Article

Discovery Medicine 2023; 35(176): 275–282
https://doi.org/10.24976/Discov.Med.202335176.28

Antifatigue Effect of Asiaticoside in Mice by Attenuating Oxidative Stress

Xinghai Liu[1,†], Tong Liu[1,†], Kunli Yang[1], Hong Liu[1,*]

[1]Institute of Basic Medicine and Forensic Medicine, North Sichuan Medical College, 637100 Nanchong, Sichuan, China
*Correspondence: 1099389691@qq.com (Hong Liu)
†These authors contributed equally.
Published: 2 June 2023

Background: Asiaticoside is one of the main components of triterpenoid saponins extracted from *Centella asiatica*. Asiaticoside has shown the effects of wound healing, osteoclastogenesis, anti-inflammatory, anti-cancer, and improving cognition in multiple human disease models. However, studies on the antifatigue effects of asiaticoside have not been explored. Therefore, the aim of this study was to investigate the potential antifatigue effect and underlying mechanism of asiaticoside administration on exhaustive exercise performance.

Methods: Male Kunming mice were divided into four groups randomly (n = 20/group). Saline (10 mL/kg) was administered to the model control group and the other three experimental groups were fed with low (10 mg/kg), medium (20 mg/kg) and high (40 mg/kg) asiaticoside once/daily for 14 days. The antifatigue effect of asiaticoside on mice was estimated by analyzing changes in body weight, weight-loaded swimming time, rotating time, lactic acid, urea nitrogen, liver/muscle glycogen, serumal superoxide dismutase, superoxide dismutase and the liver tissues of hematoxylin and eosin (H&E) staining.

Results: The results indicated that no significant differences were observed in the body weight of each group ($p > 0.05$). Compared with the model control group, supplementation of asiaticoside significantly prolonged the weight-loaded swimming time and rotating time; Decreased the blood lactic acid (LA), blood urea nitrogen (BUN), and serumal malonaldehyde (MDA); And increased the content of liver/muscle glycogen and serumal superoxide dismutase levels (SOD) ($p < 0.05$). Furthermore, the pathological results of the liver were improved greatly. The maximal effect was observed in the medium group of 20 mg/kg.

Conclusions: Asiaticoside is capable of reducing the fatigue effect by regulating energy consumption, energy metabolism and improving antioxidant activity after exercise. While there are still some shortcomings in this study, our findings provide a scientific basis for developing an asiaticoside-based antifatigue supplement.

Keywords: asiaticoside; antifatigue activity; antioxidant activity; blood lactic acid; blood urea nitrogen; glycogen

Introduction

Fatigue is a complex process of physiological changes in the human body. It is the inability of the body's physiological process to continue its function at a certain level or the failure of various organs to maintain a predetermined exercise intensity [1]. The occurrence of fatigue indicates a temporary decline in the body's original working ability or a precursor to the body's development of an injury state [2]. Due to civilization, diet, faster pace of life, and greater psychological pressure, the human body easily turns to a state of fatigue. Fatigue may cause depression, aging, Parkinsonian syndrome, multiple sclerosis, cancer, and other diseases [3]. In the United States, the male population suffering from persistent fatigue has reached 2.3%, while the figure for women is 1.9% [4]. A survey investigating the student population in Suzhou found that nearly 1% suffered from long-term fatigue symptoms [5]. The pathophysiological mechanism of fatigue is complex, including energy failure, accumulation of fatigue-related metabolites, oxidative stress damage, central transmitter imbalance, homeostasis imbalance, changes in muscle excitability and activity, and reduced muscle strength [6]. Delaying the occurrence of fatigue, quickly eliminating fatigue, and maintaining vigorous energy are essential for individuals living in a fast-paced society.

The ways to improve exercise-induced fatigue mainly include drug intervention, nutrition supplements and other means [7]. The drugs are mainly synthetic drugs (benzedrine, caffeine, some sympathomimetic stimulants, etc.) and natural plant-derived substances (ginseng, rhodiola, garlic, etc.) [8]. However, synthetic drugs have massive side effects and are unsuitable for long-term use [9,10]. Extracting antifatigue drugs from plants might be emerging as the direction [11].

Centella asiatica is the dry whole plant of *Centella asiatica (L.) Urb*, a plant of the *Umbelliferae* family. Asiaticoside is one of the main components of triterpenoid saponins extracted from *Centella asiatica*, has been widely documented and shown to be involved in a series of pharmacological processes. Asiaticoside has shown the effects of wound healing [12], osteoclastogenesis [13], anti-inflammatory [14], anti-cancer [15], and improving cognition [16] in multiple human disease models, which are related to its modulation in oxidative stress [17–19]. Although antioxidants have been reported to play a significant role in the antifatigue process [20–23], the role of asiaticoside on antifatigue activity, however, is still unknown. Based on this point, we speculated that asiaticoside may have effects on improving exercise endurance and reducing fatigue.

This study used Kunming mice as a model to elucidate the antifatigue effects of asiaticoside. After 14-day administration of asiaticoside, the weight-loaded swimming test and rota-rod test were performed on the mice to estimate the antifatigue effect of asiaticoside. The weight-loaded swimming time and rotating time of the mice and the fatigue-related biochemical parameters were determined.

Materials and Methods

Reagents

The asiaticoside (purity ≥98%) (SA8540) was purchased from Soleibao Biotechnology Company (Beijing, China). The kits for biochemical analysis of lactic acid (LA) (A019-2-1), Urea (C013-2-1), liver/muscle glycogen (A043-1-1), superoxide dismutase (SOD) (A001-1-2), malondialdehyde (MDA) (A003-1-2) were purchased from Jiancheng Bioengineering Institute (Nanjing, China). All other reagents were of analytical grade. Rota-rod (ZB-200) was purchased from Taimeng Science Technology Co., Ltd. (Chengdu, China).

Animals

Mice (Specific Pathogen Free (SPF) grade, n = 80) in this study were provided and maintained in the Experimental Animal Center of North Sichuan Medical College, license number: SCXK-(Chuan) 2018-18, SYXK-(Chuan) 2018-076. The initial body weights of the mice were 18 to 22 g. Every five mice were in a cage. All animals were kept in an air-conditioned room with a 12:12 h light-and-dark cycle, room temperature (23 °C ± 1 °C) and relative humidity (50%–60%). They had free access to laboratory standard water and diet.

Experimental Design

Five days of adaptive feeding were conducted on the mice prior to performing the tests, and then divided randomly into four groups (n = 20/group) in accordance with doses set in reference [24], being low (10 mg/kg), medium (20 mg/kg), high (40 mg/kg), and model control (saline of 10 mL/kg). Intragastric gavage was performed on the groups for 14 days.

Antifatigue Capacity Analysis 1: Weight-Loaded Swimming Test

The weight-loaded swimming test was conducted as previously described [25]. Briefly, after the last intragastric gavage, the mice of each group (n = 10) were allowed to rest for half an hour and loaded 5% of their corresponding body weight fixed to the tails. Following that, the mice were placed into a 30 cm diameter plastic pool with a depth of 30 cm water at 25 ± 2 °C for swimming individually. The swimming time was recorded from the time that mice began to swim until they failed to return to the surface within 10 s. The mice were moved onto a paper towel for drying, and then the fatigue-related biochemical parameters were determined.

Antifatigue Capacity Analysis 2: Rota-Rod Test

After the intragastric gavage, the mice of each group (n = 10) without weight-loaded swimming test had three times training on the rota-rod at 15 rpm for 1 min. During the formal test, mice were put on the rota-rod at 15 rpm. The rotating time was recorded from the mice began to rotate until they were exhausted and dropped from the rod [26].

Measurement of Fatigue-Related Biochemical Parameters and Histological Tissue Staining

After the weight-loaded swimming test, the blood of the mice was collected into eppendorf (EP) tubes immediately. Serum was obtained by centrifugation at 4000 rpm and 4 °C for 15 min and then the blood urea nitrogen (BUN), lactic acid (LA), superoxide dismutase (SOD) and malondialdehyde (MDA) were determined. Gastrocnemius muscles and liver were removed and washed in cold saline and kept at –80 °C until the determination of glycogen content was conducted.

The liver tissues of two mice in each group were hematoxylin and eosin (H&E) staining. Liver tissues were separated and fixed in 4% paraformaldehyde for 6 h, then dehydrated by different concentrations of ethanol and xylene step by step and embedded in paraffin. The 3–5 μM thick paraffin sections were dewaxed with xylene, hydrated with gradient ethanol, rinsed with tap water, soaked with hematoxylin semen for 5 min, rinsed with tap water, differentiated with hydrochloric acid alcohol, rinsed with tap water, rinsed with 1% $NaCO_3$ for 30 s, soaked with eosin solution for 2 min, and then a neutral resin was added to seal the slices after being dehydrated and transparent. The histopathological changes were observed under a light microscope.

Statistical Analysis

The data were presented by the mean ± standard deviation (M ± *SD*). Statistical significance was analyzed by SPSS 19.0 software (IBM, Armonk, NY, USA) through one-way analysis of variance (ANOVA) followed by the least significant difference. A p-value under 0.05 was statistically significant.

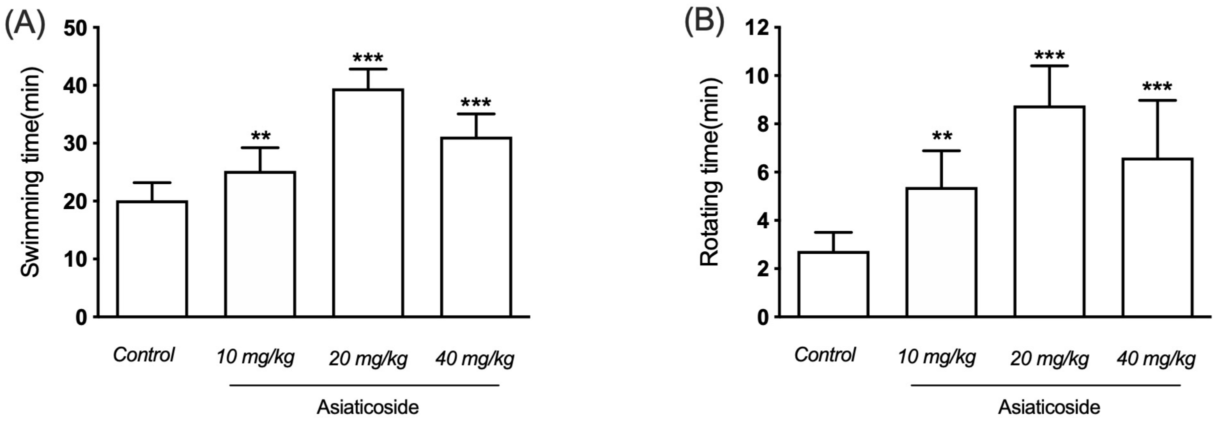

Fig. 1. Effects of asiaticoside on behavior test in mice. (A) The weight-loaded swimming time of mice (n = 10/group). (B) The rotating time of mice (n = 10/group). The data of each group were expressed as mean ± *SD*. $^{**}p < 0.01$, *vs* model control group; $^{***}p < 0.001$, *vs* model control group.

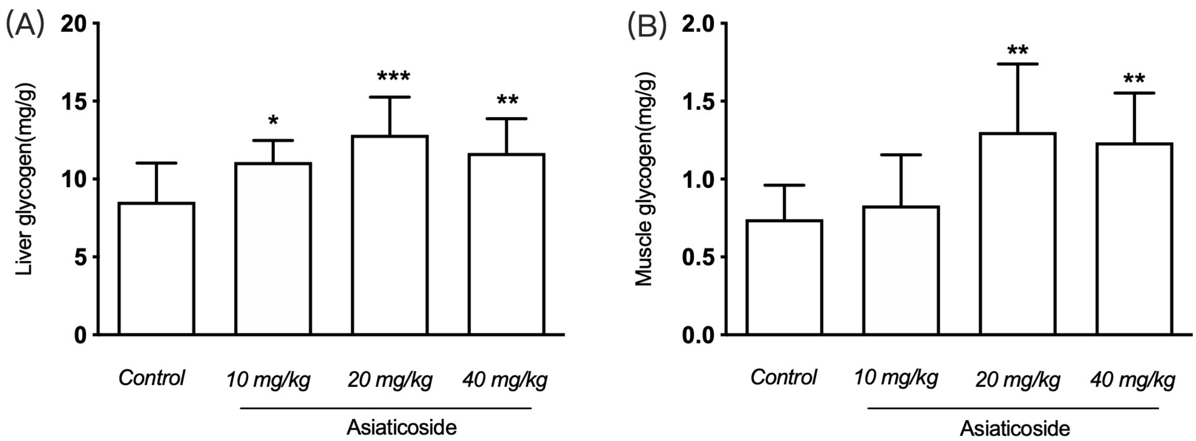

Fig. 2. Effects of asiaticoside on glycogen levels in mice. (A) The levels of liver glycogen of mice (n = 10/group). (B) The levels of muscle glycogen of mice (n = 10/group). The data of each group were expressed as mean ± *SD*. $^{*}p < 0.05$, *vs* model control group; $^{**}p < 0.01$, *vs* model control group; $^{***}p < 0.001$, *vs* model control group.

Results

Effect of Asiaticoside on Body Weight

The mice in each dose group were active, with good mental states, smooth fur, and no adverse reactions were observed. No significant differences existed in the change of body weight at the beginning or the end of the experiment in each group ($p > 0.05$) (Table 1).

Table 1. The change in body weight of mice in all groups (mean ± *SD*) (n = 20/group).

Groups	Initial (g)	Final (g)
Control	20.84 ± 0.56	35.02 ± 1.73
Low-dose asiaticoside	21.28 ± 0.59	35.80 ± 2.12
Medium-dose asiaticoside	20.74 ± 0.77	34.33 ± 1.89
High-dose asiaticoside	20.68 ± 0.82	34.14 ± 1.76

Effects of Asiaticoside on Behavior Tests

The weight-loaded swimming times in the low, medium, and high doses groups increased by 25.31% ($p < 0.01$), 95.93% ($p < 0.001$), and 54.49% ($p < 0.001$), respectively, compared with the model control group (Fig. 1A). The rotating times in the low, medium, and high doses groups were 96.35% ($p < 0.01$), 219.71% ($p < 0.001$) and 141.24% ($p < 0.001$) longer than that in the model control group, respectively (Fig. 1B).

Effects of Asiaticoside on Glycogen Levels

Compared with the model control group, the levels of liver glycogen in the asiaticoside groups of the low, medium, and high doses increased by 29.78% ($p < 0.05$),

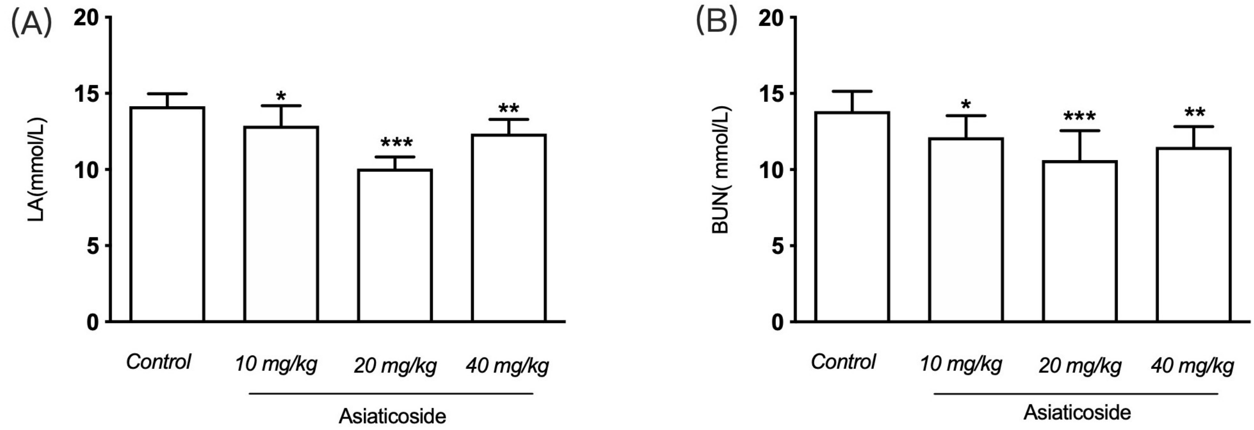

Fig. 3. Effects of asiaticoside on LA and BUN levels in mice. (A) The serum levels of LA in mice (n = 10/group). (B) The levels of BUN in mice (n = 10/group). The data of each group were expressed as mean ± *SD*. $^{*}p < 0.05$, *vs* model control group; $^{**}p < 0.01$, *vs* model control group; $^{***}p < 0.001$, *vs* model control group.

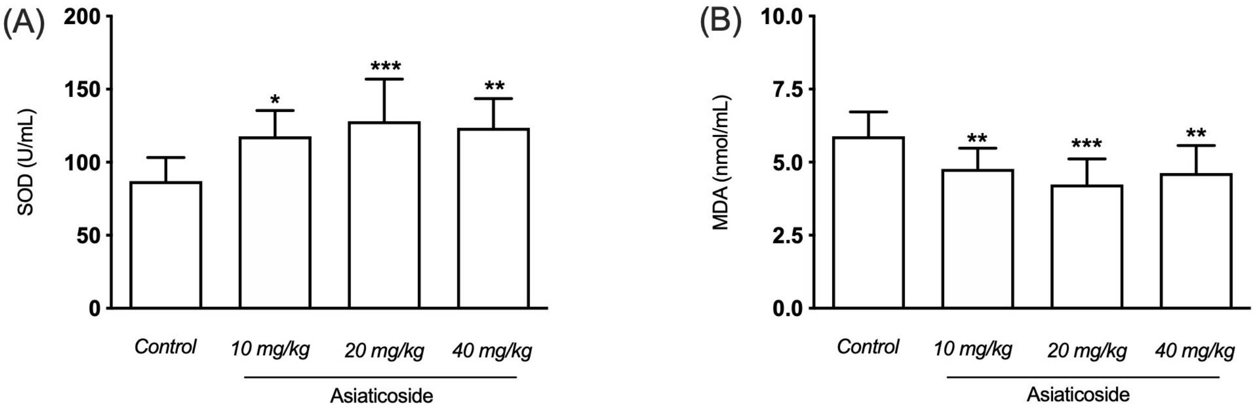

Fig. 4. Effects of asiaticoside on SOD and MDA levels in mice. (A) The levels of SOD in mice (n = 10/group). (B) The levels of MDA in mice (n = 10/group). The data of each group were expressed as mean ± *SD*. $^{*}p < 0.05$, *vs* model control group; $^{**}p < 0.01$, *vs* model control group; $^{***}p < 0.001$, *vs* model control group.

50.26% ($p < 0.001$), and 36.69% ($p < 0.01$), respectively (Fig. 2A). As for the level of muscle glycogen, the asiaticoside groups of the low, medium, and high doses increased by 11.84% ($p > 0.05$), 75.24% ($p < 0.01$), and 66.35% ($p < 0.01$), by comparison to the model control group (Fig. 2B).

Effects of Asiaticoside on LA and BUN Levels

Compared with the model control group LA levels in the asiaticoside groups of the low, medium, and high doses were lower by 9.1% ($p < 0.05$), 28.95% ($p < 0.001$), and 12.71% ($p < 0.01$), respectively (Fig. 3A). Likewise, 12.36% ($p < 0.05$), 23.19% ($p < 0.001$), and 16.98% ($p < 0.01$) reduction of the BUN levels compared to the model control group were observed (Fig. 3B).

Effect of Asiaticoside on SOD and MDA Levels

The SOD activity in all the asiaticoside groups increased, having a percentage increase of 35.29% ($p < 0.05$), 47.12% ($p < 0.001$), 41.84% ($p < 0.01$) in low, medium, and high doses groups respectively, by comparison to the model control group (Fig. 4A). Compared with the model control group, the MDA levels in the asiaticoside groups of the low, medium, and high doses decreased by 18.91% ($p < 0.01$), 27.98% ($p < 0.001$), and 21.39% ($p < 0.01$), respectively (Fig. 4B).

Histopathological Changes of Liver Tissue

The volume of liver cells of mice in the model control group was increased, there was edema between the liver cells, and some of the liver cells were necrotic (Fig. 5A). The pathological results of the liver cells in the asiaticoside groups were significantly improved and the medium dose group has the most favorable effect.

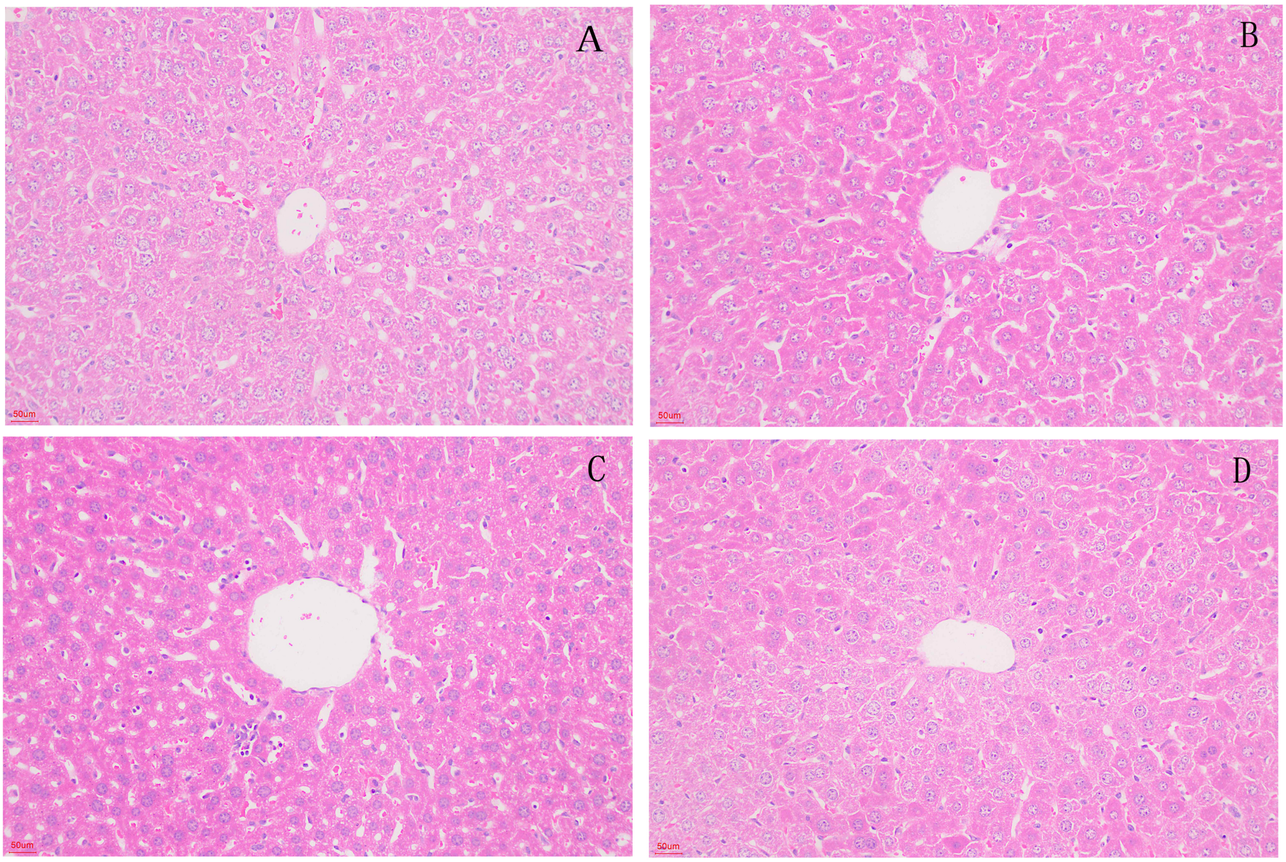

Fig. 5. The histopathological results of liver tissue of mice (200 magnification, 50 μm scale bar) (n = 2/group). (A) The model control group. (B) The low-dose asiaticoside group. (C) The medium-dose asiaticoside group. (D) The high-dose asiaticoside group.

Discussion

Fatigue can affect diet, sleep, and even energy consumption in mice, which directly affects their body weight. Therefore, it is possible to estimate the development level and physical condition of mice according to their body weight. This study shows that compared with the model control group, the weight of mice in the asiaticoside-treated group decreased, but the amount of decrease was not statistically significant, indicating that the set asiaticoside dose treatment had no significant impact on the weight of mice.

The weight-loaded swimming test and rota-rod test are reliable methods widely utilized for detecting the degree of fatigue and physical strength in laboratory animals [27,28]. The current study found that the supplementation of asiaticoside could prolong the exhaustive swimming time and rotating time in the mice, in particular, a maximal effect was observed in the 20 mg/kg/d group. The results demonstrated that the intake of asiaticoside is capable of producing antifatigue, and the medium dose to the corresponding body weight appeared to have the greatest antifatigue effect among the groups.

Glycogen is the main energy resource to the body during exercise and the storage occurs in the liver and muscles. Glycogen provides energy through its oxidation during intense exercise [29,30]. Thus, glycogen reserve can thereby directly enhance exercise capacity, and slow the occurrence of exercise fatigue [31]. After exhausting swimming, the contents of liver glycogen and muscle glycogen of mice in the asiaticoside-treated group were significantly greater than those in the model control group. These results indicated that the supplementation of asiaticoside may be beneficially delaying energy consumption during exhausted swimming.

Intensive exercise is accompanied by a large consumption of energy. Glycolytic metabolism produces large amounts of LA results from an essential step, glycolysis [32]. Excessive levels of LA reduce the pH in the body's muscle tissue and weaken the contractile function of muscles, thereby inducing fatigue [33]. Therefore, the benefit of rapid elimination of LA is seen in alleviating fatigue [34].

In addition, protein degradation occurs following intensive exercise. As the final metabolism product of protein and amino acid, raised level of BUN leads to lowering muscle contractility and causing fatigue [35]. The present study showed in mice that the supplementation of asiaticoside reduces the levels of LA and BUN following strenuous swimming. Such reduction in LA and BUN indicates that the supplementation of asiaticoside can assist in reducing lactate accumulation, inhibiting protein degradation, and relieving fatigue via regulating the body's energy metabolism.

Furthermore, strenuous swimming can promote the production and accumulation of free radicals and induce oxidative stress damage to the body [36]. As an antioxidant enzyme in the organism, SOD fights fatigue by decreasing the development of reactive oxygen species and eliminating the products of metabolism from oxidative stress reactions [37,38]. In organisms, MDA is one of the end products of the peroxidation reaction of unsaturated fatty acids in biofilms, and its content is closely related to exercise-induced fatigue. When the body exercises vigorously, it results in an accelerated oxidative phosphorylation reaction and a sharp increase in the content of free radicals in the body, which exceeds the scavenging capacity of the free radical scavenging enzyme system, leading to a lipid peroxidation reaction, reducing muscle vitality, and causing exercise-induced fatigue or sports injury [39,40].

It has been reported that asiaticoside could improve the damage to the lung and kidney, and its mechanism is related to inhibiting oxidative stress, inflammatory reaction and enhancing antioxidant capacity. Asiaticoside plays a role in improving septic lung injury in mice by upregulating the expression of peroxisome proliferator-activated receptor γ (PPAR-γ), an inhibiting signaling pathway of mitogen-activated protein kinase (MAPK) and nuclear factor kappa-B (NF-κB) [41]. Asiaticoside could ameliorate sepsis-induced acute kidney injury in mice by downregulating the expression of interleukin-6 (IL-6) in serum and nitric oxide synthase in renal tissue [42]. Asiaticoside could attenuate hyperoxia-induced lung injury in rats by reducing the levels of myeloperoxidase, MDA, tumor necrosis factor-α and IL-6 levels, increasing total antioxidant levels [43]. The current study demonstrated that the supplementation of asiaticoside significantly reduced MDA and improved SOD activity compared with the model control. These results suggested that the antifatigue effect of asiaticoside might be intimately associated with its antioxidant characteristics.

There are still some limitations in the present study. From the design and result data of this study, we only conclude that different concentrations of asiaticoside may have a certain antifatigue ability compared to Kunming mice treated with normal saline. However, it is not possible to rule out the influencing factors as to whether the experimental animals have differences before drug intervention. We need to perform more experiments to confirm this conclusion.

Conclusions

In conclusion, we revealed the antifatigue effects of asiaticoside in mice. In the present study, we proved that asiaticoside significantly increased weight-loaded swimming time and rotating time, eliminated the metabolites accumulated during the exercise by reducing the levels of LA and BUN, enhanced glycogen reserves by increasing the content of live glycogen and muscle glycogen, attenuating oxidative stress by increasing the activity of SOD and inhibiting the production of MDA. In addition, the maximal effect was the medium group of 20 mg/kg. This might imply that there is a dose-dependent effect within a particular range, also beyond that optimal range, not only does such a dose-dependent relationship no longer stand but also the assisting effect of antifatigue is weakened. Taken together, our findings suggested that asiaticoside might be a potential drug for broad application prospects in antifatigue drugs and healthy diets.

Availability of Data and Materials

The data and materials used to support the findings of this study are available from the corresponding author upon request.

Author Contributions

XHL and HL—contributed to the design of this study; XHL and TL—performed the experiments and drafted the article; HL—provided help and advice on the behavior tests; KLY—provided help and advice on the HE experiment; KLY—analyzed the data. All authors agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All authors reviewed and approved the submitted version of the manuscript.

Ethics Approval and Consent to Participate

All animal-related operations were approved by the Committee of Experimental Animal Ethics of North Sichuan Medical College (approved on 26th December 2021 with the approval number of NSMC 2021118).

Acknowledgment

Not applicable.

Funding

This work was supported by the Nanchong City school science and technology strategic cooperation project (No. 22SXQT0128) and Education Department of Sichuan Province (No. 2017ZA0167).

Conflict of Interest

The authors declare no conflict of interest.

References

[1] Li W, Luo C, Huang Y, *et al*. Evaluation of antifatigue and antioxidant activities of the marine microalgae *Isochrysis galbana* in mice. *Food Sci Biotechnol.* 2019;29(4):549–557. doi: 10.1007/s10068-019-00694-6

[2] You L, Ren J, Yang B, Regenstein J, Zhao M. Antifatigue activities of loach protein hydrolysates with different antioxidant activities. *J Agric Food Chem.* 2012;60(50):12324–12331. doi: 10.1021/jf3037825

[3] Fischer DB, William AH, Strauss AC, *et al*. Chronic Fatigue Syndrome: The Current Status and Future Potentials of Emerging Biomarkers. *Fatigue.* 2014;2(2):93–109. doi: 10.1080/21641846.2014.906066

[4] Baraniuk JN. Chronic Fatigue Syndrome prevalence is grossly overestimated using Oxford criteria compared to Centers for Disease Control (Fukuda) criteria in a U.*S.* population study. *Fatigue*. 2017;5(4):215–230. doi: 10.1080/21641846.2017.1353578

[5] Shi J, Shen J, Xie J, Zhi J, Xu Y. Chronic fatigue syndrome in Chinese middle-school students. *Medicine (Baltimore).* 2018;97(4):e9716. doi: 10.1097/MD.0000000000009716

[6] Glaister M. Multiple sprint work : physiological responses, mechanisms of fatigue and the influence of aerobic fitness. *Sports Med.* 2005;35(9):757–777. doi: 10.2165/00007256-200535090-00003

[7] Rosenthal TC, Majeroni BA, Pretorius R, Malik K. Fatigue: an overview. *Am Fam Physician.* 2008;78(10):1173–1179.

[8] Xie Q, Sun Y, Cao L, *et al*. Antifatigue and antihypoxia activities of oligosaccharides and polysaccharides from Codonopsis pilosula in mice. *Food Funct.* 2020;11(7):6352–6362. doi: 10.1039/d0fo00468e

[9] Childs E, de Wit H. Enhanced mood and psychomotor performance by a caffeine-containing energy capsule in fatigued individuals. *Exp Clin Psychopharmacol.* 2008;16(1):13–21. doi: 10.1037/1064-1297.16.1.13

[10] Shellenberg TP, Stoops WW, Lile JA, Rush CR. An update on the clinical pharmacology of methylphenidate: therapeutic efficacy, abuse potential and future considerations. *Expert Rev Clin Pharmacol.* 2020;13(8):825–833. doi: 10.1080/17512433.2020.1796636

[11] Liu Y, Li C, Shen X, Liu Y. The use of traditional Chinese medicines in relieving exercise-induced fatigue. *Front Pharmacol.* 2022;13:969827. doi: 10.3389/fphar.2022.969827

[12] Namviriyachote N, Lipipun V, Akkhawattanangkul Y, Charoonrut P, Ritthidej GC. Development of polyurethane foam dressing containing silver and asiaticoside for healing of dermal wound. *Asian J Pharm Sci.* 2019;14(1):63–77. doi: 10.1016/j.ajps.2018.09.001

[13] He L, Hong G, Zhou L, *et al*. Asiaticoside, a component of Centella asiatica attenuates RANKL-induced osteoclastogenesis via NFATc1 and NF-κB signaling pathways. *J Cell Physiol.* 2019;234(4):4267–4276. doi: 10.1002/jcp.27195

[14] Jing L, Haitao W, Qiong W, Fu Z, Nan Z, Xuezheng Z. Anti inflammatory effect of asiaticoside on human umbilical vein endothelial cells induced by ox-LDL. *Cytotechnology.* 2018;70(2):855–864. doi: 10.1007/s10616-018-0198-4

[15] Garanti T, Stasik A, Burrow AJ, Alhnan MA, Wan KW. Anti-glioma activity and the mechanism of cellular uptake of asiatic acid-loaded solid lipid nanoparticles. *Int J Pharm.* 2016;500(1-2):305–315. doi: 10.1016/j.ijpharm.2016.01.018

[16] Guo M, Xu J, Wang S, Dong B. Asiaticoside reduces autophagy and improves memory in a rat model of dementia through mTOR signaling pathway regulation. *Mol Med Rep.* 2021;24(3):645. doi: 10.3892/mmr.2021.12284

[17] Razali NNM, Ng CT, Fong LY. Cardiovascular Protective Effects of Centella asiatica and Its Triterpenes: A Review. *Planta Med.* 2019;85(16):1203–1215. doi: 10.1055/a-1008-6138

[18] Zhao J, Shi J, Shan Y, *et al*. Asiaticoside inhibits TGF-β1-induced mesothelial-mesenchymal transition and oxidative stress via the Nrf2/HO-1 signaling pathway in the human peritoneal mesothelial cell line HMrSV5. *Cell Mol Biol Lett.* 2020;25:33. doi: 10.1186/s11658-020-00226-9

[19] Zeng X, Yu J, Liu P, Liu Y, Zeng T, Li B. Asiaticoside alleviates cardiomyocyte apoptosis and oxidative stress in myocardial ischemia/reperfusion injury via activating the *PI3K-AKT-GSK3β* pathway *in vivo* and *in vitro*. *Ann Transl Med.* 2022;10(2):69. doi: 10.21037/atm-21-6667

[20] Wang X, Qu Y, Zhang Y, *et al*. Antifatigue Potential Activity of *Sarcodon imbricatus* in Acute Excise-Treated and Chronic Fatigue Syndrome in Mice via Regulation of Nrf2-Mediated Oxidative Stress. *Oxid Med Cell Longev.* 2018;2018:9140896. doi: 10.1155/2018/9140896

[21] Hu G, Gao S, Mou D. Water and alcohol extracts from Diaphragma juglandis on anti-fatigue and antioxidative effects *in vitro* and *vivo*. *J Sci Food Agric.* 2021;101(8):3132–3139. doi: 10.1002/jsfa.10942

[22] Chen Y, Wang J, Jing Z, Ordovas JM, Wang J, Shen L. Anti-fatigue and anti-oxidant effects of curcumin supplementation in exhaustive swimming mice *via* Nrf2/Keap1 signal pathway. *Curr Res Food Sci.* 2022;5:1148–1157. doi: 10.1016/j.crfs.2022.07.006

[23] Peng Y, Zhao L, Hu K, *et al*. Anti-Fatigue Effects of *Lycium barbarum* Polysaccharide and Effervescent Tablets by Regulating Oxidative Stress and Energy Metabolism in Rats. *Int J Mol Sci.* 2022;23(18):10920. doi: 10.3390/ijms231810920

[24] Zhou Y, Wang S, Zhao J, Fang P. Asiaticoside attenuates neonatal hypoxic-ischemic brain damage through inhibiting TLR4/NF-κB/STAT3 pathway. *Ann Transl Med.* 2020;8(10):641. doi: 10.21037/atm-20-3323

[25] Shao S, Wang MX, Zhang HY, *et al*. Antifatigue Activity of Glycoprotein from *Schisandra chinensis* Functions by Reducing Oxidative Stress. *Evid Based Complement Alternat Med.* 2020;2020:4231340. doi: 10.1155/2020/4231340

[26] Yang C, Yang J, Tan L, *et al*. A Novel Formula Comprising Wolfberry, Figs, White Lentils, Raspberries, and Maca (WFWRM) Induced Antifatigue Effects in a Forced Exercise Mouse Model. *Evid Based Complement Alternat Med.* 2022;2022:3784580. doi: 10.1155/2022/3784580

[27] Liu Y, Liu C. Antifatigue and increasing exercise performance of Actinidia arguta crude alkaloids in mice. *J Food Drug Anal.* 2016;24(4):738–745. doi: 10.1016/j.jfda.2016.03.001

[28] Liu C, Shao C, Du Q, *et al*. Mechanism and effects of fructose diphosphate on anti-hypoxia fatigue and learning memory ability. *Can J Physiol Pharmacol.* 2020;98(10):733–740. doi: 10.1139/cjpp-2019-0690

[29] Prats C, Graham TE, Shearer J. The dynamic life of the glycogen granule. *J Biol Chem.* 2018;293(19):7089–7098. doi: 10.1074/jbc.R117.802843

[30] Vigh-Larsen JF, Ørtenblad N, Spriet LL, Overgaard K, Mohr M. Muscle Glycogen Metabolism and High-Intensity Exercise Performance: A Narrative Review. *Sports Med.* 2021;51(9):1855–1874. doi: 10.1007/s40279-021-01475-0

[31] Park SH, Jang S, Lee SW, Park SD, Sung YY, Kim HK. Akebia quinata Decaisne aqueous extract acts as a novel anti-fatigue agent in mice exposed to chronic restraint stress. *J Ethnopharmacol.* 2018;222:270–279. doi: 10.1016/j.jep.2018.04.010

[32] Kim KM, Yu KW, Kang DH, Suh HJ. Anti-stress and anti-fatigue

effect of fermented rice bran. *J Phytother Res*. 2002;16(7):700–702. doi: 10.1002/ptr.1019

[33] Zhao P, Wang J, Zhao W, Ma X, Sun H. Antifatigue and antiaging effects of Chinese rice wine in mice. *Food Sci Nutr.* 2018;6(8):2386–2394. doi: 10.1002/fsn3.830

[34] Wang L, Zhang HL, Lu R, *et al*. The decapeptide CMS001 enhances swimming endurance in mice. *Peptides.* 2008;29(7):1176–1182. doi: 10.1016/j.peptides.2008.03.004

[35] Wang JJ, Shieh MJ, Kuo SL, Lee CL, Pan TM. Effect of red mold rice on antifatigue and exercise-related changes in lipid peroxidation in endurance exercise. *Appl Microbiol Biotechnol.* 2006;70(2):247–253. doi: 10.1007/s00253-005-0051-5

[36] Ma N, Tao H, Du H, Zhao L, Hu Q, Xiao H. Antifatigue effect of functional cookies fortified with mushroom powder (Tricholoma Matsutake) in mice. *J Food Sci.* 2020;85(12):4389–4395. doi: 10.1111/1750-3841.15510

[37] Shan X, Zhou J, Ma T, Chai Q. Lycium barbarum polysaccharides reduce exercise-induced oxidative stress. *Int J Mol Sci.* 2011;12(2):1081–1088. doi: 10.3390/ijms12021081

[38] Miao J , Liao W , Kang M , *et al*. Anti-fatigue and anti-oxidant activities of oyster (Ostrea rivularis) hydrolysate prepared by compound protease. *Food Funct.* 2018;9(12):6577–6585. doi: 10.1039/c8fo01879k

[39] Zhonghui Z, Xiaowei Z, Fang F. Ganoderma lucidum polysaccharides supplementation attenuates exercise-induced oxidative stress in skeletal muscle of mice. *Saudi J Biol Sci.* 2014;21(2):119–123. doi: 10.1016/j.sjbs.2013.04.004

[40] Xu M, Liang R, Li Y, Wang J. Anti-fatigue effects of dietary nucleotides in mice. *Food Nutr Res.* 2017;61(1):1334485. doi: 10.1080/16546628.2017.1334485

[41] Zhang LN, Zheng JJ, Zhang L, *et al*. Protective effects of asiaticoside on septic lung injury in mice. *Exp Toxicol Pathol.* 2011;63(6):519–525. doi: 10.1016/j.etp.2010.04.002

[42] Zheng J, Zhang L, Wu M, Li X, Zhang L, Wan J. [Protective effects of asiaticoside on sepsis-induced acute kidney injury in mice]. *Zhongguo Zhong Yao Za Zhi.* 2010;35(11):1482–1485.

[43] Dang JW, Lei XP, Li QP, Dong WB. Asiaticoside attenuates hyperoxia-induced lung injury *in vitro* and *in vivo*. *Iran J Basic Med Sci.* 2019;22(7):797–805. doi: 10.22038/ijbms.2019.35913.8556