extractions [28–32]. Increasing amounts of evidence have shown that enzymatic-extractable polysaccharides from edible mushrooms possess high antioxidant and biological activities. The use of these polysaccharides has many other advantages such as high extraction yields, reproducibility of results, low energy consumption, and low pollution [30, 33]. However, few reports on the exploration of the antioxidant and hepatoprotective activities of E-SMPS in alcohol-induced mice have been published.

As an organ that is sensitive to cytotoxicity during chemotherapy, the liver plays important roles in alcohol metabolism [6]. Acute alcohol consumption could induce the formation of poisonous metabolic products, causing abnormalities in alcohol metabolism, thereby leading to liver damage [34]. Clinically, serum enzymes (AST and ALT), hepatic lipids (TC and TG), and enzymes involved in alcohol metabolism (ADH, ALDH, and CYP2E1) are commonly used as biochemical markers for early diagnosis of hepatic injury. Serum ALT and AST activities are elevated when hepatic injury occurs since ALT and AST could leach out of hepatocytes into the blood circulation. This is associated with

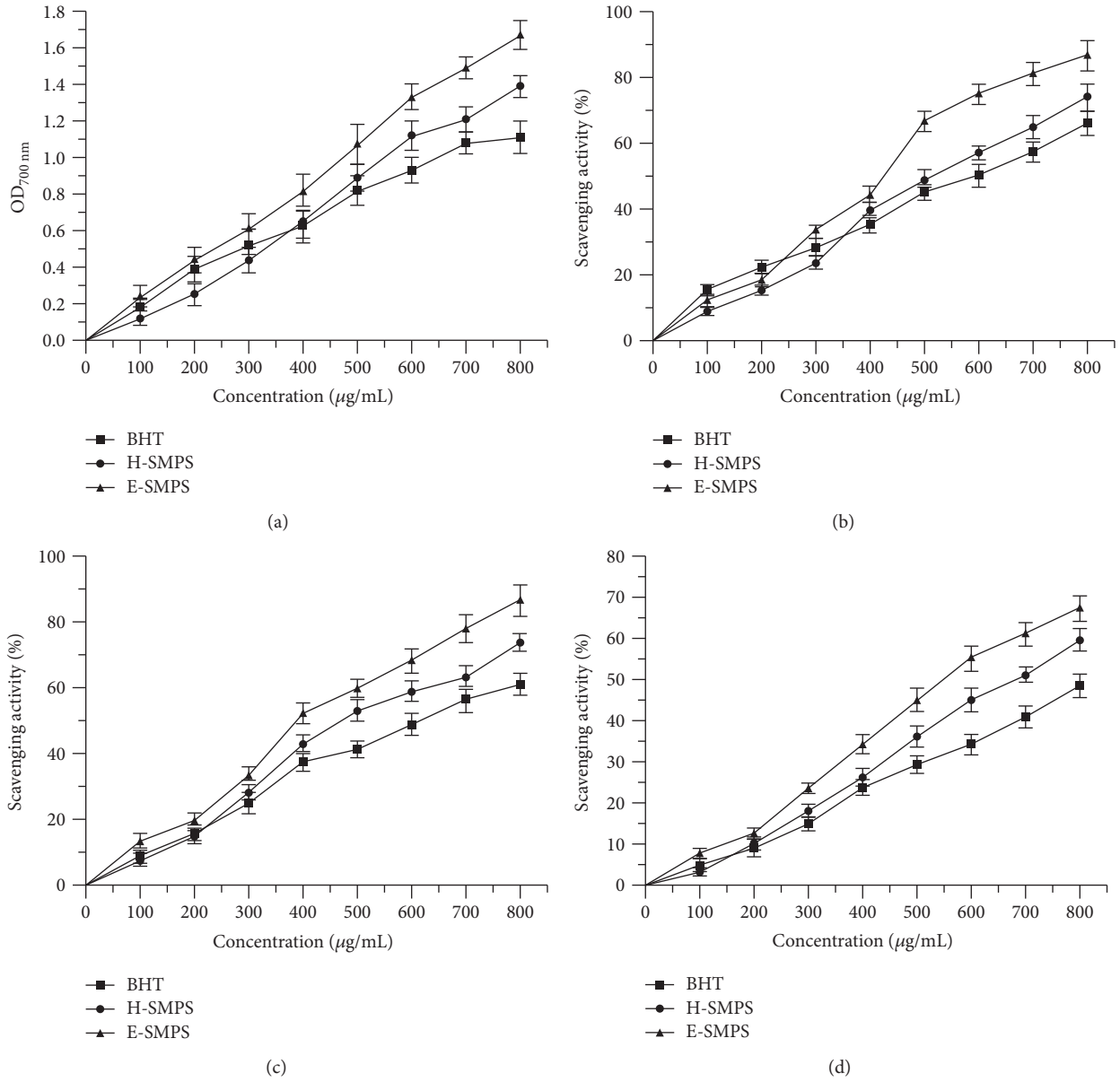

Figure 6: *In vitro* antioxidant activities of H-SMPS and E-SMPS. Reducing power (a) and scavenging activities on hydroxyl radicals (b), DPPH radicals (c), and superoxide anion radicals (d).

ballooning degeneration, massive centrilobular necrosis, and cellular infiltration [35, 36]. Furthermore, acute alcohol-induced lipid metabolism disorders, including accumulation of fat and lipochondrion on the surface of hepatocytes, could be reflected in abnormal changes of hepatic TC and TG levels. *In vivo*, alcohol metabolism mainly consists of two pathways, the alcohol dehydrogenase oxidation system and the microsome alcohol oxidation system, catalyzed by the metabolic enzymes ADH, ALDH, and CYP2E1 [6]. During alcohol consumption, about 90% of the alcohol can be metabolized into acetaldehyde by ADH, which is further metabolized into acetic acid by ALDH, and eventually decomposed into carbon dioxide and water. About 8–10% of alcohol is metabolized by CYP2E1 in the tricarboxylic acid cycle [37, 38]. The activities of ADH and ALDH, two crucial enzymes in regulating alcohol metabolism, could be remarkably suppressed when acute alcohol consumption occurs [5]. The activity of CYP2E1, an important metabolic enzyme in catalyzing exogenous and endogenous compounds that plays a role in hepatic alcohol metabolism, could be increased when alcohol accumulation activates the microsome alcohol oxidation system [34]. In the present study, the ALD mice in the MC group showed serious liver damage as evidenced by significant increases in serum enzyme activities (AST and ALT), hepatic CYP2E1 levels, and hepatic lipid levels (TC and TG), as well as decreases in hepatic ADH and ALDH activities, when compared with the mice in the NC group. The abnormal changes were remedied by the supplementation of H-SMPS and E-SMPS, indicating that polysaccharides from the SMS of *L. sulphureus* have potential

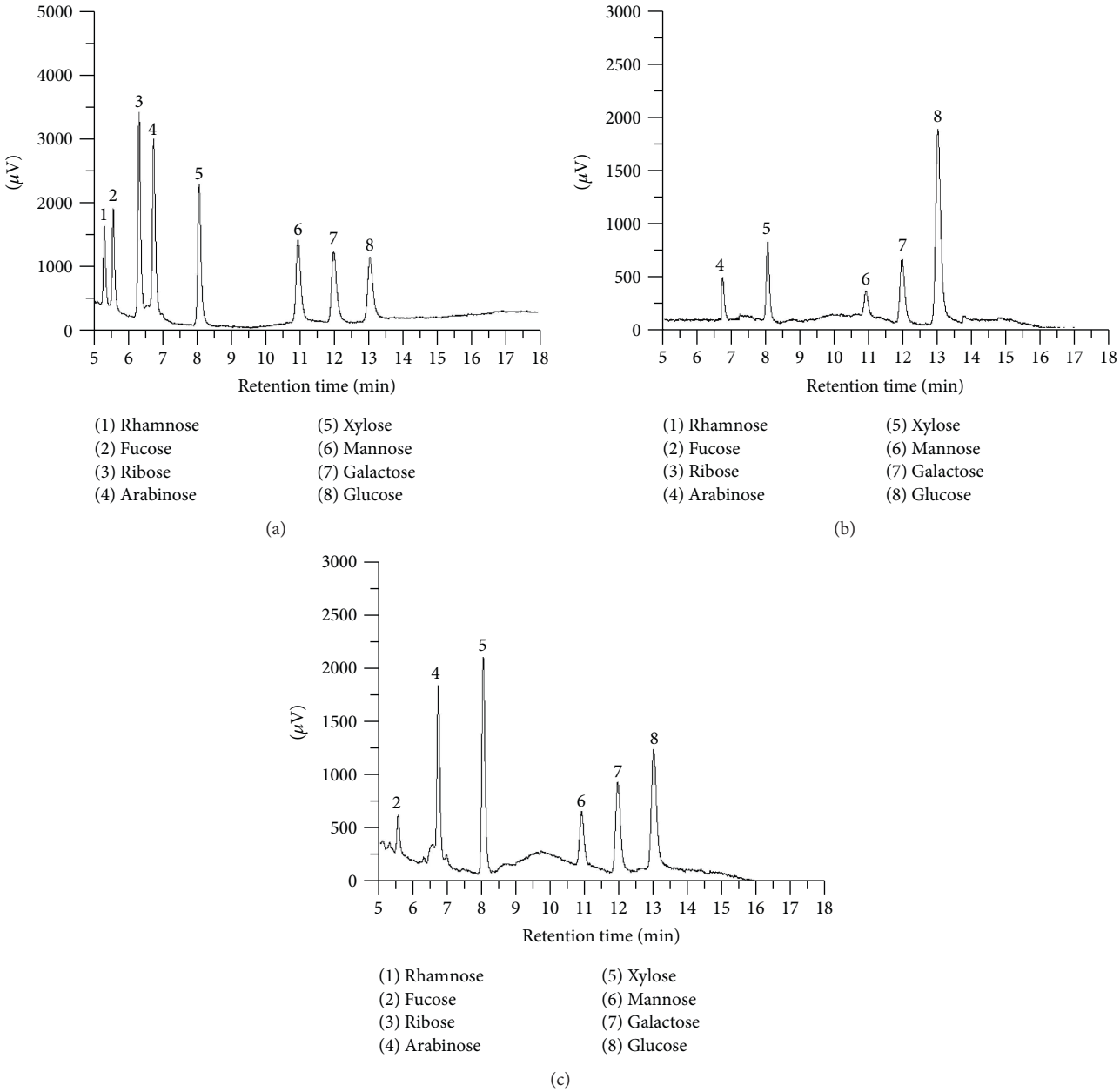

Figure 7: GC chromatograms of standard monosaccharides (a), H-SMPS (b), and E-SMPS (c).

protective effects against toxicity induced by acute alcohol consumption. Furthermore, the hepatoprotective effects of H-SMPS and E-SMPS were confirmed by observation of histopathological sections.

It has been reported that acute alcohol consumption can induce oxidative stress *in vivo*, leading to the overproduction of ROS. This plays an important role in the development of ALD due to the toxic effects of ROS on lipids, enzymes, nucleic acids, and proteins [39–41]. Hence, it seems necessary to increase the scavenging abilities against ROS. Previous literature has indicated that reducing power, which reflects the electron donation capacity of a compound, is one of the most important indicators for evaluating antioxidant activity [42]. The hydroxyl radicals, which are the major type of radicals, have been proven to be involved in the toxicity against biomolecules and in the induction of lipid peroxidation. Hence, there is great potential in discovering natural antioxidant agents with higher hydroxyl radical scavenging activities for the development of treatments against radical-induced diseases [43]. Scavenging activities against DPPH radicals, which are stable free radicals, are a wide indicator and allow a rapid method for assessing antioxidant activities [44]. Superoxide anion radicals, which are relatively weak oxidants, are one of the precursors of singlet oxygen and hydroxyl radicals and, as such, could indirectly activate lipid peroxidation and amplify cellular damage [45]. Based on the present results, both H-SMPS and E-SMPS showed significant reducing power and scavenging activities against

hydroxyl, DPPH, and superoxide anion radicals. Previous studies have proposed that antioxidant enzymes, including SOD, GSH-Px, and CAT, are the primary defense in eliminating ROS-induced oxidative stress *in vivo* [46]. The possible underlying mechanism may be the degradation of superoxides into H_2O_2 by SOD and the subsequent decomposition to O_2 and H_2O by CAT and GSH-Px, which results in the suppression of ROS generation [47]. Moreover, accumulated studies have proven that ALD is also connected with lipid peroxidation, which is also regarded as an indicator of oxidative damage [48, 49]. In our *in vivo* assays, significant decreases in SOD, GSH-Px, and CAT activities as well as remarkable increases in MDA and LPO levels were observed after alcohol consumption, indicating that liver function had been damaged and the antioxidant defense mechanism had been disabled [50]. The polysaccharide supplements significantly remedied the decreased enzyme activities and increased lipid peroxidation, demonstrating that both H-SMPS and E-SMPS have the potential to alleviate acute alcohol-induced liver damage.

The monosaccharide compositions of polysaccharides from *Catathelasma ventricosum* [46], *Lentinus edodes* [12], *T. albuminosus* [27], and *Flammulina velutipes* [14] were found to be different from the monosaccharide compositions of H-SMPS and E-SMPS from the SMS of *L. sulphureus* determined in the present study. This difference may be attributed to the differences in strains, culture methods, and extraction conditions used. Furthermore, previous studies have demonstrated that the biological functions of polysaccharides were mainly determined by their monosaccharide compositions [51]. Only fucose could be observed in E-SMPS, indicating that fucose may play an important role in conferring higher biological activities. Additionally, Schneider et al. also revealed that fucose plays a vital role in maintaining biological functions such as immunoregulation and anticancer activities in mammals [52].

5. Conclusions

The *in vitro* and *in vivo* analyses in this study demonstrate that both H-SMPS and E-SMPS confer effective hepatoprotection against acute alcohol-induced ALD, possibly by reducing oxidative stress. E-SMPS showed superior effects, indicating that enzymatic hydrolysis has a potential effect on enhancing bioactivities. The results may provide a mechanistic basis for the use of polysaccharides from *L. sulphureus* as a potential natural and functional food supplement for the prevention and alleviation of ALD and its complications.

Abbreviations

ADH: Alcohol dehydrogenase
ALT: Alanine aminotransferase
ALD: Alcoholic liver disease
ALDH: Aldehyde dehydrogenase
AST: Aspartate aminotransferase
BHT: Butylated hydroxytoluene
CAT: Catalase
CYP2E1: Cytochrome P4502E1
E-SMPS: Enzymatic-extractable polysaccharides
FID: Flame ionization detector
GSH-Px: Glutathione peroxidase
H-SMPS: Hot-water-extractable polysaccharides
LPO: Lipid peroxidation
MC: Model control
MDA: Malondialdehyde
NC: Normal control
PC: Positive control
ROS: Reactive oxygen species
SD: Standard deviations
SMS: Spent mushroom substrates
SOD: Superoxide dismutase
TC: Total cholesterol
TG: Triglyceride.

Conflicts of Interest

The authors declared no conflicts of interest.

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References

[1] J. Wang, Y. Zhang, R. Liu, X. Li, Y. Cui, and L. Qu, "Geniposide protects against acute alcohol-induced liver injury in mice *via* up-regulating the expression of the main antioxidant enzymes," *Canadian Journal of Physiology and Pharmacology*, vol. 93, no. 4, pp. 261–267, 2015.

[2] J. Altamirano and R. Bataller, "Alcoholic liver disease: pathogenesis and new targets for therapy," *Nature Reviews Gastroenterology & Hepatology*, vol. 8, no. 9, pp. 491–501, 2011.

[3] P. Mathurin and R. Bataller, "Trends in the management and burden of alcoholic liver disease," *Journal of Hepatology*, vol. 62, no. 1, pp. S38–S46, 2015.

[4] C. S. Lieber, "Alcohol and the liver: 1994 update," *Gastroenterology*, vol. 106, no. 4, pp. 1085–1105, 1994.

[5] C. S. Lieber, "Ethanol metabolism, cirrhosis and alcoholism," *Clinica Chimica Acta*, vol. 257, no. 1, pp. 59–84, 1997.

[6] D. C. Wen, X. Y. Hu, Y. Y. Wang et al., "Effects of aqueous extracts from *Panax ginseng* and *Hippophae rhamnoides* on acute alcohol intoxication: an experimental study using mouse model," *Journal of Ethnopharmacology*, vol. 192, pp. 67–73, 2016.

[7] Y. Y. Chen, C. L. Zhang, X. L. Zhao, K. Q. Xie, and T. Zeng, "Inhibition of cytochrome P4502E1 by chlormethiazole attenuated acute ethanol-induced fatty liver," *Chemico-Biological Interactions*, vol. 222, pp. 18–26, 2014.

[8] M. Jin, A. Ande, A. Kumar, and S. Kumar, "Regulation of cytochrome P4502e1 expression by ethanol: role of oxidative stress-mediated pkc/jnk/sp1 pathway," *Cell Death & Disease*, vol. 4, no. 3, article e554, 2013.

[9] D. Wu and A. I. Cederbaum, "Oxidative stress and alcoholic liver disease," *Seminars in Liver Disease*, vol. 29, no. 02, pp. 141–154, 2009.

[10] B. Gao and R. Bataller, "Alcoholic liver disease: pathogenesis and new therapeutic targets," *Gastroenterology*, vol. 141, no. 5, pp. 1572–1585, 2011.

[11] H. Wang, G. Li, W. Zhang, C. Han, X. Xu, and Y. P. Li, "The protective effect of *Agaricus blazei* murrill, submerged culture using the optimized medium composition, on alcohol-induced liver injury," *Biomed Research International*, vol. 2014, Article ID 573978, 9 pages, 2014.

[12] H. J. Zhu, K. Sheng, E. F. Yan, J. J. Qiao, and F. Lv, "Extraction, purification and antibacterial activities of a polysaccharide from spent mushroom substrate," *International Journal of Biological Macromolecules*, vol. 50, no. 3, pp. 840–843, 2012.

[13] C. Paredes, E. Medina, R. Moral et al., "Characterization of the different organic matter fractions of spent mushroom substrate," *Communications in Soil Science and Plant Analysis*, vol. 40, no. 1-6, pp. 150–161, 2009.

[14] L. Lin, F. Y. Cui, J. J. Zhang et al., "Antioxidative and renoprotective effects of residue polysaccharides from *Flammulina velutipes*," *Carbohydrate Polymers*, vol. 146, pp. 388–395, 2016.

[15] L. Wang, N. Xu, J. J. Zhang et al., "Antihyperlipidemic and hepatoprotective activities of residue polysaccharide from *Cordyceps militaris* SU-12," *Carbohydrate Polymers*, vol. 131, pp. 355–362, 2015.

[16] J. Petrović, J. Glamočlija, D. S. Stojković et al., "*Laetiporus sulphureus*, edible mushroom from Serbia: investigation on volatile compounds, *in vitro* antimicrobial activity and *in situ* control of *Aspergillus flavus* in tomato paste," *Food and Chemical Toxicology*, vol. 59, no. 8, pp. 297–302, 2013.

[17] R. G. Jayasooriya, C. H. Kang, M. J. Seo, Y. H. Choi, Y. K. Jeong, and G. Y. Kim, "Exopolysaccharide of *Laetiporus sulphureus* var. *miniatus* downregulates LPS-induced production of NO, PGE_2, and TNF-α in BV2 microglia cells *via* suppression of the NF-κB pathway," *Food and Chemical Toxicology*, vol. 49, no. 11, pp. 2758–2764, 2011.

[18] M. Y. Lung and W. Z. Huang, "Production, purification and tumor necrosis factor-α (TNF-α) release capability of exopolysaccharide from *Laetiporus sulphureus*, (Bulliard: Fries) Bondartsev & Singer in submerged cultures," *Process Biochemistry*, vol. 46, no. 2, pp. 433–439, 2011.

[19] M. Y. Lung and W. Z. Huang, "Antioxidant properties of polysaccharides from *Laetiporus sulphureus* in submerged cultures," *African Journal of Biotechnology*, vol. 11, no. 23, pp. 6350–6358, 2012.

[20] A. M. Staub, "Removal of proteins from polysaccharides methods," *Carbohydrate Chemistry*, vol. 5, no. 5, pp. 5-6, 1965.

[21] S. Song, S. Li, N. Su, J. Li, F. Shi, and M. Ye, "Structural characterization, molecular modification and hepatoprotective effect of melanin from *Lachnum* YM226 on acute alcohol-induced liver injury in mice," *Food and Function*, vol. 7, no. 8, pp. 3617–3627, 2016.

[22] M. Wang, P. Zhu, C. Jiang, L. Ma, Z. Zhang, and X. Zeng, "Preliminary characterization, antioxidant activity *in vitro* and hepatoprotective effect on acute alcohol-induced liver injury in mice of polysaccharides from the peduncles of *Hovenia dulcis*," *Food and Chemical Toxicology*, vol. 50, no. 9, pp. 2964–2970, 2012.

[23] M. Oyaizu, "Studies on products of browning reactions: antioxidative activities of products of browning reaction prepared from glucosamine," *Japanese Journal of Nutrition*, vol. 44, no. 6, pp. 307–315, 1986.

[24] C. C. Winterbourn and H. C. Button, "Hydroxyl radical production from hydrogen peroxide and enzymatically generated paraquat radicals: catalytic requirements and oxygen dependence," *Archives of Biochemistry and Biophysics*, vol. 235, no. 1, pp. 116–126, 1984.

[25] N. Cheng, N. Ren, H. Gao, X. Lei, J. Zheng, and W. Cao, "Antioxidant and hepatoprotective effects of *Schisandra chinensis* pollen extract on CCl_4-induced acute liver damage in mice," *Food and Chemical Toxicology*, vol. 55, no. 3, pp. 234–240, 2013.

[26] Q. Wei, X. H. Ma, S. S. Zhang, and S. C. Liu, "Antioxidant activities and chemical profiles of pyroligneous acids from walnut shell," *Journal of Analytical and Applied Pyrolysis*, vol. 88, no. 2, pp. 149–154, 2010.

[27] H. J. Zhao, S. S. Li, J. J. Zhang et al., "The antihyperlipidemic activities of enzymatic and acidic intracellular polysaccharides by *Termitomyces albuminosus*," *Carbohydrate Polymers*, vol. 151, pp. 1227–1234, 2016.

[28] L. Zheng, M. Liu, G. Y. Zhai, Z. Ma, L. Q. Wang, and L. Jia, "Antioxidant and anti-ageing activities of mycelia zinc polysaccharide from *Pholiota nameko* SW-03," *Journal of the Science of Food and Agriculture*, vol. 95, no. 15, pp. 3117–3126, 2015.

[29] G. L. Song and Q. Z. Du, "Structure characterization and antitumor activity of an α β-glucan polysaccharide from *Auricularia polytricha*," *Food Research International*, vol. 45, no. 1, pp. 381–387, 2012.

[30] J. J. Zhang, G. Y. Meng, C. Zhang et al., "The antioxidative effects of acidic-, alkalic-, and enzymatic-extractable mycelium zinc polysaccharides by *Pleurotus djamor* on liver and kidney of streptozocin-induced diabetic mice," *BMC Complementary and Alternative Medicine*, vol. 15, no. 1, pp. 440–452, 2015.

[31] J. Tang and Y. Q. Ma, "The application of ultrasonic technology in the *Auricularia auricula* polysaccharide extractions," *Food and Machinery*, vol. 21, no. 1, pp. 28-29, 2005.

[32] L. S. Fan, S. H. Zhang, and X. G. Wu, "Studies on microwave assisted extractions of *Auricularia auricula* polysaccharides," *Food and Fermentation Industries*, vol. 31, no. 10, pp. 142–144, 2005.

[33] N. Xu, Z. Gao, J. Zhang et al., "Hepatoprotection of enzymatic-extractable mycelia zinc polysaccharides by *Pleurotus eryngii* var. *tuoliensis*," *Carbohydrate Polymers*, vol. 157, pp. 196–206, 2017.

[34] R. Brůha, K. Dvorák, M. Dousa, J. Petrtýl, and T. Svestka, "Alcoholic liver disease," *Prague Medical Report*, vol. 110, no. 3, pp. 181–190, 2009.

[35] J. Ozer, M. Ratner, M. Shaw, W. Bailey, and S. Schomaker, "The current state of serum biomarkers of hepatotoxicity," *Toxicology*, vol. 245, no. 3, pp. 194–205, 2008.

[36] G. J. Huang, J. S. Deng, S. S. Huang, Y. Y. Shao, C. C. Chen, and Y. H. Kuo, "Protective effect of antrosterol from *Antrodia camphorata* submerged whole broth against carbon tetrachloride-induced acute liver injury in mice," *Food Chemistry*, vol. 132, no. 2, pp. 709–716, 2012.

[37] C. S. Lieber, "Hepatic, metabolic and toxic effects of ethanol: 1991 update," *Alcoholism Clinical and Experimental Research*, vol. 15, no. 4, pp. 573–592, 1991.

[38] A. I. Cederbaum, "Alcohol metabolism," *Clinics in Liver Disease*, vol. 16, no. 4, pp. 667–685, 2012.

[39] J. G. O'Grady and R. Williams, "Classification of acute liver failure," *Lancet*, vol. 342, no. 8873, p. 743, 1993.

[40] R. S. O'Shea, S. Dasarathy, and A. J. McCullough, "Alcoholic liver disease," *Hepatology*, vol. 51, no. 1, pp. 307–328, 2010.

[41] C. Zhang, Z. Gao, C. L. Hu et al., "Antioxidant, antibacterial and anti-aging activities of intracellular zinc polysaccharides from *Grifola frondosa* SH-05," *International Journal of Biological Macromolecules*, vol. 95, pp. 778–787, 2016.

[42] J. Liu, J. Luo, H. Ye, and X. Zeng, "Preparation, antioxidant and antitumor activities *in vitro* of different derivatives of levan from endophytic bacterium *Paenibacillus polymyxa* EJS-3," *Food and Chemical Toxicology*, vol. 50, no. 3-4, pp. 767–772, 2012.

[43] B. Halliwell and J. M. C. Gutteridge, "Role of free radicals and catalytic metal ions in human disease: an overview," *Methods in Enzymology*, vol. 186, no. 1, pp. 1–85, 1990.

[44] C. L. Ke, D. L. Qiao, D. Gan, Y. Sun, H. Ye, and X. X. Zeng, "Antioxidant activity in vitro and in vivo of the capsule polysaccharides from *Streptococcus equi* subsp. *zooepidemicus*," *Carbohydrate Polymers*, vol. 75, no. 4, pp. 677–682, 2009.

[45] X. Liu, B. Zhou, R. Lin et al., "Extraction and antioxidant activities of intracellular polysaccharide from *Pleurotus* sp. mycelium," *International Journal of Biological Macromolecules*, vol. 47, no. 2, pp. 116–119, 2010.

[46] Y. Liu, J. Sun, S. Rao, Y. Su, and Y. Yang, "Antihyperglycemic, antihyperlipidemic and antioxidant activities of polysaccharides from *Catathelasma ventricosum* in streptozotocin-induced diabetic mice," *Food and Chemical Toxicology*, vol. 57, no. 12, pp. 39–45, 2013.

[47] D. Yao, W. Shi, Y. Gou et al., "Fatty acid-mediated intracellular iron translocation: a synergistic mechanism of oxidative injury," *Free Radical Biology & Medicine*, vol. 39, no. 10, pp. 1385–1398, 2005.

[48] V. Vande, J. Sedman, and A. Russin, "Lipid analysis by vibrational spectroscopy," *European Journal of Lipid Science Technology*, vol. 103, pp. 15–25, 2001.

[49] Y. Cui, Q. Ye, H. Wang, Y. Li, W. Yao, and H. Qian, "Hepatoprotective potential of *Aloe vera* polysaccharides against chronic alcohol-induced hepatotoxicity in mice," *Journal of the Science of Food and Agriculture*, vol. 94, no. 9, pp. 1764–1771, 2014.

[50] Y. F. Sun, X. B. Yang, X. S. Lu, D. Y. Wang, and Y. Zhao, "Protective effects of Keemun black tea polysaccharides on acute carbon tetrachloride-caused oxidative hepatotoxicity in mice," *Food and Chemical Toxicology*, vol. 58, pp. 184–192, 2013.

[51] Q. Han, Q. Yu, J. Shi, C. Xiong, Z. Ling, and P. He, "Structural characterization and antioxidant activities of 2 water-soluble polysaccharide fractions purified from tea (*Camellia sinensis*) flower," *Journal of Food Science*, vol. 76, no. 3, pp. C462–C471, 2011.

[52] M. Schneider, E. Al-Shareffi, and R. S. Haltiwanger, "Biological functions of fucose in mammals," *Glycobiology*, vol. 27, no. 7, pp. 601–618, 2017.

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Research Article

Radical Scavenger Capacity of Jabuticaba Fruit (*Myrciaria cauliflora*) and Its Biological Effects in Hypertensive Rats

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Jabuticaba is an exotic fruit native to Brazil that has been arousing medicinal interest. Using chemical (HPLC-PDA, resonance mass spectra, and NMR), electroanalytical (differential pulse voltammetry, radical scavenging assay), and pharmacological (*in vivo* and *in vitro*) approaches, we have identified its bioactive compounds and hypotensive effects on hypertensive rats. The hydroalcoholic extract of jabuticaba (HEJ) presents a great quantity of phenolic compounds, and several molecules with hydroxyl groups present high efficiency as an antioxidant. The treatment with HEJ (100 and 300 mg/kg/day, for four weeks) presented hypotensive effects on L-NAME-induced hypertensive rats, possibly improving the nitric oxide bioavailability because of its high antioxidant potential. Furthermore, renal and cardiac hypertrophies were also attenuated after the HEJ treatment. Moreover, the vascular responses to contractile and dilating agonists were improved with the HEJ treatment, which is also able to induce nitric oxide production in endothelial cells.

1. Introduction

Hypertension is the main risk factor for the development of cardiovascular diseases, accounting for approximately 10 million deaths per year [1]. To control vascular tone and consequently regulate blood pressure, the endothelium produces several substances with vasodilatory action. The main one is nitric oxide (NO), produced through the reaction catalyzed by the enzyme NO synthase (NOS). NO controls various physiological and pathological processes in the cardiovascular system, in addition to contributing directly to the regulation of blood pressure [2]. Any inhibition of NO synthesis or bioactivity impairs vascular relaxation and blood pressure control, leading to systemic hypertension. In experimental models, NO levels can be suppressed using NOS inhibitors (such as L-NAME), which cause systemic hypertension comparable to hypertension observed in humans, causing several cardiovascular changes and increasing reactive oxygen species (ROS) [3].

The use of natural substances has been gaining more attention in the treatment of cardiovascular diseases, and several plant species have shown important hypotensive results with few adverse effects [4, 5]. Jabuticaba is a native Brazilian fruit (Myrtaceae) that has the same shape and size as grapes and a color that ranges from a deep purple to black. Like grapes, jabuticaba also perishes quickly; hence,

it must be consumed shortly after harvest. This fruit is very popular in Brazil and is often used in the production of jams, ice creams, juices, and alcoholic beverages, which have been increasing in consumption in Brazil and abroad [6, 7]. It has a pleasant flavor, and its taste is sweet with a slightly sour accent. In addition to its use as a food and beverage, jabuticaba is used in folk medicine (mainly in south Brazil) for its antioxidant benefits and for treating spasmodic vasomotor disturbances [8, 9].

Several phenolic compounds, flavonoids, and anthocyanins are present in the peel of the fruit, which may be responsible for a number of biological effects of this species and has been of great medicinal interest [10, 11]. These compounds are capable of complexing free radicals and inhibiting the initiation of the chain of propagation of oxidative reactions, including lipid peroxidation [12].

Our research group has shown important effects of this species on the cardiovascular system *in vitro*, such as the vascular dilatatory effect acting on the endothelial cells of the vessels [13] or a direct relaxing effect on vascular smooth muscle, activating K^+ channels and inhibiting Ca^{2+} influx [14]. In this work, we chemically studied the electroanalytical profile of this species and the antioxidant capacities and effects of chronic treatment with jabuticaba berry in hypertensive animals to associate the cardiovascular effects with the typical phytochemistry groups present in jabuticaba.

2. Materials and Methods

2.1. Plant Material and Preparation of Extract. Mature jabuticaba fruits (*Myrciaria cauliflora* (Mart.) O. Berg) were donated by "Jaboticabal" winery in the city of Hidrolândia, GO, Brazil (16°57′57″S and 49°13′35″W). A voucher specimen (number 21140) has been deposited in the herbarium of the ICB/UFG Botany Department. The seedless fruits were air-dried (40°C), pulverized in a knife mill, and passed through a 60-mesh sieve in the Laboratory of Natural Products Research, Faculty of Pharmacy, Federal University of Goiás. The obtained powder product underwent exhaustive percolation with ethanol : water (55 : 45, *v*/*v*). The extract obtained was filtered and evaporated under reduced pressure in a rotary evaporator at 40°C to provide the hydroalcoholic extract of jabuticaba (HEJ) with a yield of ~8.56%. The HEJ was dissolved in distilled water at a concentration of 120 mg/mL and stored at −20°C without contact with the clarity until the experiments.

2.2. Phytochemical Analyses. For HPLC-PDA analyses of the HEJ, ellagic acid was used as the internal phytochemical standard [6, 15]. These analyses were carried out using the Waters LC system (Milford, Massachusetts, USA) comprising a quaternary pump, an online degasser, an autosampler, and a photodiode array detector model 2998. Empower 2.0 software was used for the control of the HPLC equipment and for the acquisition and treatment of data. Chromatographic separation was carried out with a C18 reverse phase column (250 × 4.6 mm, 5 μm) purchased from Phenomenex (Phenomenex Inc., Torrance, CA, USA). The detection wavelength was 252 nm at a flow rate of 0.5 mL/min at 25°C in 10 min (mobile phase was composed of methanol : water (60 : 40, *v*/*v*)).

A colorimetric test was carried out to verify the total phenolic compounds in HEJ, using ferric chloride in aqueous extract solution under alkaline conditions to result in a colored complex with phenolic compounds, read at 510 nm [16].

In order to elucidate the compounds present in the extract, the HEJ was analyzed by ESI FT-ICR mass spectra. Briefly, the HEJ sample was diluted to ≈0.25 mg/mL in water/methanol (1 : 1, *v*/*v*) which contained 0.1% (*m*/*v*) of NH_4OH for ESI in a negative mode. The resulting solution was directly infused at a flow rate of 3 μL/min into the ESI source. The mass spectrometer (model 9.4T Solarix, Bruker Daltonics, Bremen, Germany) was set to operate over a mass range of *m*/*z* 150–2000. The ESI source conditions were as follows: a nebulizer gas pressure of 3 bar, a capillary voltage of 3.5 kV, and a transfer capillary temperature of 250°C. The ions are accumulated in the hexapolar collision cell with a time of 5.10^{-3} s followed by transport to the analyzer cell (ICR) through the multipole ion guide system (another hexapole). The time of flight in the hexapole was 0.5 ms. Each spectrum was acquired by accumulating 200 scans of time-domain transient signals in four megapoint time-domain data sets. All mass spectra were externally calibrated using sodium trifluoroacetate solutions (*m*/*z* from 200 to 2000). A resolving power, $m/\Delta m50\% \cong 730{,}000$, in which $\Delta m50\%$ is the full peak width at the half-maximum peak height, of *m*/*z* 6400 and a mass accuracy of <1 ppm provided the unambiguous molecular formula assignments for singly charged molecular ions. The mass spectra were acquired and processed using data analysis software (Bruker Daltonics, Bremen, Germany). The MS data were processed, and the elemental compositions of the compounds were determined by measuring the *m*/*z* values. The proposed structures for each formula were assigned using the ChemSpider (http://www.chemspider.com) database.

Nuclear magnetic resonance (NMR) analyses were also performed. All ^1H NMR analyses were performed on a Bruker Avance III 500 11.75 Tesla spectrometer, at 298 K, using a 5 mm inverse probe head. The spectra were obtained at 500.13 MHz for ^1H, using lyophilized HEJ and a solution of 600 μL of D_2O and 0.1% sodium-3-trimethylsilylpropionate (TMSP-2,2,3,3-d4) (*m*/*v*). D_2O/TMSP was used as field frequency lock and internal standard. Sixty-four pulses were employed on the acquisition of the spectra, with an acquisition time of 3.28 s, a spectral width of 10,000 Hz, and a relaxation delay of 1 s. The NOESYGPPR1D sequence was applied for water signal suppression, with a mixing time of 150 ms. For quantitative analysis, 0.3 μL of dimethylformamide, 600 μL of D_2O/TMSP solution, and 18.6 mg of sample were used. The experiments were done in triplicate. The signal area of DMF was measured and compared with those of the compounds in the extract to determine the absolute concentrations of those compounds. The relaxation delay was set to five times the value of the longest spin–lattice relaxation time T1 of the integrated resonance signals in order to ensure full relaxation of the corresponding protons.

2.3. Antioxidant Activity

2.3.1. Electroanalytical Assay (Differential Pulse Voltammetry (DPV)). These experiments were carried out according to previously standardized methods [17]. Voltammetric experiments were performed in a potentiostat/galvanostat μAutolab III® integrated to the GPES 4.9® software (Eco Chemie, Utrecht, The Netherlands). Measurements were performed using 50 μL of HEMC (120 mg/mL) in 0.1 M phosphate buffer solution (pH 6.0) in a 5.0 mL one-compartment electrochemical cell, with a three-electrode system consisting of a carbon paste electrode (prepared as a piston-driven holder containing 70% of graphite powder and 30% of purified mineral oil, $\varnothing = 2$ mm), a Pt wire, and the $Ag/AgCl/KCl_{sat}$ (both purchased from Analyser, São Paulo, Brazil), representing the working electrode, the counter electrode, and the reference electrode, respectively. The surface of the carbon paste electrode was mechanically renewed before the start of a new set of experiments by extruding approximately 0.5 mm of carbon paste out of the electrode holder and smoothing it with a filter paper. This procedure ensured very reproducible experimental results.

The experimental conditions for differential pulse voltammetry (DPV) were as follows: pulse amplitude, 50 mV; pulse width, 0.4 s; and scan rate, 5 $mV \cdot s^{-1}$. All experiments were done at room temperature ($21 \pm 1^{\circ}C$) in triplicate ($n = 3$) and treated with the software Origin 8®.

2.3.2. DPPH Radical Scavenging Assay. The antioxidant capacity of different extracts was evaluated by means of the conversion (decolorization) of stable 1,1-diphenyl-2-picrylhydrazyl (DPPH·) radical into its reduced form (DPPH) in accordance with the procedure revised and described by Lino et al. [17]. Briefly, a reaction solution consisting of 2.5 mL of 0.1 mM DPPH ethanolic solution was mixed to an aliquot of 0.5 mL of ethanol in order to reach A~0.7 at $\lambda = 517$ nm, whereas the ethanol, the solvent used to prepare all solutions, was used in order to adjust the baseline ($A = 0.000$). In turn, the same amount of sample and standard ethanolic solutions were added to other reaction systems, and the antioxidant activity was expressed as gallic acid equivalents.

2.4. Animals. Male Wistar rats (200–230 g) from the Central Animal House, at the Federal University of Goiás, were used for the experiments. The animals were housed in a temperature- and light-controlled room ($22 \pm 2^{\circ}C$; 12 h light/dark cycle) with free access to water and rodent chow and acclimatized for a period of at least one week before starting the experiment. They were handled in accordance with the internationally accepted standard guidelines for the use of animals. All procedures were approved by the Animal Research Ethics Committee at the Federal University of Goiás, Goiânia, Brazil (protocol: 015/2014).

The hypertension was induced by oral administration with L-NAME (60 mg/kg/day in drinking water) for six weeks. HEJ was administered orally (100 or 300 mg/kg/day by gavage in distilled water) starting at the second week along with oral treatment with L-NAME until the end of the sixth week. The control group received only drinking water and vehicle (distilled water). The rats were weighed weekly, and the systolic blood pressure (SBP) and heart rate (beats per min (bpm)) were also measured once a week using noninvasive tail-cuff plethysmography (Panlab Harvard Apparatus, Barcelona, Spain).

2.5. Studies in Isolated Arteries. Following the hemodynamic measurements in the sixth week, rats were anaesthetized (inhaled isoflurane) and killed by cervical dislocation. The heart and left kidney were isolated and weighed in relation to the corporal weight. The thoracic aorta was isolated, cleaned, and cut into rings approximately 4 mm in length; placed in an organ bath between two stainless-steel stirrups; and connected to a computerized system and a WinDaq Resource (DATAQ Instruments, Akron, OH, USA) data acquisition unit to measure isometric tension in the preparations. The aortic rings were placed in a 10 mL organ chamber containing a Krebs solution of the following compositions: 130 mM NaCl, 4.7 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 14.9 mM $NaHCO_3$, 5.5 mM glucose, and 1.6 mM $CaCl_2$ (pH 7.4), at 36°C and gassed with 95% O_2 and 5% CO_2. The rings were initially stretched to a basal tension of 1.5 g before allowing them to equilibrate in the bathing medium. Each rat supplied only one aortic ring for different protocols.

In some preparations, the endothelial cells were mechanically removed by rubbing the internal artery surface with a fine metallic wire and the effectiveness of the removal was demonstrated by the absence of relaxation to acetylcholine (ACh) (1 μM) after being precontracted with phenylephrine (Phe) (0.1 μM). For studies in preparations with the endothelium, the rings were discarded when the relaxation to ACh was less than 80%. Each ring was sequentially washed, reequilibrated, and then left to relax. Again, the aortic rings with or without the endothelium were contracted with Phe (0.1 μM), and cumulative concentration-response curves for ACh (0.1 nM to 10 μM) or sodium nitroprusside (SNP) (0.01 nM to 1 μM) were carried out. In another series of experiments, cumulative concentration-response curves were carried out for phenylephrine (Phe) (0.1 nM a 10 μM) in aortic rings without the endothelium.

2.6. Evaluation of Cytosolic Nitric Oxide ($[NO]_c$) Levels in Endothelial Cells. After isolation, the arteries were sectioned longitudinally and the endothelial cells were mechanically removed from the vessels by gentle rubbing with a plastic rod in Hanks solution of the following compositions (composition in mmol/L): $CaCl_2$ (1.6), $MgSO_4$ (1.0), NaCl (145.0), KCl (5.0), NaH_2PO_4 (0.5), dextrose (10.0), and HEPES (10.0), maintained at pH 7.4 and constant temperature of $37 \pm 1^{\circ}C$. The endothelial cells (1×10^6 cells/mL) were divided in polypropylene tubes, centrifuged at 1500 rpm for 5 minutes, suspended in 500 μL of Hanks solution containing 10 μM DAF-2/DA (specific fluorescent dye for NO detection), and incubated in a humidified atmosphere of 5% CO_2 in air at 37°C for 20 minutes. After incubation, the cells were treated with HEJ (EC_{50}), Ach (EC_{50}), or distilled water in the same volume and incubated again for 5 minutes. Then, $[NO]_c$ levels were quantified by a

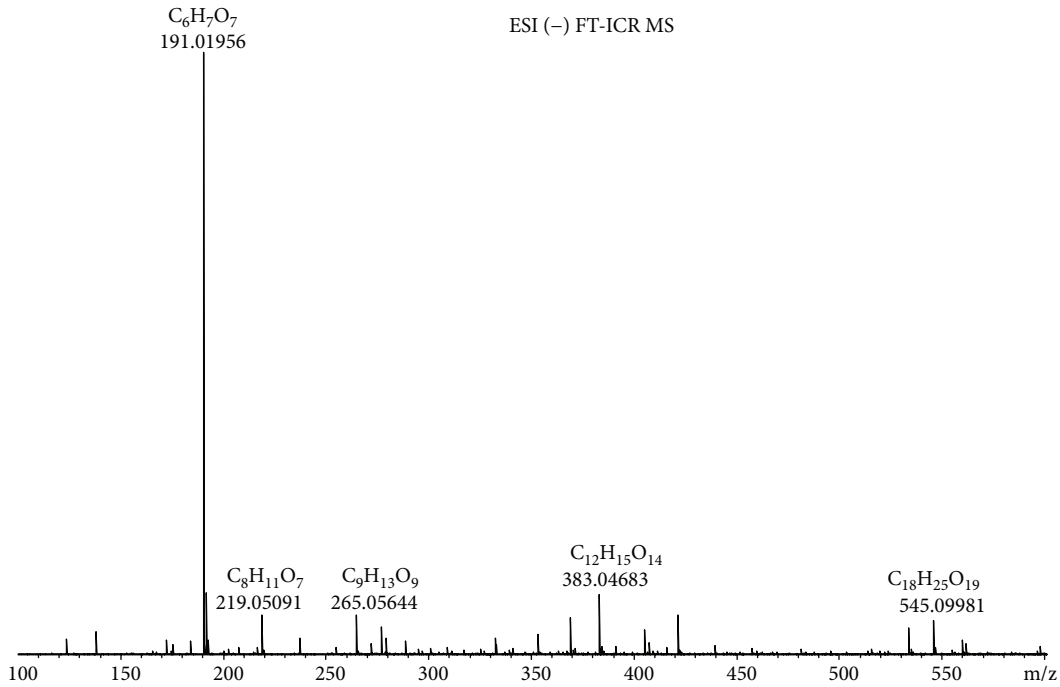


Figure 1: ESI(−) FT-ICR MS mass spectrum of the hydroalcoholic extract of jabuticaba (HEJ). The peaks show the phenolic compounds present in the jabuticaba extract. For peak identification, the molecular structure can be seen in Table 1.

flow cytometer (BD FACSCanto II, Biosciences, USA) recording 10,000 events for each five independent analyses.

2.7. Reagents. All chemicals of reagent grade (acetylcholine, ellagic acid, L-NAME, phenylephrine, and sodium nitroprusside) were obtained from Sigma (Sigma-Aldrich Inc., St. Louis, MO, USA). All other chemicals used in the present study were commercially available and of reagent grade. The purity of all substances was >97%. The concentrations given are the final concentrations in the bath solution.

2.8. Statistical Analysis. In the graphics, the data are presented as means ± SEM. The statistical analysis was performed using GraphPad Prism version 5.0 (GraphPad Software Corporation). Comparisons among groups were performed using ANOVA (post hoc test: Newman–Keuls), and values of $p < 0.05$ were considered to be significant.

3. Results and Discussion

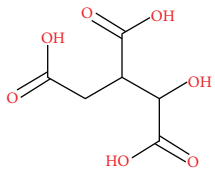
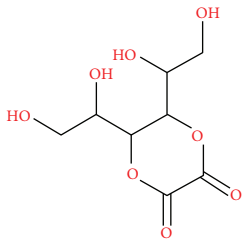
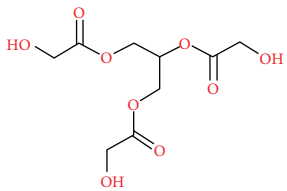
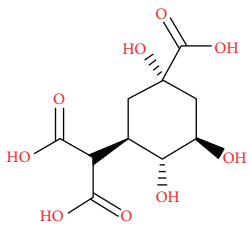
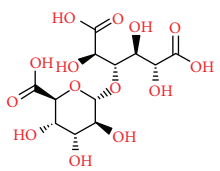
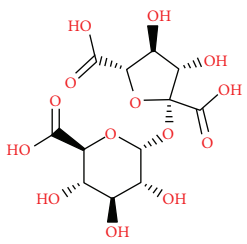
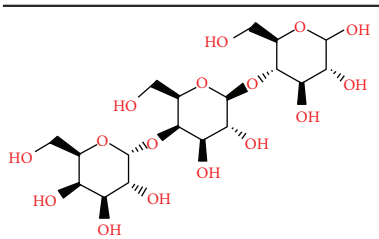
3.1. Chemical Characterization of Extract. The concentration of total phenolic compounds in the HEJ (calculated on the total solid content in the extract) was 17.85%. In the HPLC analyses, HEJ showed the presence of ellagic acid (Rt: 7.826 min). The level of ellagic acid (chemical marker) found in HEJ was 0.23% (*w*/*w*), identified by comparison with external standard (Sigma-Aldrich Co., St. Louis, MO, USA). The ellagitannins present in foods change to free ellagic acid and its derivatives during digestion [18]. Ellagic acid has a high efficiency as an antioxidant compound due to the presence of several hydroxyl groups, which are responsible for the strong potential to donate a hydrogen atom and support the unpaired electron [19]. According to literature, the content of total ellagic acid in jabuticaba fruits (*M. cauliflora*) varied from 0.0215% to 0.311% (*w*/*w*). The level of ellagic acid found in HEJ was 0.23% (*w*/*w*); thus, the concentration of this compound in the HEJ is in agreement with the content in fruits [6]. There has been an increasing interest in this chemical marker due to its powerful antioxidant activities and other properties such as cardioprotective effects and angiotensin-converting enzymes [18, 20].

We acquired the ESI(−) FT-ICR mass spectra of the jabuticaba berry extract (Figure 1). The ESI(−) FT-ICR mass spectra shows that the ions of *m*/*z* 191.01956, 219.05091, 265.05644, 383.04683, and 533.13619 are the most abundant species, detected as sodium adducts: $[C_6H_8O_7\text{-}H]^-$, $[C_8H_{12}O_8\text{-}H]^-$, $[C_9H_{14}O_9\text{-}H]^-$, $[C_{12}H_{16}O_{14}\text{-}H]^-$, and $[C_{18}H_{30}O_{18}\text{-}H]^-$, respectively. The majority compounds and derivatives in the HEJ were revealed by ESI(−) FT-ICR mass spectrometry as illustrated in Table 1.

In the one-dimensional spectrum, the NMR analyses identified five groups of signals representing phenolic compounds (200 μg/mL), like anthocyanins, tannins, flavonoids, and phenolic acids. Coumarins were also observed in a very similar concentration (~200 μg/mL). Other compounds were identified in the spectrum: rhamnose (390 μg/mL), cinnamic acid (40 μg/mL), fructose (170 μg/mL), glycerol (2.17 mg/mL), lactate (120 μg/mL), α,β-glucose (140 μg/mL), and succinic acid (1.11 mg/mL).

We introduce the ESI(−) Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) combined with NMR to characterize HEJ without prior extraction or separation. FT-ICR MS routinely provides ultrahigh mass resolving power, $m/\Delta m50\% > 400{,}000$, and mass accuracy better than

Table 1: Elemental compositions assigned to peaks in the negative-ion ESI FT-ICR mass spectrum of the hydroalcoholic extract of jabuticaba (HEJ).

Possible structures	Measured *m/z*	Molecular formula	Error (ppm)
	191.01956	$[C_6H_8O_7\text{-}H]^-$	0.87
	219.05091	$[C_8H_{12}O_8\text{-}H]^-$	0.55
	265.05644	$[C_9H_{14}O_9\text{-}H]^-$	0.26
	277.05492	$[C_{10}H_{14}O_9\text{-}H]^-$	0.86
	369.06751	$[C_{12}H_{18}O_{14}\text{-}H]^-$	0.14
	383.04683	$[C_{12}H_{16}O_{14}\text{-}H]^-$	0.28
	533.13619	$[C_{18}H_{30}O_{18}\text{-}H]^-$	0.48

1 ppm. These high specifications mean that FT-ICR is ideal for analyzing complex mixtures [21]. Moreover, it becomes possible to assign molecular formulas (CcHhNnOoSs) unambiguously by mass measurement from singly charged ions such as $[M+H]^+$, $[M+Na]^+$, or $[M-H]^-$, where "M" corresponds to neutral molecules. Furthermore, NMR methods provide information on a wide range of compounds present in the HEJ matrix in a single experiment, offering advantages in terms of simplicity of sample preparation and brevity of analyses while providing quantitative information about the compounds detected in the jabuticaba berry extract. This study demonstrates that HEJ contain high concentration of phenol compounds and possess high-quality antioxidant properties.

3.2. Antioxidant Activity. From the DPV, it was possible to observe the presence of electroactive compounds in the HEJ, which presented three oxidation peaks, 1a, 2a, and 3a, at $E_{p1a} \pm 0.33$ V, $E_{p2a} \pm 0.45$ V, and $E_{p3a} \pm 0.71$ V (Figure 2). It is well established that peaks below 0.5 V (pH 5.0) are related to compounds presenting high reducing power [17, 22].

Moreover, the high radical scavenging power of HEJ was also evidenced by the DPPH assay, in which it was found that each milligram of the HEJ present the antioxidant power equivalent to 7.08 mg gallic acid.

Antiradical compounds are normally reduced in living organisms at 0.8 V. Therefore, electroactive compounds exhibiting peak potentials lower than 0.7 V (pH 7.0) will be able to scavenge the free radicals. Furthermore, since ascorbic acid and tocopherol present reduction potential below 0.5 V (pH 7.0), the jabuticaba extract could restore such endogenous antioxidants [22, 23]. As antioxidants are electroactive compounds, the electroanalytical methods are indicated and very suitable to their study. It has been demonstrated that the HEJ present high antioxidant potential.

3.3. Cardiovascular Assessment and Body Weight. Before the experiments, the baseline SBP and body weight were similar in the four experimental groups. The L-NAME administration induced a rapid gradual rise in SBP, reaching 225.8 ± 11.2 mmHg in the sixth week (Figure 3, Table 2). In the L-NAME + HEJ- (100 and 300 mg/kg) treated group, SBP values were determined to decrease significantly at the fourth week of HEJ treatment in comparison with the those in the group receiving L-NAME alone. Alternatively, in the control group, SBP values remained stable all through the six-week period. No significant differences in body weight were observed among the experimental groups. Another condition closely related to hypertension is oxidative stress, which plays an important role in the pathogenesis of hypertension [24, 25]. The reactive oxygen species (ROS) react with NO, decreasing its bioavailability. It is probable that treatment with HEJ, which showed a high antioxidant capacity, could neutralize the ROS and increase the NO bioavailability, attenuating hypertension. Another possibility is the direct relaxing effect of the HEJ on vascular smooth muscle (as shown by our research group) [14], which could reduce the peripheral vascular resistance and, consequently, the blood pressure.

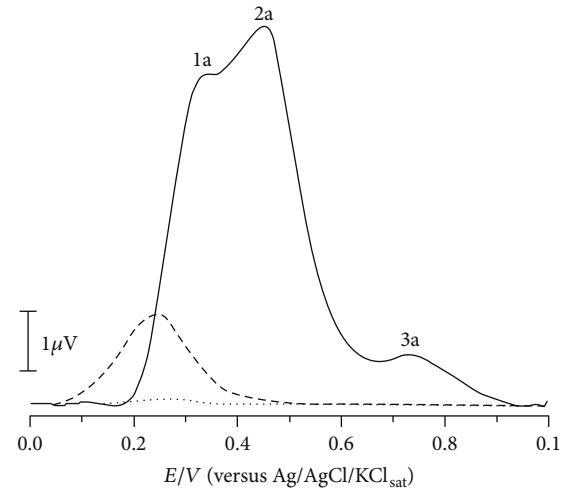


Figure 2: Average DP voltammograms obtained for 50 μL of HEJ (12%; continuous line), standard ascorbic acid (positive control, 10 μM; dashed line), and blank (dotted line) in 5 mL of 0.1 M phosphate buffer (pH 6.0) solution characterized at carbon paste electrodes ($\varnothing = 2$ mm). Other parameters included a pulse width of 5 mV, a pulse amplitude of 50 mV, and a scan rate of 5 mV·s^{-1}.

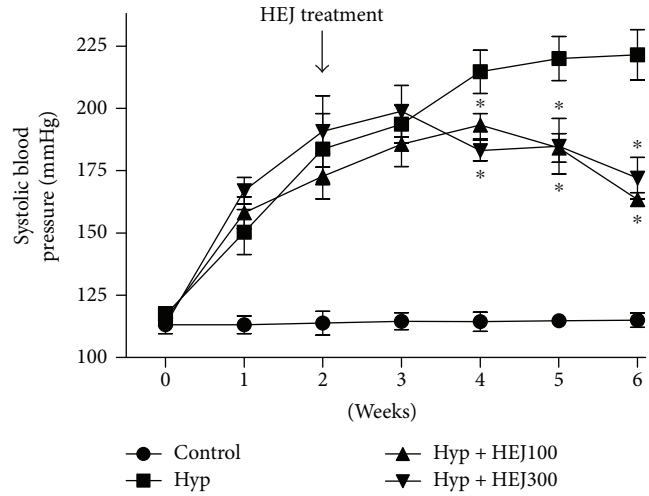


Figure 3: Systolic blood pressure (mmHg) measured by a tail-cuff method in the four experimental groups (control, hypertensive, hypertensive treated with HEJ (100 mg/kg/day), or hypertensive treated with HEJ (300 mg/kg/day)) during the study period ($n = 5 - 7$ per group). Data are shown as means ± SEM and were analyzed using one-way ANOVA followed by the post hoc Newman–Keuls test. $^*p < 0.05$ versus hypertensives. From the first week, all groups are statistically different in relation to the control.

Treatment with HEJ also attenuated the increases in the heart rate (bpm) in hypertensive rats (Table 2). Tachycardia is a compensatory physiologic phenomenon that is a common phenomenon during hypertension [26].

In the heart and kidneys, the increase in blood pressure has a direct relationship to damage and hypertrophy, which is characterized by an increase in organ volume and weight [27]. The weight of the heart and kidneys also was measured

Table 2: Systolic blood pressure and general parameters with the treatment with the hydroalcoholic extract of jabuticaba (HEJ).

Parameters	Control	Hypert	Hypert + HEJ100	Hypert + HEJ300
SBP (mmHg)	115.1 ± 5.8	225.8 ± 11.2*	163.4 ± 8.7*#	172.1 ± 9.3*#
HR (beats/min)	364.2 ± 5.4	407.1 ± 13.5*	370.5 ± 15.3#	368.7 ± 9.2#
Body weight (g)	308.8 ± 7.7	298.9 ± 6.3	302.5 ± 9.4	305.2 ± 9.9
Heart weight	0.291 ± 0.011	0.384 ± 0.025*	0.339 ± 0.019*#	0.349 ± 0.015*#
Kidney weight	0.358 ± 0.011	0.405 ± 0.016*	0.340 ± 0.021#	0.344 ± 0.008#

Systolic blood pressure (SBP), heart rate (HR) (beats/min), body weight (g), and heart and left kidney weight (% body weight) in control rats, hypertensive rats, and HEJ- (100 and 300 mg/kg) treated hypertensive rats. Data are represented as mean ± SEM ($n = 5$–7 rats) and were analyzed using one-way ANOVA followed by the post hoc Newman–Keuls test. $^{*}p < 0.05$ versus control and $^{\#}p < 0.05$ versus hypertensive.

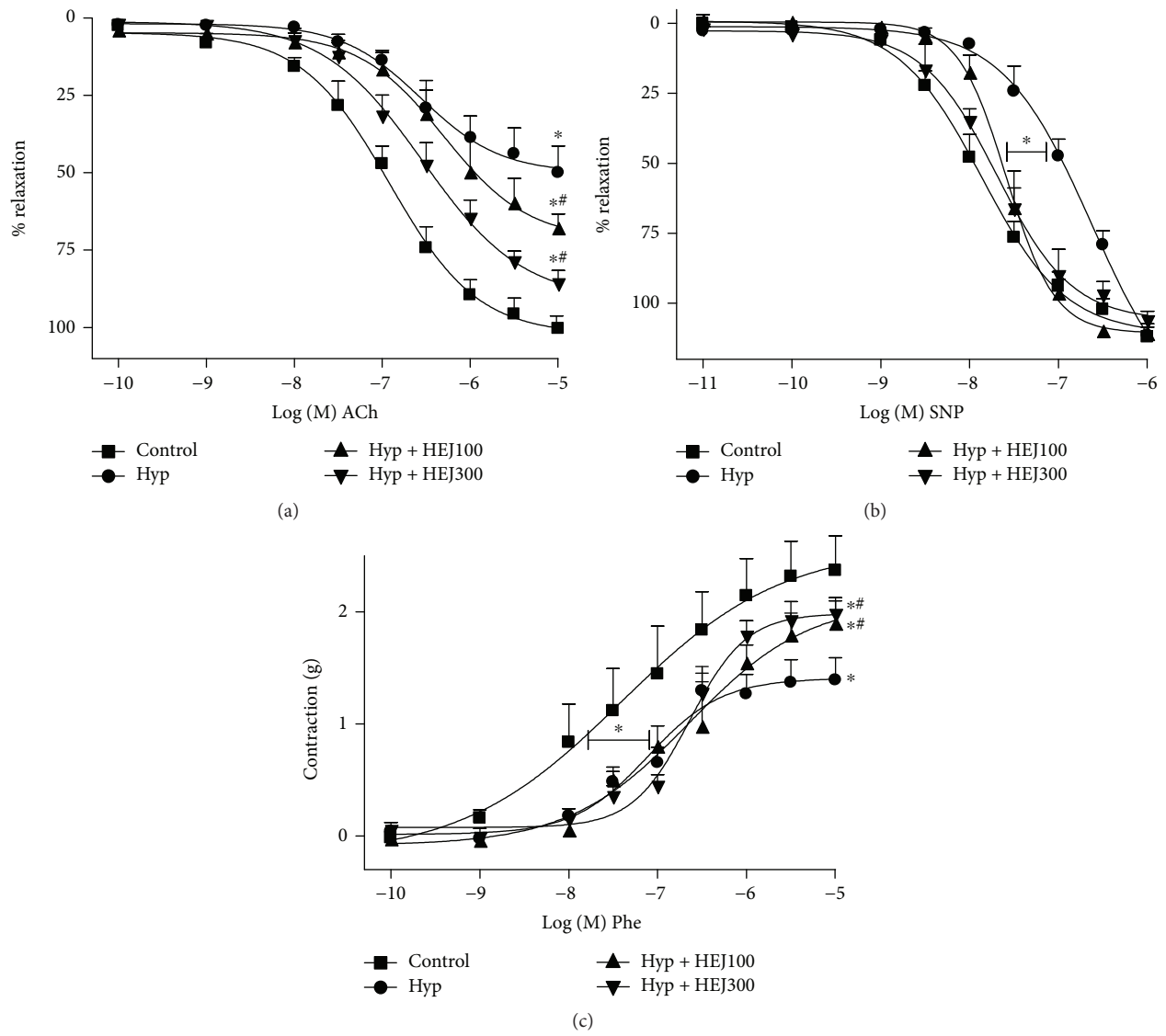


Figure 4: Vascular reactivity studies in aortic artery rings in the four experimental groups (control, hypertensive, hypertensive treated with HEJ (100 mg/kg/day) or hypertensive treated with HEJ (300 mg/kg/day)) after six-week treatment ($n = 6 - 7$ per group). (a) Endothelium-dependent relaxation in response to ACh. (b) Endothelium-independent relaxation in response to sodium nitroprusside (SNP). (c) Cumulative concentration-response curve to phenylephrine in arteries without the endothelium. Data are represented as mean ± SEM and were analyzed using one-way ANOVA followed by the post hoc Newman–Keuls test. $^{*}p < 0.05$ versus control and $^{\#}p < 0.05$ versus hypertensives.

after six weeks of experiments. Both organs in the rats treated with HEJ recovered the weight (hypertrophic index) raised by hypertension (Table 2). The antiproliferative [28] and anti-inflammatory [9] activity of jabuticaba fruit could be responsible for this cardiovascular and renal effects, since some lesions induced by hypertension are hypertrophic and inflammatory in origin.

3.4. Vascular Function. Short-term systemic blood pressure control is carried out by a sophisticated physiological system involving hormonal and neural regulations that strongly influence the blood vessel tone and heart function. Thus, a single substance that interferes with the function of arteries, veins, or the heart will quickly alter blood pressure [29].

The endothelium-dependent vasorelaxation and endothelium-independent vasorelaxation were induced by ACh and SNP, respectively, and can be observed in Figures 4(a) and 4(b). The hypertension was associated with the significant impairment of the ACh maximum response (50.1 ± 6.6%, $n = 8$) as compared to the control group (100.3 ± 4.1%, $n = 8$). The treatment with HEJ (100 and 300 mg/kg) significantly improved the relaxation by ACh to 67.7 ± 5.6% ($n = 6$) and 85.9 ± 5.3% ($n = 6$), respectively. The reduction of SBP by HEJ could be related to antioxidant-induced improvement of vascular relaxation. In isolated arteries, the endothelium-dependent relaxation stimulated by ACh was significantly impaired in the hypertensive group when compared to the control group, as reported in previous studies using hypertensive animals [30, 31]. Herein, the impaired endothelial reactivity in arteries of hypertensive animals was attenuated by the HEJ treatment in both doses (Figure 4(a)).

In the same way, the hypertension was also associated with the significant impairment in the pD_2 ($-\log EC_{50}$) response to SNP (6.39 ± 0.39, $n = 7$), as compared to the control group (7.80 ± 0.14, $n = 9$). This fact also was observed by other works [32, 33]. The treatment with HEJ (100 and 300 mg/kg) significantly improved the relaxation (pD_2 values) by SNP to 7.56 ± 0.12 and 7.60 ± 0.16, respectively. Some authors have attributed this impairment to an increased ROS production in the arteries from hypertensive rats, since NO reacts with ROS, reducing NO bioavailability [33, 34] (Figure 4(b)).

The contractile response to vascular tissues after adrenergic stimulation is generally controversial in arteries from hypertensive rats. There are doubts about whether the hypertension induces hypo- or hyperactivity to adrenergic contractile stimulus, since experiments have shown that vascular responsiveness in hypertensive rat arteries was increased [35], decreased [33], or unchanged [36]. In our work, the hypertension caused impairment in the contraction induced by Phe (adrenergic agonist) in arteries. The treatment with HEJ was able to restore and improve the constriction when compared to the hypertensive and control groups. The E_{max} observed for the hypertensive group was lower (1.39 ± 0.12 g, $n = 7$) than that observed for the control group (2.36 ± 0.31 g, $n = 9$). The treatment with HEJ (100 and 300 mg/kg) improved the contraction induced by Phe (E_{max}) to 1.89 ± 0.17 g ($n = 5$) and 1.97 ± 0.19 g ($n = 5$), respectively (Figure 4(c)).

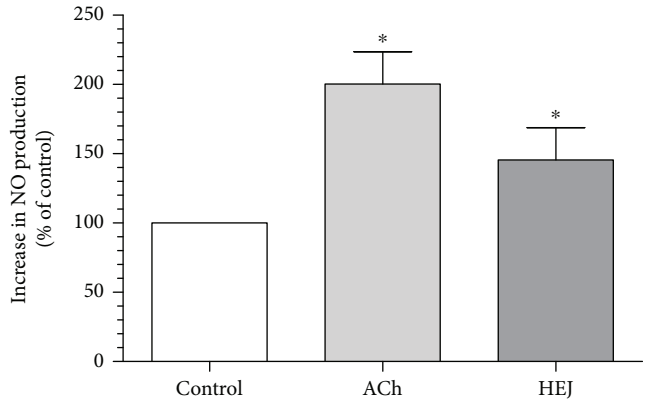


Figure 5: Effects of HEJ and ACh on $[NO]_c$ production in endothelial cells obtained from the aorta of rats. After incubation with DAF-2/DA, the cells were treated with HEJ or ACh for 10 min and $[NO]_c$ levels were quantified by a flow cytometer. Each bar presents mean ± SEM of each five independent analyses ($^*p < 0.05$ versus control. ANOVA followed by the post hoc Newman–Keuls test, $p < 0.05$).

3.5. Cytosolic Concentration of NO ($[NO]_c$) in Endothelial Cells. Endothelial cells can modulate the vascular smooth muscle response to different contractile or relaxant stimuli [37]. Since it was first demonstrated that the acetylcholine-induced relaxation of contracted arteries is mediated by NO, the role of the endothelium in regulating the vascular tone has been well established. NO is a potent vasodilator, which lowers blood pressure by several cellular pathways [32, 33]. As shown in Figure 5, ACh promoted a significant increase of 203.3 ± 21.2% in the $[NO]_c$ production in endothelial cells when compared to the basal levels of the control group. Similarly, the treatment with HEJ also promoted a significant increase in $[NO]_c$ levels (148.9 ± 13.7%). Both stimuli increased NO production from the endothelial cells, highlighting the capacity of the HEJ to produce endothelial NO.

4. Conclusions

In conclusion, this study demonstrated that the extract obtained from the fruits of *M. cauliflora* presents a high antioxidant potential. The treatment with HEJ attenuated hypertension possibly improving the NO biodisponibility. The endothelium-dependent and endothelium-independent vascular contraction and relaxation were impaired by hypertension and improved after treatment with HEJ. Further, the HEJ has the ability to stimulate the production of NO from endothelial cells. Therefore, this work will contribute to the body of knowledge about jabuticaba-derived compounds and their use as a medicinal plant allied for cardiovascular and oxidative illness prevention and health.

Abbreviations

ACh: Acetylcholine
HEJ: Hydroalcoholic extract of jabuticaba
L-NAME: Nω-Nitro-L-arginine methyl ester hydrochloride

NO: Nitric oxide
NOS: Nitric oxide synthase
Phe: Phenylephrine
ROS: Reactive oxygen specie
SBP: Systolic blood pressure
SNP: Sodium nitroprusside.

Disclosure

This manuscript is a part from a master's dissertation of Camila Gabriela de Souza and Juliana Bahia Reis Jordão, at Federal University of Goiás, Brazil. An earlier version of this work was presented as an abstract in the "9th World Congress on Pharmacology," 2017.

Conflicts of Interest

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

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References

[1] A. V. Chobanian, "Guidelines for the management of hypertension," *The Medical Clinics of North America*, vol. 101, no. 1, pp. 219–227, 2017.

[2] S. Moncada, A. G. Herman, E. A. Higgs, and J. R. Vane, "Differential formation of prostacyclin (PGX or PGI2) by layers of the arterial wall. An explanation for the antithrombotic properties of vascular endothelium," *Thrombosis Research*, vol. 11, no. 3, pp. 323–344, 1977.

[3] M. O. Ribeiro, E. Antunes, G. de Nucci, S. M. Lovisolo, and R. Zatz, "Chronic inhibition of nitric oxide synthesis. A new model of arterial hypertension," *Hypertension*, vol. 20, no. 3, pp. 298–303, 1992.

[4] F. Greenway, Z. Liu, Y. Yu, and A. Gupta, "A clinical trial testing the safety and efficacy of a standardized *Eucommia ulmoides* Oliver bark extract to treat hypertension," *Alternative Medicine Review*, vol. 16, no. 4, pp. 338–347, 2011.

[5] S. Kumar, P. Prahalathan, and B. Raja, "Vanillic acid: a potential inhibitor of cardiac and aortic wall remodeling in L-NAME induced hypertension through upregulation of endothelial nitric oxide synthase," *Environmental Toxicology and Pharmacology*, vol. 38, no. 2, pp. 643–652, 2014.

[6] L. T. Abe, F. M. Lajolo, and M. I. Genovese, "Potential dietary sources of ellagic acid and other antioxidants among fruits consumed in Brazil: jabuticaba (M*yrciaria jaboticaba* (Vell.) Berg)," *Journal of the Science of Food and Agriculture*, vol. 92, no. 8, pp. 1679–1687, 2012.

[7] G. A. C. Fortes, S. S. Naves, and F. F. F. Godoi, "Assessment of a maturity index in jabuticaba fruit by the evaluation of phenolic compounds, essential oil components, sugar content and total acidity," *American Journal of Food Technology*, vol. 6, no. 11, pp. 974–984, 2011.

[8] O. H. Boscolo and L. S. Valle, "Plantas de uso medicinal em Quissamã, Rio de Janeiro, Brasil," *Iheringia Série Botânica*, vol. 63, pp. 263–277, 2008.

[9] M. Giraldi and N. Hanazaki, "Use and traditional knowledge of medicinal plants at Sertão do Ribeirão, Florianópolis, Santa Catarina State, Brazil," *Acta Botanica Brasilica*, vol. 24, no. 2, pp. 395–406, 2010.

[10] K. A. Reynertson, A. M. Wallace, S. Adachi et al., "Bioactive depsides and anthocyanins from jabuticaba (*Myrciaria cauliflora*)," *Journal of Natural Products*, vol. 69, no. 8, pp. 1228–1230, 2006.

[11] J. C. Baldin, E. C. Michelin, Y. J. Polizer et al., "Microencapsulated jabuticaba (*Myrciaria cauliflora*) extract added to fresh sausage as natural dye with antioxidant and antimicrobial activity," *Meat Science*, vol. 118, pp. 15–21, 2016.

[12] P. L. Almeida, S. N. Lima, L. L. Costa, C. C. Oliveira, K. A. Damasceno, and B. A. Santos, "Effect of jabuticaba peel extract on lipid oxidation, microbial stability and sensory properties of Bologna-type sausages during refrigerated storage," *Meat Science*, vol. 110, pp. 9–14, 2015.

[13] D. M. L. de Andrade, C. F. Reis, P. F. Castro et al., "Vasorelaxant and hypotensive effects of jaboticaba fruit (*Myrciaria cauliflora*) extract in rats," *Evidence-Based Complementary Alternative Medicine*, vol. 2015, Article ID 696135, 8 pages, 2015.

[14] D. M. L. de Andrade, L. L. Borges, I. M. S. Torres, E. C. Conceição, and M. L. Rocha, "Jabuticaba-induced endothelium-independent vasodilating effect on isolated arteries," *Arquivos Brasileiros de Cardiologia*, vol. 107, no. 3, pp. 223–229, 2016.

[15] L. A. J. Boari, C. A. Duarte, and A. A. P. Carvalho, "Chemical characterization of the jabuticaba fruits (*Myrciaria cauliflora* Berg) and their fractions," *Archivos Latinoamericanos de Nutrición*, vol. 58, no. 4, pp. 416–421, 2008.

[16] P. G. Waterman and S. A. Mole, "A critical analysis of techniques for measuring tannins in ecological studies. I. Techniques for chemically defining tannins," *Oecologia*, vol. 72, no. 1, pp. 137–147, 1987.

[17] F. M. A. Lino, L. A. Sá, I. M. S. Torres et al., "Voltammetric and spectrometric determination of antioxidant capacity of selected wines," *Electrochimica Acta*, vol. 128, pp. 25–31, 2014.

[18] M. Larrosa, M. T. García-Conesa, J. C. Espín, and F. A. Tomás-Barberán, "Ellagitannins, ellagic acid and vascular health," *Molecular Aspects of Medicine*, vol. 31, no. 6, pp. 513–539, 2010.

[19] M. C. Nicoli, M. Anese, and M. Parpinel, "Influence of processing on the antioxidant properties of fruit and vegetables," *Trends in Food Science & Technology*, vol. 10, no. 3, pp. 94–100, 1999.

[20] T. Berkban, P. Boonprom, and S. Bunbupha, "Ellagic acid prevents L-NAME induced hypertension via restoration of eNOS and $p47^{phox}$ expression in rats," *Nutrients*, vol. 7, no. 7, pp. 5265–5280, 2015.

[21] K. A. P. Colati, G. P. Dalmaschio, E. V. R. Castro, A. O. Gomes, B. G. Vaz, and W. Romão, "Monitoring the liquid/liquid extraction of naphtenic acids in Brazilian crude oil using electrospray ionization FT-ICR mass spectrometry (ESI FT-ICR MS)," *Fuel*, vol. 108, pp. 647–655, 2013.

[22] E. S. Gil, A. T. Enache, and A. M. Oliveira-Brett, "Anodic behaviour of flavonoids orientin, eriodictyol and robinin at a glassy carbon electrode," *Electroanalysis*, vol. 24, no. 7, pp. 576–583, 2012.

[23] T. Madrakian, E. Haghshenas, and A. Afkhami, "Simultaneous determination of tyrosine, acetaminophen and ascorbic acid using gold nanoparticles/multiwalled carbon nanotube/glassy carbon electrode by differential pulse voltammetric method," *Sensors and Actuators B: Chemical*, vol. 193, pp. 451–460, 2014.

[24] M. M. Castro, E. Rizzi, G. J. Rodrigues et al., "Antioxidant treatment reduces matrix metalloproteinase-2-induced vascular changes in renovascular hypertension," *Free Radical Biology & Medicine*, vol. 46, no. 9, pp. 1298–1307, 2009.

[25] L. Nabha, J. C. Garbern, C. L. Buller, and J. R. Charpie, "Vascular oxidative stress precedes high blood pressure in spontaneously hypertensive rats," *Clinical and Experimental Hypertension*, vol. 27, no. 1, pp. 71–82, 2005.

[26] S. Reule and P. E. Drawz, "Heart rate and blood pressure: any possible implications for management of hypertension?," *Current Hypertension Reports*, vol. 14, no. 6, pp. 478–484, 2012.

[27] Y. Ma, H. Huang, J. Jiang et al., "AVE0991 attenuates cardiac hypertrophy through reducing oxidative stress," *Biochemical and Biophysical Research Communications*, vol. 291, pp. 30567–30572, 2015.

[28] W. Wang, Y. Tyan, Z. Chen et al., "Evaluation of the antioxidant activity and antiproliferative effect of the jaboticaba (*Myrciaria cauliflora*) seed extracts in oral carcinoma cells," *BioMed Research International*, vol. 2014, Article ID 185946, 7 pages, 2014.

[29] T. N. Thrasher, "Baroreceptors and the long-term control of blood pressure," *Experimental Physiology*, vol. 84, pp. 331–341, 2004.

[30] F. Sekiguchi, Y. Miyake, A. Hirakawa et al., "Hypertension and impairment of endothelium-dependent relaxation of arteries from spontaneously hypertensive and L-NAME-treated Wistar rats," *Journal of Smooth Muscle Research*, vol. 37, no. 2, pp. 67–79, 2001.

[31] F. I. Alp Yildirim, D. Eker Kizilay, B. Ergin et al., "Barnidipine ameliorates the vascular and renal injury in L-NAME-induced hypertensive rats," *European Journal of Pharmacology*, vol. 764, pp. 433–442, 2015.

[32] D. Bonaventura, F. S. Oliveira, R. S. da Silva, and L. M. Bendhack, "Decreased vasodilation induced by a new nitric oxide donor in two kidney, one clip hypertensive rats is due to impaired K^+ channel activation," *Clinical and Experimental Pharmacology & Physiology*, vol. 32, no. 5-6, pp. 478–481, 2005.

[33] G. J. Rodrigues, C. B. Restini, and C. N. Lunardi, "Caveolae dysfunction contributes to impaired relaxation induced by nitric oxide donor in aorta from renal hypertensive rats," *The Journal of Pharmacology and Experimental Therapeutics*, vol. 323, no. 3, pp. 831–837, 2007.

[34] X. Chen, R. M. Touyz, J. B. Park, and E. L. Schiffrin, "Antioxidant effects of vitamins C and E are associated with altered activation of vascular NADPH oxidase and superoxide dismutase in stroke-prone SHR," *Hypertension*, vol. 38, pp. 606–611, 2011.

[35] F. J. Dowell, D. Henrion, M. Duriez, and J. B. Michel, "Vascular reactivity in mesenteric resistance arteries following chronic nitric oxide synthase inhibition in Wistar rats," *British Journal of Pharmacology*, vol. 117, no. 2, pp. 341–346, 1996.

[36] C. F. Küng, P. Moreau, H. Takase, and T. F. Lüscher, "L-NAME hypertension alters endothelial and smooth muscle function in rat aorta. Prevention by trandolapril and verapamil," *Hypertension*, vol. 26, no. 5, pp. 744–751, 1995.

[37] S. Hyslop and G. de Nucci, "The mechanisms and significance of the coupled release of endothelium-derived relaxing factor (EDRF) and prostacyclin (PGI2) fromendothelial cells," *Wiener Klinische Wochenschrift*, vol. 103, no. 14, pp. 422–434, 1991.