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Research Article
Protective Effect of Dandelion Polysaccharide on Extensive Exercise-Induced Liver Injury

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Abstract

Background and Objective: Strenuous and excessive exercise or training may cause muscle and internal organ injuries, of which liver injury is a typical pathological feature of sports tissue injury. Liver injury can affect the performance of sports and jeopardize the health of athletes or sports enthusiasts. Studies have shown that exercise-related injuries are associated with oxidative stress and inflammation. Therefore, the pathway of enhancing the antioxidant and anti-inflammatory capacity of the body can be attempted to ameliorate exercise-related liver injury during excessive exercise. Studies showed that dandelion polysaccharide (DP), a natural product present in dandelion, has obvious anti-inflammatory and antioxidant activities. The study aimed to investigate the protective effects of DP on liver injury induced by excessive exercise. **Materials and Methods:** Overexercise model was performed with Kunming mice, during the training, the DP 100 and DP 200 groups received 100 and 200 mg/kg of DP daily, while the control groups (Normal and Model groups) received equal volume of saline. After 4 weeks of excessive exercise, mice were executed and tested for liver injury. **Results:** Compared with Model group, ALT and AST levels were significantly reduced in the DP groups and cell morphology was more regular and aligned in liver tissues; the antioxidant ability of liver was significantly increased and the level of the lipid oxidation product MDA was significantly decreased in liver; and pro-inflammatory factors, such as TNF-α, significantly decreased and the levels of anti-inflammatory factors increased significantly in serum and liver; Nrf2 and HO-1 proteins in Keap1/Nrf2/HO-1 signaling pathway increased significantly and phosphorylation ratio of protein JAK2 and STAT3 in JAK2/STAT3 pathway and the protein expression of HMGB1, TLR4 and p-NF-κB p65 in HMGB1/TLR4/NF-κB signaling pathways decreased significantly. **Conclusion:** The DP intervention can ameliorate excessive exercise-induced liver injury and this protective mechanism is involved in activating Keap1/Nrf2/HO-1 antioxidant pathway and inhibiting JAK2/STAT3 and HMGB1/TLR4/NF-κB pro-inflammatory signaling pathway.

Key words: Dandelion polysaccharide, exercise-induced liver injury, antioxidant pathway, pro-inflammatory pathway, hepatoprotective effect

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Proper exercise or training is beneficial to cardiorespiratory fitness, improves athletic performance and is helpful in the management of chronic diseases[1]. However, strenuous and excessive exercise or training may cause muscle and internal organ injuries[2,3], of which liver injury is a typical pathological feature of sports tissue injury[4]. Liver injury can affect the performance of sports and jeopardize the health of athletes or sports enthusiasts. Therefore, prolonged over-exercise should be avoided as much as possible, but in order to improve performance, maintain physical fitness and sharpen skills, athletes, military personnel or firefighters often need to undergo a large number of prolonged exercise training[5] and the potential health risks associated with this type of over-exercise deserve special attention.

Studies have shown that exercise-related injuries are associated with oxidative stress and inflammation[6]. During excessive exercise, in order to meet the energy demand, the liver, as the center of energy metabolism of the body, significantly enhances the metabolism, but the excessive metabolism leads to a significant increase in the levels of reactive oxygen species (ROS) free radicals in the liver, such as superoxide anion (O^{2-}), Hydrogen Peroxide (H_2O_2) and hydroxyl radicals (OH^-). While the body's antioxidant capacity is limited, which leads to untimely scavenging of ROS, causing oxidative stress and ultimately resulting in hepatic tissue inflammation and injury[7-9]. In addition to the inflammatory response caused by excessive accumulation of ROS, excessive exercise also leads to an increase in the secretion of inflammatory factors, such as TNF-α, which was found to induce hepatic inflammatory injury and accelerate the accumulation of ROS and aggravate the oxidative stress response[10]. In contrast, when the antioxidant and anti-inflammatory capacity of the body is increased through exogenous pathways during exercise, liver injury can be significantly ameliorated[11-13]. Therefore, the pathway of enhancing the antioxidant and anti-inflammatory capacity of the body can be attempted to ameliorate exercise-related liver injury during excessive exercise.

Traditional anti-inflammatory or antioxidant drugs have significant side effects in the treatment of athletes with sports-related liver injury and they may also prevent athletes from passing doping tests due to drug residues, thus affecting athletes' career development[14]. Recent studies have shown that natural plant products with antioxidant and anti-inflammatory properties, such as quercetin, radicicchioidin and dendrobium, have very obvious protective effects against sports liver injury[13,15] and use of non-toxic or low-toxicity natural products with few side effects against sports liver injury has become one of the hot topics in the field of sports medicine and pharmacology research. Dandelion polysaccharide (DP), a natural product present in dandelion, has obvious anti-inflammatory and antioxidant activities[16,17], but its protective effects against liver injury caused by excessive exercise have not been reported in detail.

Therefore, in the present study, the mouse exercise liver injury model was prepared by prolonged and load-increasing swimming training and evaluated the protective effects of DP supplementation on exercise-induced liver injury in mice by detecting the liver injury markers, liver tissue morphology, oxidative stress levels and inflammatory factor levels and by analyzing the expression of key proteins in the Keap1/Nrf2/HO-1 antioxidant pathway, JAK2/STAT3 and HMGB1/TLR4/NF-κB pro-inflammatory pathway, to preliminarily reveal the hepatoprotective mechanism of DP. The study will provide a partial scientific basis for further expanding the potential application and promotion of DP in the field of sports medicine and liver injury.

MATERIALS AND METHODS

Study area: The experiments were carried out in the College of Physical Education at Yuxi Normal University in Yunnan Province of China, from February, 2023 to October, 2023.

Ethics statement: The experiment described in this paper were designed according to the international regulations on the ethical treatment of animals and approved by the Animal Care and Use Committee of Yuxi Normal University (YNU 2022115).

Animals: As 42-48 days old SPF-grade male Kunming mice, with an average weight of 20±1 g, were purchased from Changchun Yis Laboratory Animal Technology Co., Ltd. They were housed in a thermostatic animal laboratory at 23±2, with 12 hrs of light exposure per day, free access to food and water.

Materials and reagents: The DP was purchased from Shanghai Yuanye Biotechnology Co., Ltd. (No. S28147). The content assay kit of Glutathione (GSH), superoxide dismutase (SOD), malondialdehyde (MDA), Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) was purchased from Nanjing Jiancheng Bioengineering Institute Co., Ltd.

Enzyme-Linked Immunosorbent Assay (ELISA) kits of TNF-α, IL-1β, IL-6, IL-18, IL-4 and IL-10 were purchased from BioLegend company; The antibodies against key proteins in the Keap1/Nrf2/HO-1, JAK2/STAT3 and HMGB1/TLR4/NF-κB signaling pathways, as well as iNOS and COX-2 proteins for western blot were purchased from Cell Signaling Technology Ltd.

Swimming exercise experiments: After 1 week of acclimatization in the animal house, mice were pre-trained in swimming for 3 days (20 min of training per day) and the unsuitable individuals were screened and eliminated. Forty-eight mice were randomly divided into 4 groups (4 mice each): The quiet control group (Normal), excessive exercise model group (Model), excessive exercise model+100 mg/kg low-dose DP treatment group (DP 100) and excessive exercise model+200 mg/kg high-dose DP treatment group (DP 200).

The increased load excessive swimming was designed based on the methods of previous research[18-21]. Briefly, the mice in the Model, DP 100 and DP 200 groups were subjected to swimming training for 4 weeks. In the first week, mice undergone swimming training for 30 min per day without any weight bearing; in the second week, mice undergone swimming training for 30 min per day with 3% body weight bearing; in the third week, mice undergone swimming training for 40 min per day with 5% body weight bearing and in the fourth week, all the mice swam until exhaustion with 5% body weight bearing. During the training period, mice in the DP 100 and DP 200 groups were gavaged once a day with a gavage needle 1 hr before training, with 100 mg/kg of DP in the DP 100 group, 200 mg/kg of DP in the DP 200 group, in a volume of 5 mL/kg and an equal volume of saline in the Normal and Model groups.

The size of the swimming pool is 1.5×0.8×1.0 m and it has a smooth inner wall. The water depth of the pool is 0.5 m and the water temperature is 30~33°C.

Measurement of the indices of inflammatory cytokines, liver function and oxidative stress in the serum and liver tissue: The mice were executed by necking 12 hrs after the training, blood was collected from the eyeballs and serum was prepared and the livers were also collected. Then the levels of two liver function indexes, ALT and AST, the pro-inflammatory factors TNF-α, IL-1β, IL-6, IL-18 and the anti-inflammatory factors IL-4 and IL-10 in the serum with ELISA kits and the levels of three oxidative stress-related indexes, in the liver tissues, were detected according to the instructions of the MDA, GSH and SOD-specific test kit.

Hematoxylin and Eosin (H&E) staining: The structural and pathological changes of liver tissues were measured with H&E staining. In brief, the liver tissues were fixed in formalin over 48 hrs, then they were embedded in wax and cut into 5 um thick slices and further stained with Hematoxylin and Eosin (H&E). The structural and pathological changes were observed under a light microscope (Olympus Corporation of Asia Pacific Limited., Tokyo, Japan).

Western blot analysis: Liver tissues from 2.5 were cut into pieces and lysed with lysate on the ice, then the samples were centrifuged at 12,000×g with a centrifuge (Eppendorf Inc., Hamburg, Germany) and the supernatant was boiled and prepared for use. The protein concentration was determined using the BCA method. After the operations of loading, electrophoresis, membrane transfer and closure, the sample were incubated with the primary antibodies for Nrf2, Keap1, HO-1, p-JAK2, p-STAT3, JAK2, STAT3, SOCS3, HMGB1, TLR4, MYD88, p-TAK1, CD68, p-NF-κB p65, iNOS and COX-2 overnight, after extensive washing, the samples were further incubated with the corresponding secondary antibodies. Subsequently, after extensive washing, the visualization of protein bands was performed using the ECL method and the optical density of the protein bands was analyzed using Image Lab software Bio-Rad (Bio-Rad, Richmond, California, USA) to assess the protein expression.

Statistical analysis: Data were statistically analyzed using SPSS 25.0 software (SPSS Inc., Chicago, Illinois, USA), where Tukey's method in one-way ANOVA was used to analyze the significance of the differences between multiple groups and student's t-test was used to analyze the significance of the differences between the two groups and the values are expressed as Mean±Standard Deviation, with $p<0.05$ indicates a significant difference.

RESULTS

Protective effects of DP on liver injury after excessive exercise in mice: The protective effect of DP on exercise-induced liver injury in mice was comprehensively evaluated by detecting the changes in the levels of liver function indexes ALT and AST, as well as observing the pathological changes of liver tissues through H&E staining. As shown in Fig. 1, the serum levels of ALT and AST increased significantly in the over-exercise model group compared with the quiet group (Fig. 1a-b, Model vs Normal, $p<0.01$). After DP treatment, ALT and AST were significantly decreased in DP 100 and DP 200 groups compared with the over-exercise model group (Fig. 1a-b, DP 100 or 200 vs Model, $p<0.01$).

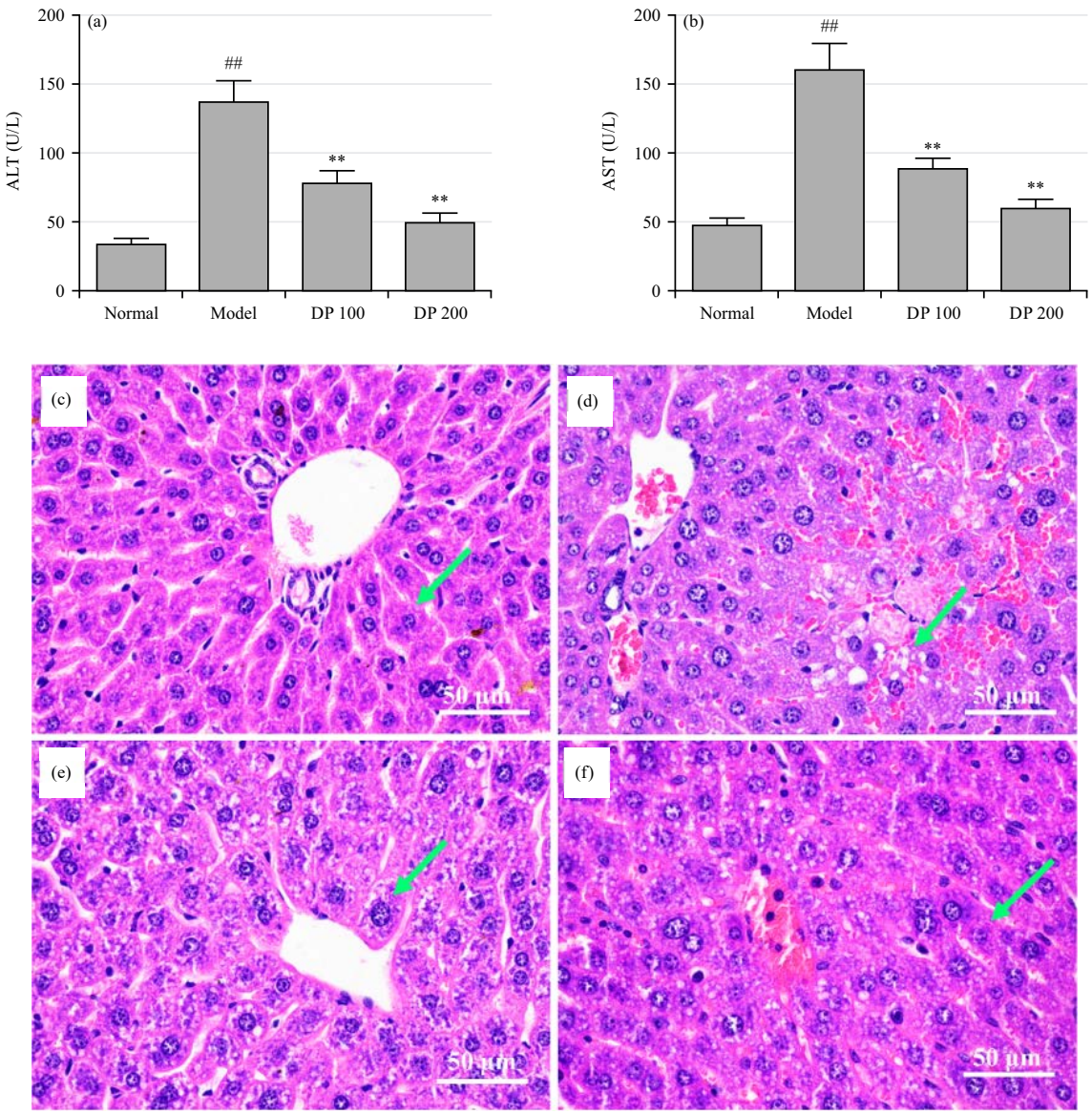

Fig. 1(a-f): Effects of dandelion polysaccharide on the levels of markers and histopathological changes of exercise liver injury in mice, (a) ALT levels in different groups, (b) AST levels in different groups, (c) H&E staining of liver tissue in Normal group, (d) H&E staining of liver tissue in Model group, (e) H&E staining of liver tissue in DP 100 group and (f) H&E staining of liver tissue in DP 200 group

Data are represented as Means±SD, ##p<0.01vs Normal and **p<0.01vs Model

The H&E staining showed that the hepatocytes in the quiet Control group had normal morphology, normal arrangement and uniform cytoplasmic staining (Fig. 1c), while the hepatocytes in the exercise Model group had irregular morphology, disorganized arrangement, uneven cytoplasmic staining and obvious vacuoles (Fig. 1d). But the hepatocytes became more regular in arrangement and uniform in staining after DP 100 or 200 vs Model (Fig. 1e-f). These observations suggested that DP could effectively alleviate overexercise-induced liver tissue injury in mice.

DP improves the antioxidant capacity and reduces the oxidative stress level in the liver of mice after excessive exercise: As shown in Fig. 2, compared with the quiet Control group, the levels of antioxidant enzymes GSH and SOD in the liver of the exercise Model group decreased significantly (Fig. 2a-b, Model vs Normal, $p<0.01$), while the content of oxidative stress product MDA increased significantly (Fig. 2c, Model vs Normal, $p<0.01$). In contrast, with DP treatment, the levels of antioxidant enzymes GSH and SOD were significantly increased in the DP 100 and DP 200 groups

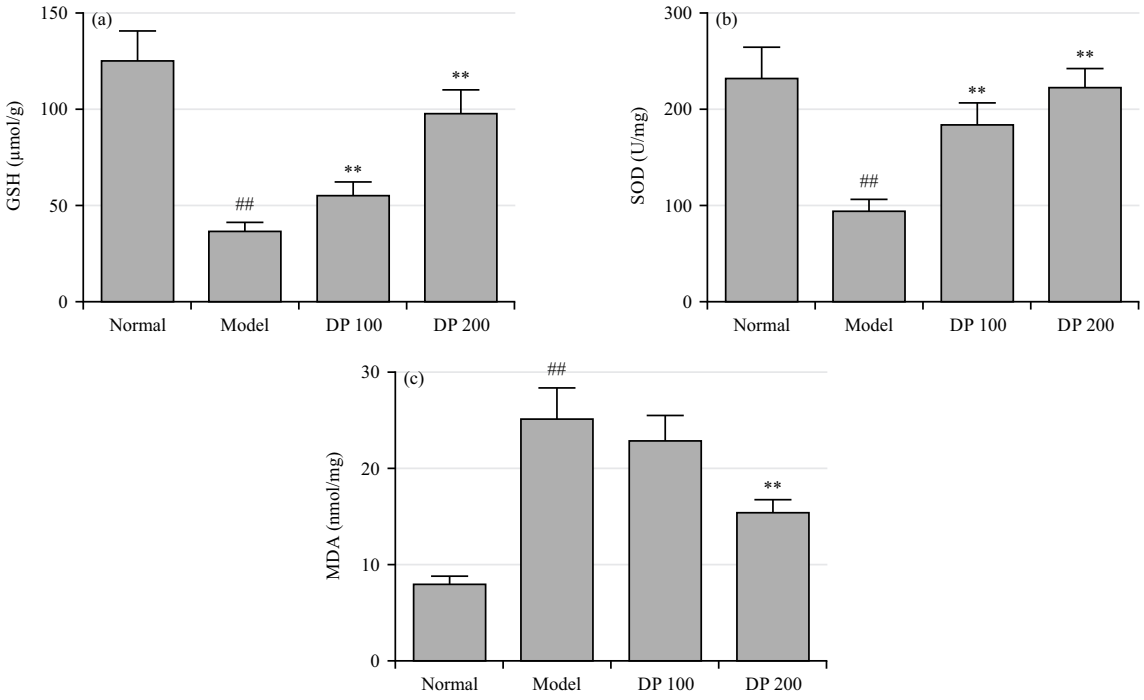

Fig. 2(a-c): Effects of dandelion polysaccharide on the levels of (a) GSH, (b) SOD and (c) MDA in liver of mice after excessive exercise

Data are represented as Means±SD, ##p<0.01vs Normal and **p<0.01vs Model

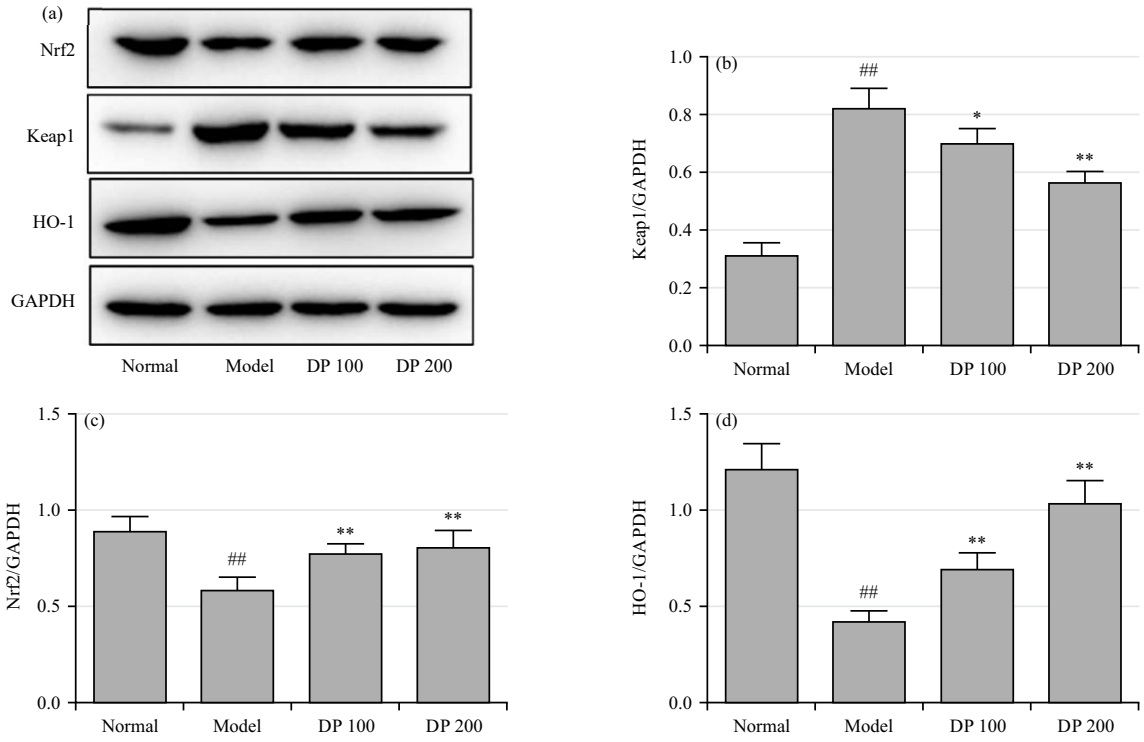

Fig. 3(a-d): Effect of dandelion polysaccharide on the expression of key proteins in Keap1/Nrf2/HO-1 pathway in liver of mice after excessive exercise, (a) Western blot image of key proteins in Keap1/Nrf2/HO-1 signaling pathway, (b) Protein level of Keap1 in different groups, (c) Protein level of Nrf2 in different groups and (d) Protein level of HO-1 in different groups

Data are represented as Means±SD, ##p<0.01vs Normal and *p<0.05, **p<0.01vs Model

compared with the exercise Model group (Fig. 2a-b, DP 100 or 200 vs Model, p<0.01) and the content of the oxidative stress indicator MDA was significantly down-regulated in DP 200 groups (Fig. 2c, DP 200 vs Model, p<0.01). Together, these findings indicated that DP could improve the antioxidant capacity and reduce the oxidative stress level in the liver of overexercise mice.

DP enhances the Keap1/Nrf2/HO-1 anti-oxidative stress signaling pathway in the liver tissue of mice after excessive exercise: As shown in Fig. 3, Keap1, the key protein in the Keap1/Nrf2/HO-1 pathway, was significantly up-regulated in the liver tissues of the overexercise Model group compared with the quiet Control group (Fig. 3a-d, Model vs Normal, p<0.01) and the expressions of both Nrf2 and HO-1 were significantly down-regulated. After the addition of DP, the expression of Keap1 was significantly decreased, while the expression of Nrf2 and HO-1 was significantly increased (Fig. 3a-d, DP 100 or DP 200 vs Model, p<0.05). These observations suggested that DP could enhance the Keap1/Nrf2/HO-1 anti-oxidative stress signaling pathway in the liver tissue of mice after excessive exercise.

DP attenuates inflammation response in the liver tissue of mice after excessive exercise: As shown in Fig. 4 and 5, compared with the quiet Control group, the levels of pro-inflammatory factors TNF-α, IL-1β, IL-6, IL-18, iNOS and COX-2 in serum and liver tissues of the exercise Model group

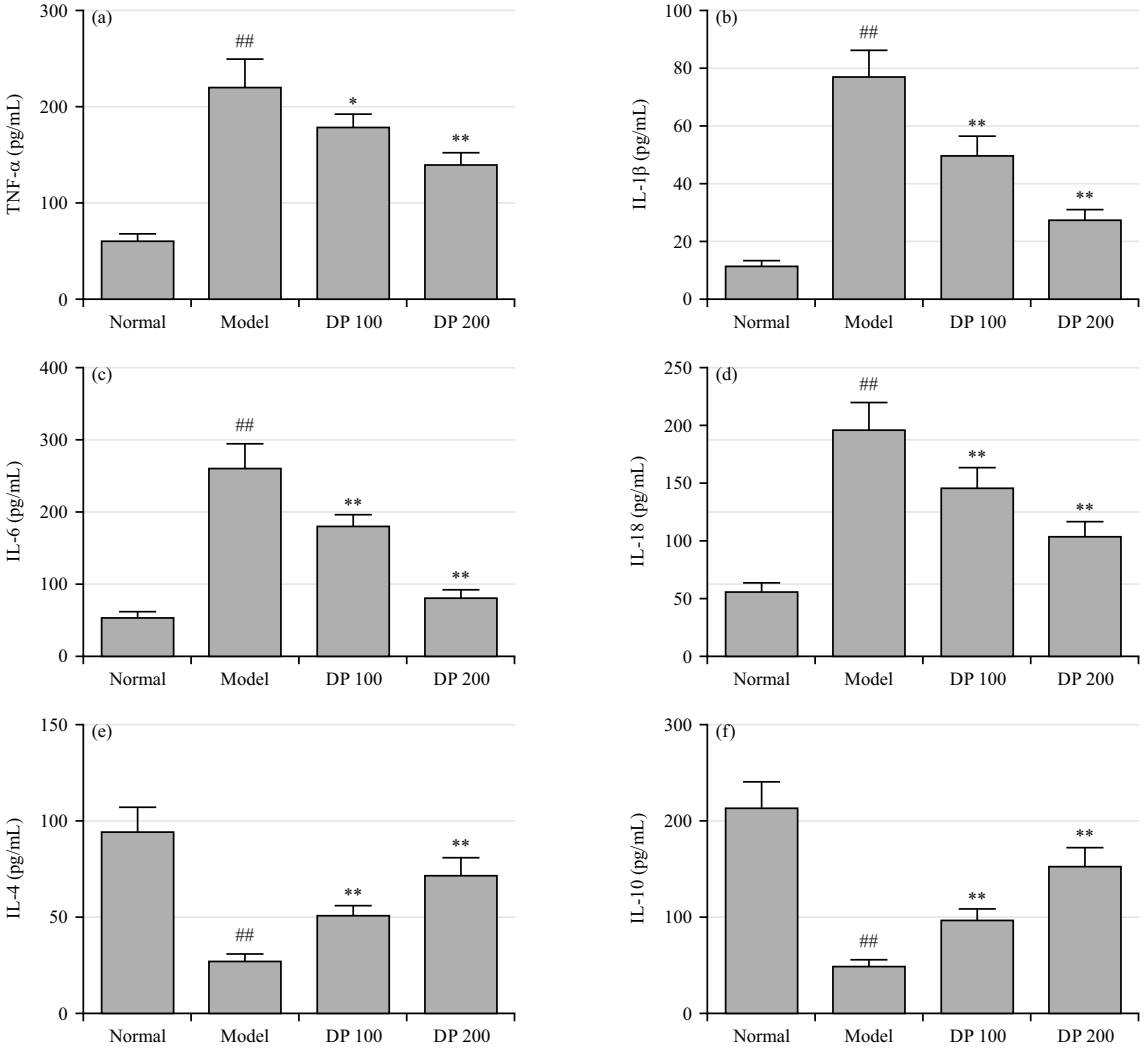

Fig. 4(a-f): Effects of dandelion polysaccharides on the levels of pro-inflammatory factors and anti-inflammatory factors in the serum of mice after excessive exercise. Level of (a) TNF-α, (b) IL-1β, (c) IL-6, (d) IL-18, (e) IL-4 and (f) IL-10
Data are represented as Means±SD, ##p<0.01vs Normal and *p<0.05, **p<0.01vs Model

were significantly increased (Fig. 4a-d, Fig. 5a-c, Model vs Normal, p<0.01), while the levels of anti-inflammatory factors IL-4 and IL-10 were significantly decreased (Fig. 4e-f, Model vs Normal, p<0.01). After the addition of DP, the levels of pro-inflammatory factors were significantly down-regulated in the DP 200 group compared with the overexercise Model group (Fig. 4a-d, Fig. 5a-c, DP 200 vs Model, p<0.01), while the levels of anti-inflammatory factors IL-4 and IL-10 were significantly up-regulated (Fig. 4e-f, DP 100 or 200 vs Model, p<0.01). These findings indicated that DP could attenuate the inflammatory response in the liver tissue of mice after excessive exercise.

DP inhibits the JAK2/STAT3 pro-inflammation signaling pathway in the liver tissue of mice after excessive exercise: As shown in Fig. 6, compared with the quiet Control group, the phosphorylation proportion of JAK2 and STAT3 protein in the JAK2/STAT3 inflammatory signaling pathway was significantly higher in the liver tissues of the overexercise Model group and the expression of SOCS3 was significantly lower (Fig. 6a-d, Model vs Normal, p<0.01). While in the DP 100 and DP 200 groups, the phosphorylation proportion of JAK2 and STAT3 protein were significantly reduced, whereas SOCS3 expression was significantly elevated (Fig. 6a-d, DP 100 or 200 vs Model, p<0.05). These findings indicated that DP could suppress the JAK2/STAT3 pro-inflammation signaling pathway in the liver tissue of mice after excessive exercise.

DP inhibits the HMGB1/TLR4/NF-κB pro-inflammation signaling pathway in the liver tissue of mice after excessive exercise: As shown in Fig. 7, the protein expression of HMGB1, TLR4, MYD88, p-TAK1, CD68 and p-NF-κB p65 in the HMGB1/TLR4/NF-κB inflammatory signaling pathway in the liver tissues of the exercise Model group was significantly higher compared with that of the quiet Control group (Fig. 7a-g, Model vs Normal, p<0.01); whereas, after the addition of DP, the expression of all these key proteins was significantly down-regulated in the DP 200 group (Fig. 7a-g, DP 200 vs Model, p<0.05). These findings indicated that DP could suppress the HMGB1/TLR4/NF-κB pro-inflammation signaling pathway in the liver tissue of mice after excessive exercise.

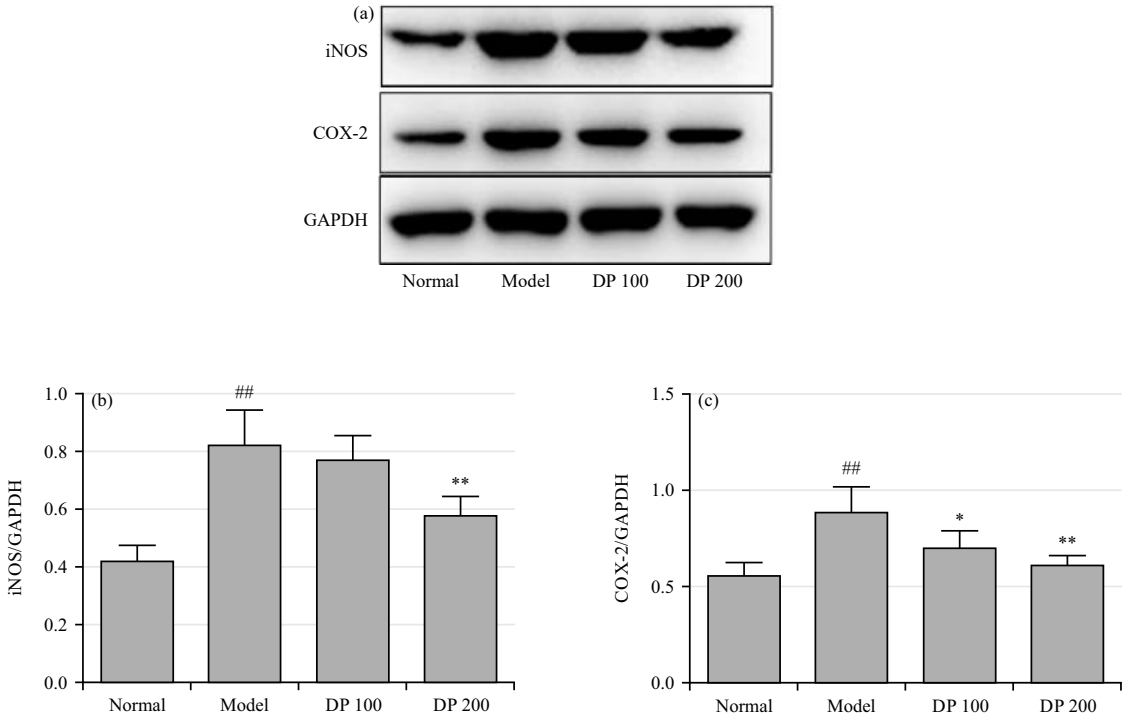

Fig. 5(a-c): Effects of dandelion polysaccharide on the levels of inflammation factor iNOS and COX-2 in liver of mice after excessive exercise, (a) Western blot image of iNOS and COX-2 proteins, (b) Protein expression analysis of iNOS and (c) Protein enzyme analysis of COX-2 via the densitometry of the band in (a)
Data are represented as Means±SD, ##p<0.01vs Normal and *p<0.05, **p<0.01vs Model

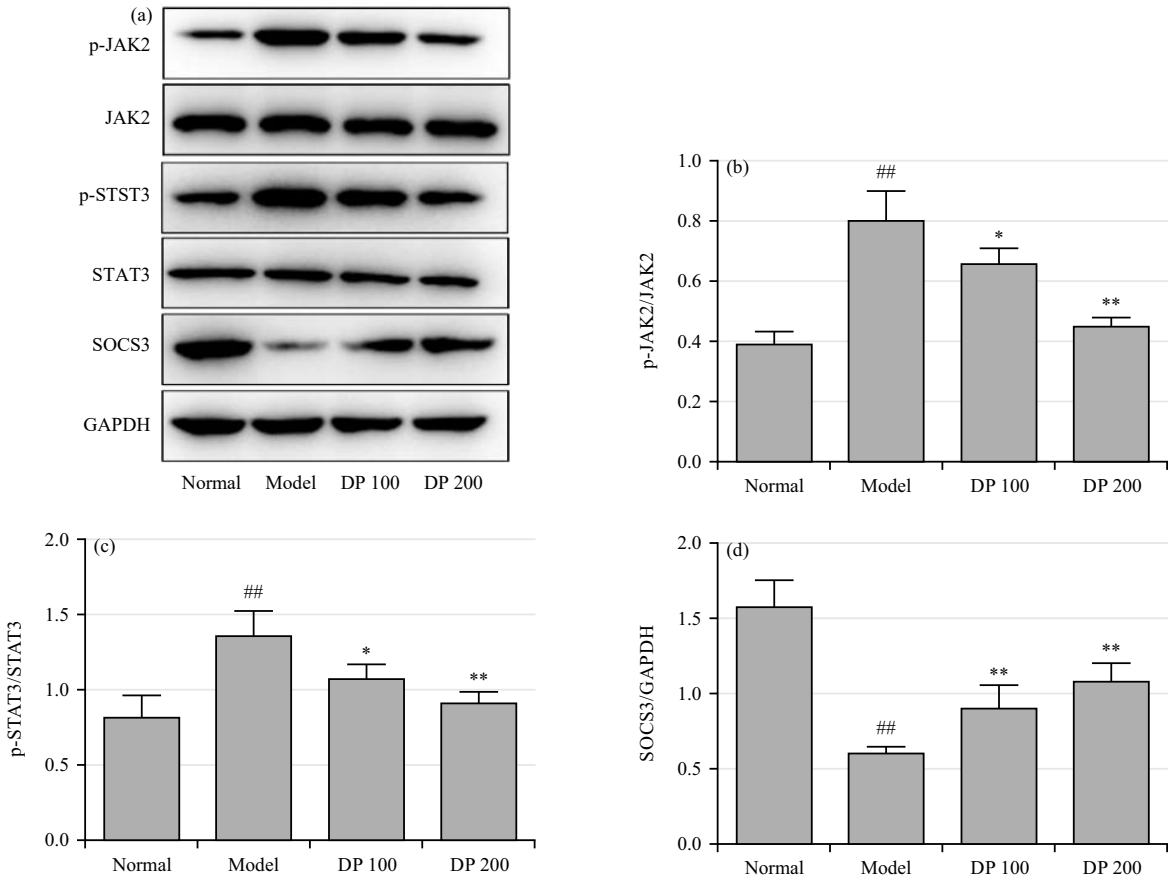

Fig. 6(a-d): Effect of dandelion polysaccharide on the expression of key proteins in JAK2/STAT3 pathway in liver of mice after excessive exercise, (a) Western blot image of key proteins in JAK2/STAT3 signaling pathway, (b) Proportion of JAK2 protein phosphorylation and COX-2, (c) Proportion of STAT3 protein phosphorylation and (d) Protein level of SOCS3 in different groups

Data are represented as Means±SD, ##p<0.01vs Normal and *p<0.05, **p<0.01vs Model

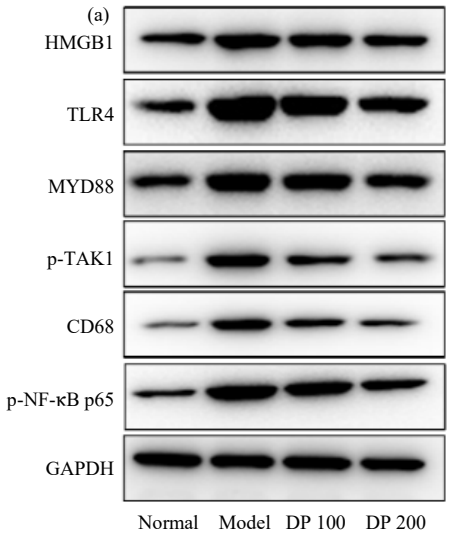

Fig. 7(a-g): Continue

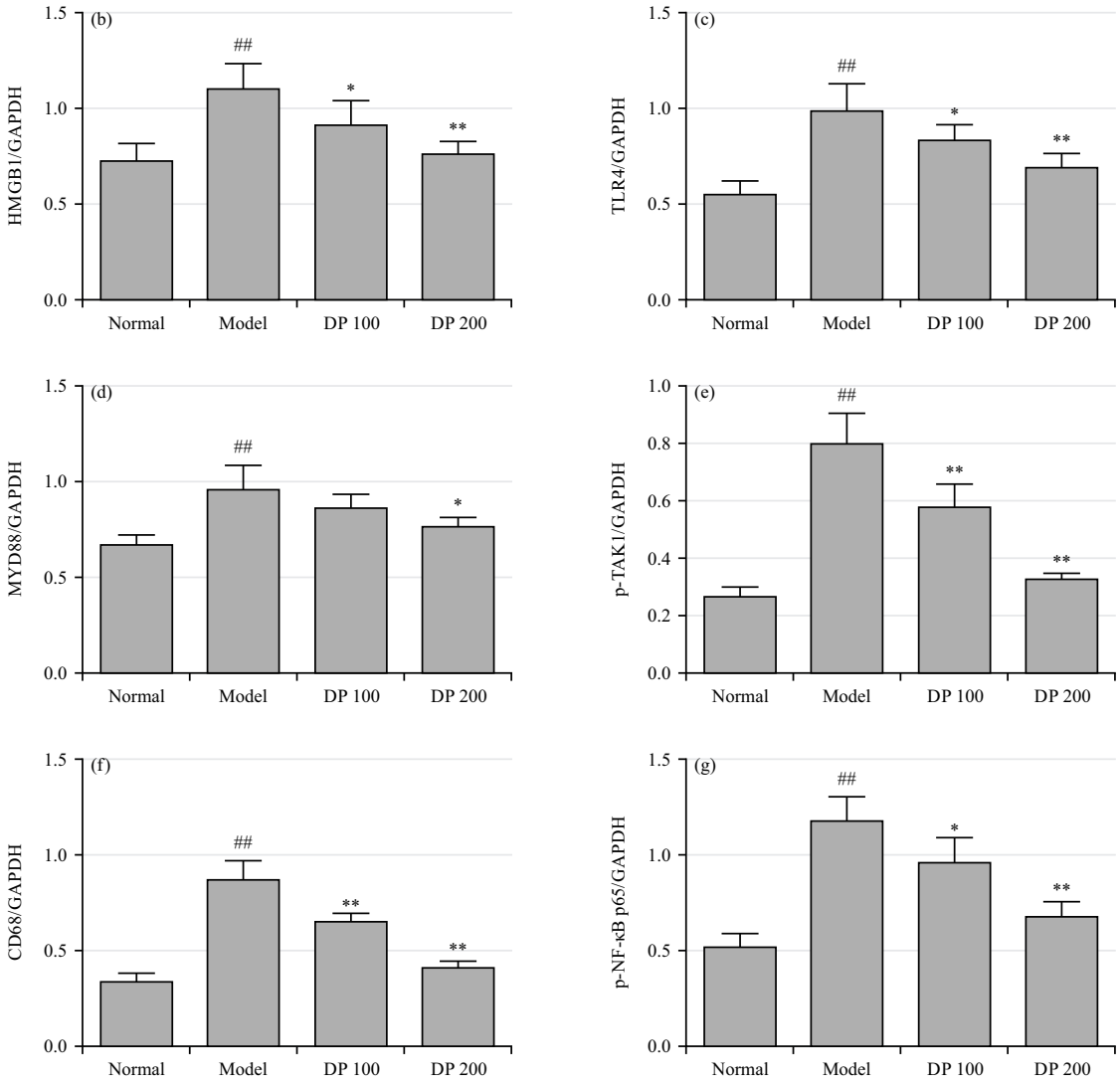

Fig. 7(a-g): Effect of dandelion polysaccharide on the expression of key proteins in HMGB1/TLR4/NF-κB pathway in liver after excessive exercise in mice, (a) Western blot image of key proteins in HMGB1/TLR4/NF-κB signaling pathway, (b) Protein level of HMGB1 in different groups, (c) Protein level of TLR4 in different groups, (d) Protein level of MYD88, (e) Protein level of p-TAK1 in different groups, (f) Protein level of CD68 in different groups and (g) Protein level of p-NF-κB p65 in different groups

Data are represented as Means±SD, ##p<0.01vs Normal and *p<0.05, **p<0.01vs Model

DISCUSSION

Prolonged overloaded exercise or training can cause fatigue of the body and result in sports injuries[22,23], especially in liver tissue[24,25]. In this study, the prolonged overexercise model was made by using mice with long-time load swimming training and the protective effect of DP on exercise-induced liver injury was observed. The results of the study showed that the arrangement of cells in the liver tissues of mice subjected to excessive exercise became disordered and irregular and areas of inflammatory infiltration appeared in the tissues, whereas the liver cells of mice became more regular and the levels of ALT and AST, markers of liver injury, were significantly reduced after dosing 100 or 200 mg/kg of DP during training, which suggests that DP significantly reduced liver injury caused by excessive exercise. These findings suggest that DP can significantly reduce liver injury caused by excessive exercise.

In addition, the protective effect of DP on the liver was also demonstrated by changes in oxidative stress indicators and inflammatory response indicators in serum and liver of mice in the exercise model and DP-treated group, which showed a significant increase in the content of the antioxidant enzymes GSH and SOD, a significant decrease in the lipid oxidation damage product MDA, a significant decrease in the pro-inflammatory factor TNF-α, etc. and a significant increase in the anti-inflammatory factor IL-10, etc., in the DP treated group. In addition to the protective effects against exercise liver injury observed in this study, DP and dandelion extracts have shown significant protective effects in some other types of liver injury[26]. Ren *et al.*[27] found that DP could inhibit hepatocellular carcinoma angiogenesis and exert anti-hepatocellular carcinoma effects by regulating the expression of vascular endothelial growth factor and hypoxia-inducible factor 1α. Davaatseren *et al.*[28] found that dandelion leaf extract ameliorated liver injury in mice induced by methionine and choline deficient diet. In addition, dandelion and its extracts are capable of inhibiting cirrhosis, preventing liver failure and ameliorating parasitic liver injury[29-31]. The results of this study further confirmed the hepatoprotective effect of dandelion, which makes its indications in the clinic more extensive and extends the application of dandelion to the improvement of athletic injuries, which is also helpful for its application and promotion in sports medicine.

The hepatoprotective effects of DP are related to its antioxidant and anti-inflammatory activities[32]. The results of this study showed that the antioxidant signaling pathway Keap1/Nrf2/HO-1 was significantly activated after DP supplementation and the antioxidant HO-1 and GSH and SOD contents were significantly up-regulated, which suggests that the antioxidant capacity of the liver was significantly improved and oxidative stress was effectively inhibited. The DP's hepatoprotective effects is associated with its ability to regulate the antioxidant signaling pathway. The Nrf2/HO-1 is a common antioxidant pathway in the body, however, Keap1 protein often inhibits this antioxidant pathway by binding to Nrf2. In exercise-induced liver injury, Keap1 is significantly increased, which results in more Nrf2 being in the bound state, thus failing to activate the expression of the antioxidant HO-1, while DP could partially reverse this harm by decreasing Keap1 expression. Indeed, alleviation of liver injury through activation of the Keap1/Nrf2/HO-1 pathway is a common feature of a variety of natural plant products and Ma *et al.*[33] found that red algae astaxanthin ameliorates chemotherapeutic drug adriamycin-induced hepatic injury in mice through the pathway and chlorogenic acid, quercetin, coenzyme Q10 and silymarin attenuate acute thioacetamide-induced also via Keap1/Nrf2/HO-1 pathway hepatotoxicity[34].

In addition, the results showed that the pro-inflammatory pathways JAK2/STAT3 and HMGB1/TLR4/NF-κB were significantly inhibited in the liver of mice in the exercise model after supplementation with DP. The JAK2/STAT3 is a common signaling pathway in hepatic inflammatory response[35]. When JAK2 and STAT3 proteins are phosphorylated, the pathway is in an activated state and could promote the production and release of inflammatory factors. Inhibiting the JAK2/STAT3 pro-inflammatory pathway is a commonly used strategy for the treatment of liver injury and Xiong *et al.*[36] found that *Rhodiola rosea* glycoside exerts a protective effect on hypoxia-induced inflammatory responses through inhibiting the JAK2/STAT3 signaling pathway. The studies of Zhou *et al.*[37] indicated that walnut glycosides inhibit fructose-induced hepatic inflammation and apoptosis by regulating the TLR4 and JAK2/STAT3 signaling pathways. The HMGB1/TLR4/NF-κB is another common pro-inflammation signaling pathway in hepatic injury and studies have demonstrated that inhibition of this pathway ameliorates the ischemia/reperfusion injury caused by liver grafts as well as *Toxoplasma gondii* infection-induced liver injury[38,39] and attenuates hepatic ischemia-reperfusion injury[40]. These findings revealed the inhibitory effects of DP on these two pro-inflammatory pathways and proved its ameliorative effects on inflammation in exercise-induced liver injury, which provides a preliminary basis for subsequent in-depth studies related to the anti-inflammatory effects of DP in sports injury.

Notably, although the present study initially revealed that the protective effect of DP against exercise-related liver injury was associated with activation of the Keap1/Nrf2/HO-1 antioxidant pathway and inhibition of the JAK2/STAT3 and HMGB1/TLR4/NF-κB pro-inflammatory signaling pathways. It did not provide enough insights into the dynamics of protein expression in these pathways, nor did it reveal a closely linked between these antioxidant and pro-inflammation pathways. In the future research, more attention will be paid to the related studies in order to reveal the protective mechanism of DP against exercise-related liver injury in more detail. In addition, natural products such as ganoderma tsugae, melatonin, sulforaphane, freshwater clam extract have also shown significant protective effects against exercise-related liver injury[15,41-43], so recent advances in these studies may also provide new ideas for exploring the protective effects of DP against exercise-related liver injury. Moreover, the combination of dandelion extract with antioxidants such as selenium may also exert stronger antioxidant effects and enhance immunomodulatory effects[44]. Therefore, exploring the protocol of DP in combination with other anti-inflammatory and antioxidant agents is also one of the potential avenues to fully utilize its protective effects against exercise-related injuries in future studies.

CONCLUSION

The present study demonstrated that DP attenuated liver injury caused by excessive exercise in mice, which was related to its activation of Keap1/Nrf2/HO-1 antioxidant pathway and inhibition of JAK2/STAT3 and HMGB1/TLR4/NF-κB pro-inflammatory signaling pathways. The present study may provide a theoretical basis for further revealing the detailed protective mechanism of DP against exercise liver injury and provide a reference for the continued exploration to discover the protective value of DP in exercise injury, which may help to provide some basic data for the future development of dandelion or DP-rich natural products into functional foods for assisting exercise.

SIGNIFICANCE STATEMENT

The study was designed to investigate the protective effects of dandelion polysaccharide supplementation on liver injury that induced by extensive exercise. Results showed that dandelion polysaccharide could reduce liver injury and this hepatoprotective effect is involved in activating the Keap1/Nrf2/HO-1 antioxidant pathway and inhibiting JAK2/STAT3, HMGB1/TLR4/NF-κB pro-inflammatory signaling pathway. These findings will provide a partial scientific basis for further expanding the potential application and promotion of dandelion polysaccharides in the field of sports medicine and liver injury.

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REFERENCES

1. Pedersen, B.K. and B. Saltin, 2015. Exercise as medicine-evidence for prescribing exercise as therapy in 26 different chronic diseases. Scand. J. Med. Sci. Sports, 25: 1-72.
2. Armstrong, R.B., G.L. Warren and J.A. Warren, 1991. Mechanisms of exercise-induced muscle fibre injury. Sports Med., 12: 184-207.
3. Baumert, P., M.J. Lake, C.E. Stewart, B. Drust and R.M. Erskine, 2016. Genetic variation and exercise-induced muscle damage: Implications for athletic performance, injury and ageing. Eur. J. Appl. Physiol., 116: 1595-1625.
4. Hassan, A. and R.J. Fontana, 2018. Liver injury associated with sporting activities. Semin. Liver Dis., 38: 357-365.
5. Schelling, X. and L. Torres, 2016. Accelerometer load profiles for basketball-specific drills in elite players. J. Sports Sci. Med., 15: 585-591.
6. Chevion, S., D.S. Moran, Y. Heled, Y. Shani and G. Regev *et al*., 2003. Plasma antioxidant status and cell injury after severe physical exercise. Proc. Natl. Acad. Sci. U.S.A., 100: 5119-5123.
7. Powers, S.K., R. Deminice, M. Ozdemir, T. Yoshihara, M.P. Bomkamp and H. Hyatt, 2020. Exercise-induced oxidative stress: Friend or foe? J. Sport Health Sci., 9: 415-425.
8. VinÑa, J., M.C. Gomez-Cabrera, A. Lloret, R. Marquez, J.B. Miñana, F.V. Pallardó and J. Sastre, 2000. Free radicals in exhaustive physical exercise: Mechanism of production, and protection by antioxidants. IUBMB Life, 50: 271-277.
9. Huang, C.C., T.J. Lin, C.C. Chen and W.T. Lin, 2009. Endurance training accelerates exhaustive exercise-induced mitochondrial DNA deletion and apoptosis of left ventricle myocardium in rats. Eur. J. Appl. Physiol., 107: 697-706.
10. Wang, T., M. Yu, H. Li, S. Qin and W. Ren *et al*., 2023. FNDC5/irisin inhibits the inflammatory response and mediates the aerobic exercise-induced improvement of liver injury after myocardial infarction. Int. J. Mol. Sci., Vol. 24. 10.3390/ijms24044159.
11. Korivi, M., C.W. Hou, C.Y. Huang, S.D. Lee and M.F. Hsu *et al*., 2012. Ginsenoside-Rg1 protects the liver against exhaustive exercise-induced oxidative stress in rats. Evidence-Based Complementary Altern. Med., Vol. 2012. 10.1155/2012/932165.
12. Nogueira, J.E., P. Passaglia, C.M.D. Mota, B.M. Santos, M.E. Batalhão, E.C. Carnio and L.G.S. Branco, 2018. Molecular hydrogen reduces acute exercise-induced inflammatory and oxidative stress status. Free Radical Biol. Med., 129: 186-193.
13. Tang, Y., J. Li, C. Gao, Y. Xu and Y. Li *et al*., 2016. Hepatoprotective effect of quercetin on endoplasmic reticulum stress and inflammation after intense exercise in mice through phosphoinositide 3-kinase and nuclear factor-kappa B. Oxid. Med. Cell. Longevity, Vol. 2016. 10.1155/2016/8696587.
14. Thevis, M., T. Kuuranne, M. Fedoruk and H. Geyer, 2021. Sports drug testing and the athletes' exposome. Drug Test. Anal., 13: 1814-1821.
15. Yang, J., X. Guo, T. Li, Y. Xie, D. Wang, L. Yi and M. Mi, 2023. Sulforaphane inhibits exhaustive exercise-induced liver injury and transcriptome-based mechanism analysis. Nutrients, Vol. 15. 10.3390/nu15143220.
16. Guo, H., W. Zhang, Y. Jiang, H. Wang, G. Chen and M. Guo, 2019. Physicochemical, structural, and biological properties of polysaccharides from dandelion. Molecules, Vol. 24. 10.3390/molecules24081485.

17. Cai, L., B. Chen, F. Yi and S. Zou, 2019. Optimization of extraction of polysaccharide from dandelion root by response surface methodology: Structural characterization and antioxidant activity. Int. J. Biol. Macromol., 140: 907-919.
18. Lima, F.D., D.N. Stamm, I.D. Della-Pace, F. Dobrachinski and N.R. de Carvalho *et al*., 2013. Swimming training induces liver mitochondrial adaptations to oxidative stress in rats submitted to repeated exhaustive swimming bouts. PLoS ONE, Vol. 8. 10.1371/journal.pone.0055668.
19. Qi, B., L. Zhang, Z. Zhang, J. Ouyang and H. Huang, 2014. Effects of ginsenosides-Rb_1 on exercise-induced oxidative stress in forced swimming mice. Pharmacogn. Mag., 10: 458-463.
20. Chen, Q. and P. Wei, 2013. Icariin supplementation protects mice from exercise-induced oxidant stress in liver. Food Sci. Biotechnol., 22: 1-5.
21. Li, Q., X. Tuo, B. Li, Z. Deng, Y. Qiu and H. Xie, 2020. Semaglutide attenuates excessive exercise-induced myocardial injury through inhibiting oxidative stress and inflammation in rats. Life Sci., Vol. 250. 10.1016/j.lfs.2020.117531.
22. Taimela, S., U.M. Kujala and K. Osterman, 1990. Intrinsic risk factors and athletic injuries. Sports Med., 9: 205-215.
23. Flockhart, M., L.C. Nilsson, S. Tais, B. Ekblom, W. Apró and F.J. Larsen, 2021. Excessive exercise training causes mitochondrial functional impairment and decreases glucose tolerance in healthy volunteers. Cell Metab., 33: 957-970.E6.
24. Latour, M.G., A. Brault, P.M. Huet and J.M. Lavoie, 1999. Effects of acute physical exercise on hepatocyte volume and function in rat. Am. J. Physiol. Regul. Integr. Comp. Physiol., 276: R1258-R1264.
25. Shephard, R.J. and N. Johnson, 2015. Effects of physical activity upon the liver. Eur. J. Appl. Physiol., 115: 1-46.
26. Zheng, Y., L. Lei, S. Liang, J. Ai and X. Deng *et al*., 2022. Protective effect of fresh/dry dandelion extracts on APAP-overdose-induced acute liver injury. Chin. J. Integr. Med., 28: 683-692.
27. Ren, F., K. Wu, Y. Yang, Y. Yang, Y. Wang and J. Li, 2020. Dandelion polysaccharide exerts anti-angiogenesis effect on hepatocellular carcinoma by regulating VEGF/HIF-1α expression. Front. Pharmacol., Vol. 11. 10.3389/fphar.2020.00460.
28. Davaatseren, M., H.J. Hur, H.J. Yang, J.T. Hwang and J.H. Park *et al*., 2013. Dandelion leaf extract protects against liver injury induced by methionine-and choline-deficient diet in mice. J. Med. Food, 16: 26-33.
29. Amer, N.S. and A.I. El-Refaiy, 2022. Ameliorative effect of dandelion leaves extract against liver injury induced by schistosomiasis in mice. J. Pharm. Negat. Results, 13: 2554-2569.
30. Hamza, A.A., M.G. Mohamed, F.M. Lashin and A. Amin, 2020. Dandelion prevents liver fibrosis, inflammatory response, and oxidative stress in rats. J. Basic Appl. Zool., Vol. 81. 10.1186/s41936-020-00177-9.
31. Pfingstgraf, I.O., M. Taulescu, R.M. Pop, R. Orăsan and L. Vlase *et al*., 2021. Protective effects of *Taraxacum officinale* L. (dandelion) root extract in experimental acute on chronic liver failure. Antioxidants, Vol. 10. 10.3390/antiox10040504.
32. Mahboubi, M. and M. Mahboubi, 2020. Hepatoprotection by dandelion (*Taraxacum officinale*) and mechanisms. Asian Pac. J. Trop. Biomed., 10: 1-10.
33. Ma, H., S. Chen, H. Xiong, M. Wang and W. Hang *et al*., 2020. Astaxanthin from *Haematococcus pluvialis* ameliorates the chemotherapeutic drug (doxorubicin) induced liver injury through the Keap1/Nrf2/HO-1 pathway in mice. Food Funct., 11: 4659-4671.
34. Hussein, R.M., D.M. Sawy, M.A. Kandeil and H.S. Farghaly, 2021. Chlorogenic acid, quercetin, coenzyme Q10 and silymarin modulate Keap1-Nrf2/heme oxygenase-1 signaling in thioacetamide-induced acute liver toxicity. Life Sci., Vol. 277. 10.1016/j.lfs.2021.119460.
35. Li, M., X. Zhang, B. Wang, X. Xu, X. Wu, M. Guo and F. Wang, 2018. Effect of JAK2/STAT3 signaling pathway on liver injury associated with severe acute pancreatitis in rats. Exp. Ther. Med., 16: 2013-2021.
36. Xiong, Y., Y. Wang, Y. Xiong and L. Teng, 2021. Protective effect of salidroside on hypoxia-related liver oxidative stress and inflammation via Nrf2 and JAK2/STAT3 signaling pathways. Food Sci. Nutr., 9: 5060-5069.
37. Zhou, G.Y., Y.X. Yi, L.X. Jin, W. Lin and P.P. Fang *et al*., 2016. The protective effect of juglanin on fructose-induced hepatitis by inhibiting inflammation and apoptosis through TLR4 and JAK2/STAT3 signaling pathways in fructose-fed rats. Biomed. Pharmacother., 81: 318-328.
38. Lu, J.M., G.N. Jin, Y.N. Lu, X.D. Zhao and H.W. Lan *et al*., 2021. Resveratrol modulates *Toxoplasma gondii* infection induced liver injury by intervening in the HMGB1/TLR4/NF-κB signaling pathway. Eur. J. Pharmacol., Vol. 910. 10.1016/j.ejphar.2021.174497.
39. Xuanfei, L., W. Yakun, Z. Wenfeng, G. Jianping and C. Yao, 2017. Pre-conditioning with tanshinone IIA attenuates the ischemia/reperfusion injury caused by liver grafts via regulation of HMGB1 in rat Kupffer cells. Biomed. Pharmacother., 89: 1392-1400.
40. Xu, L., F. Ge, Y. Hu, Y. Yu, K. Guo and C. Miao, 2021. Sevoflurane postconditioning attenuates hepatic ischemia-reperfusion injury by limiting HMGB1/TLR4/NF-κB pathway via modulating microRNA-142 *in vivo* and *in vitro*. Front. Pharmacol., Vol. 12. 10.3389/fphar.2021.646307.

41. Huang, C.C., W.C. Huang, S.C. Chan, C.C. Yang and W.T. Lin, 2013. *Ganoderma tsugae* hepatoprotection against exhaustive exercise-induced liver injury in rats. Molecules, 18: 1741-1754.
42. Huang, K.C., W.T. Wu, F.L. Yang, Y.H. Chiu and T.C. Peng *et al*., 2013. Effects of freshwater clam extract supplementation on time to exhaustion, muscle damage, pro/anti-inflammatory cytokines, and liver injury in rats after exhaustive exercise. Molecules, 18: 3825-3838.
43. Farjallah, M.A., A. Graja, K. Ghattassi, L.B. Mahmoud and H. Elleuch *et al*., 2023. Melatonin ingestion prevents liver damage and improves biomarkers of renal function following a maximal exercise. Res. Q. Exercise Sport, 94: 869-879.
44. Wang, L., L. Li, J. Gao, J. Huang and Y. Yang *et al*., 2021. Characterization, antioxidant and immunomodulatory effects of selenized polysaccharides from dandelion roots. Carbohydr. Polym., Vol. 260. 10.1016/j.carbpol.2021.117796.