

Comparison of Antifatigue Activity of Five Sea Cucumber Species in a Mouse Model of Intense Exercise

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Abstract Background: Sea cucumber (class Holothuroidea, phylum Echinodermata) is a marine food rich in valuable nutrients and is confirmed to have antifatigue effects. This study aimed to compare the antifatigue activity of five sea cucumber species from China and Thailand. Materials and Methods: A mouse model of effective intense exercise was used. Blood levels of lactic acid (LA), and creatine kinase (CK), liver and muscle glycogen content, malondialdehyde (MDA) content and blood superoxide dismutase (SOD) activity were investigated. Results: *Holothuria scabra* (Thailand) supplementation decreased blood LA and CK levels from 9.17 ± 0.59 and 0.52 ± 0.03 to 7.82 ± 0.97 mmol/L and 0.40 ± 0.08 U/ml, respectively and increased liver and muscle glycogen levels after intense exercise from 10.70 ± 0.18 and 0.93 ± 0.02 to 14.57 ± 1.61 and 1.02 ± 0.06 mg/g, respectively. Identical results were also obtained in the popular Chinese species *A. japonicas*. Furthermore, *A. japonicus* and *Thenenota ananas* supplementation decreased MDA content significantly ($p < 0.01$ and $p < 0.05$, respectively). *A. japonicus*, *H. scabra*, *Acaudina molpadioides* and *Cucumaria frondosa* supplementation significantly increased SOD level. Conclusion: The five sea cucumber species, especially *A. japonicus* and *H. scabra*, display good antifatigue activities.

Keywords: *Holothuroidea*, *antifatigue*, *antioxidant*, *exercise performance*, *mouse model*

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1. Introduction

Fatigue defined as physical and/or mental tiredness and a failure to maintain the required or expected vitality, is a common symptom of stress [1,2,3]. To date, many studies have reported the link between fatigue and various illnesses, such as aging, cancer, depression, HIV infection, multiple sclerosis and Parkinson's disease [4]. Furthermore, fatigue can interfere with exercise performance. During exercise, fatigue is caused by a lack of energy sources, decrease in blood glucose levels and liver glycogen consumption including accumulation of end products of fatigue, such as blood lactate, disruption of the internal environment of the cell, enzymes and hormones [5]. When athletes fail to recover from training, they become progressively more fatigued and suffer from prolonged underperformance [6]. Accordingly, fatigue plays an important role in athletic performance [7]. Many studies have reported that a variety of nutritional supplements are used to improve athletic performance and reduce fatigue [8]. However, effective pharmacological therapies that reduce fatigue have not yet been identified and various research groups are aiming to identify natural bioactive products that can improve physical fitness,

postpone and reduce fatigue and have few side effects [9,10]. Many studies have focused on antifatigue effects of herbs, such as ginseng (*Panax sp.*) [11], *Ixora parviflora*, Rubiaceae [12], fenugreek [13], *P. Quinquefolium* [4], and functional foods, such as jellyfish collagen hydrolysate [14], pumpkin [15], naked oat [16], glutinous rice [17] and chicken essence [3]. Furthermore, a recent report in mice has also shown that antioxidant supplementation could improve grip strength and endurance during swimming [18].

Sea cucumber is a marine food that is rich in valuable nutrients, such as vitamins, minerals and numerous bioactive and antioxidant compounds [19,20,21]. Studies have demonstrated that sea cucumbers can improve immunity and disease resistance and that they have anticoagulant, anti-hypertensive, anti-inflammatory, antimicrobial, antioxidant, antithrombotic, antitumor and anticancer properties [19,21,22]. Furthermore, numerous studies have shown that sea cucumbers have antifatigue effects, especially *A. japonicas* species. Sea cucumber polypeptides have been shown to have significant antifatigue effects in mice, as they significantly prolonged the time of weighted swimming and rolling stick in post-exercise mice [23,24]. The antifatigue effects of sea cucumber oral liquid have been investigated by

ChangHeng *et al.* [25], who have shown that sea cucumber oral liquid significantly prolonged the weighted swimming time, demonstrating that sea cucumber has antifatigue effects. In addition, Bing *et al.* [26] studied the bioactive composition of cultured sea cucumber (*A. japonicus*) and its antifatigue effects in mice. The study of Bing *et al.* [26] indicated that administration of *A. japonicus* for 30 consecutive days prolonged the duration of exhaustive swimming in mice weighted with approximately 5% of body weight. These findings demonstrate that *A. japonicus* has an antifatigue effect and improves exercise endurance in mice. However, little is known regarding antifatigue activity of other sea cucumber species.

Some species of sea cucumber are commonly consumed [19]. The flavour and texture differ between sea cucumber species, and nutrient composition has been shown to vary between different habitats. The most extensively studied sea cucumber species and their bioactive compounds are glycosaminoglycan, lectin, sulphated polysaccharide, peptides and mucopolysaccharide of *A. (Stichopus) japonicas*; triterpene glycoside (saponin), chondroitin sulphates (fucosylated), glycosaminoglycan and polyunsaturated fatty acids of *T. ananas*; triterpene glycoside (saponin), sterol (glycosides, sulphates), peptides, phenols, sulphated polysaccharide and flavonoids of *C. frondosa*; glycosaminoglycan, lectin, phenols and flavonoids of *H. scabra*; bioactive peptides, sulphated polysaccharides, fucoidan of *A. molpadioides* [19,27,28,29,30,31,32,33]. Although many sea cucumber species have been studied for their physiological functions and their bioactive compounds [21], the information about antifatigue activity of many popularly consumed sea cucumber species is still scarce. Consumption of sea cucumber species from Thailand and China is particularly popular. Because these species differ in their habitats and may have different effects on fatigue due to their different compositions. In this study, we used a mouse model of effective intense long term/high intensity exercise in order to examine the potential antifatigue activity of five most commonly consumed sea cucumber species, namely *A. japonicas*, *T. ananas*, *C. frondosa*, *A. molpadioides* from China and *H. scabra* from Thailand. Findings of this study will provide the basis for use of highly valued sea cucumber species and provide novel uses of these species to people engaging in high intensity exercise. Furthermore, these results will support the development of sea cucumber industry in China and Thailand.

2. Materials and Methods

2.1. Materials

A. japonicas was collected from Qingdao, China and *T. ananas*, *C. frondosa* and *A. molpadioides* were purchased from Nanshan markets in Qingdao, China and *H. scabra* was collected from Andaman Sea, Trang, Thailand. All sea cucumbers were dried by hot-air process at 80°C for 8 h. The dried sea cucumbers were soaked in distilled water for 2 days and rinsed thoroughly with water. The sea cucumbers were medium cooked at 60°C until boiling then slightly cooked at 40°C for 30 min. The sea

cucumbers were cooled to room temperature and stored at 4 °C in distilled water for two days. The sea cucumbers were cut into small pieces and lyophilised at - 60°C, pressure 30 Pa [34].

2.2. Animal Maintenance

Male BALB/c mice (18-20 g, 4 weeks old) were purchased from Vital River Laboratory Animal Technology Company (Beijing, China). The mice were housed on a 12/12-h light-dark cycle at 24 °C and allowed free access to distilled water and standard laboratory pellet chow (The Chinese national standard GB 13078 and GB 14924.2, Kangda, Jinan, China. Ingredients: corn, bean pulp, fish meal, flour, bran, salt, calcium hydrophosphate, vitamins, microelements, amino acid, etc.) during the feeding period.

2.3. Experimental Design

Fifty-six mice were randomly divided into 7 groups with 8 mice per group (body weight 18-20 g) for oral gavage treatment daily for 6 weeks as follows: (1) sedentary control (S), (2) exercise control (E), (3) *A. japonicas* (A.j), (4) *T. ananas* (T.a), (5) *H. scabra* (H.s), (6) *A. molpadioides* (A.m) and (7) *C. frondosa* (C.f). Mice in the exercise control group were running on a patented motor-driven wheel-track treadmill to induce intense exercise. S and E groups received normal saline, while A.j, T.a, H.s, A.m and C.f groups received 512 mg kg⁻¹ sea cucumber-lyophilised powder. This dose was equal to consumption of 1/2 sea cucumber weighted (the lyophilised powder was 3 g per person of 60 kg daily, the conversion factor between person and mouse is 12.33) [34]. Body weight and food intake were recorded every day during the 6 weeks of experimental period. This study was conducted according to the guidelines provided by the ethical committee of experimental animal care in Ocean University of China (Qingdao, China).

2.4. Exercise Protocol

A patented motor-driven wheel-track treadmill (YLS-10B, Shandong Academy of Medical Sciences, Jinan, China) was used in this study. All groups except the S group were submitted to an intense exercise protocol (Figure 1A). Briefly, the animals were kept running at 20 rpm, 120 min every day for the first 2 weeks of the study, and then at 30 rpm, 120 min every day for next 2 weeks, which resulted in a daily distance of 1,440 m and induced fatigue. During the exercise, when the mice stopped running, they will be immediately shocked by electricity conducted by the platform and the electric shock times and running distance are automatically recorded by the system of the patented motor-driven wheel-track treadmill.

2.5. Biochemical, Antifatigue and Antioxidant Activity Analyses

After the last exercise session, mice were fasted for 12 h and anaesthetised with diethyl ether. Blood was sampled from the eye and mice were sacrificed by cervical dislocation. Liver and gastrocnemius muscle were collected, weighed and frozen immediately in liquid nitrogen and stored at -80°C for future analysis. For

antifatigue activity analysis, blood lactic acid (LA) and creatine kinase (CK) activities were assayed using LA (millimoles per litre serum, mmol/L) and CK (units per millilitre serum, U/ml) kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China), respectively and liver and muscle glycogen content (glycogen milligram per gram tissue, mg/g) were assayed using kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). For antioxidant activity analysis, malondialdehyde (MDA) and blood superoxide dismutase (SOD) activities were assayed using MDA (nanomoles per millilitre serum, nmol/ml) and SOD (units per millilitre serum, U/ml) kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). All activities were tested according to the manufacturer's instructions.

2.6. Statistical Analysis

Statistical analysis was performed using an one-way ANOVA, followed by Tukey's post hoc test using SPSS v18.0 to compare the differences between groups. Statistical significance was defined as p value less than 0.05. All values in the tables and figures are expressed as the mean \pm standard error of the mean (SEM).

3. Results

3.1. Effect of Supplementation with Five Sea Cucumber Species on Food Intake and Body Weight

A trend of increasing food intake was observed from the beginning to the end of the experiment. Food intake of E group was significantly higher compared to that in S, A.j, T.a, H.s and A.m groups ($p < 0.01$). On week 6, food intake of A.m and C.f groups was significantly higher compared to S groups ($p < 0.05$ and $p < 0.01$, respectively) and food intake of E group was significantly higher compared to S, A.j, and T.a groups ($p < 0.01$, $p < 0.05$ and $p < 0.05$, respectively, Figure 1B). The body weight of A.j and H.s groups approached that of S group from week 4 to week 6 and was significantly lower compared to that of E group ($p < 0.01$ and $p < 0.05$) on week 6, (Figure 1C). These results suggest that sea cucumber supplementation affects the body weight and food intake in mice.

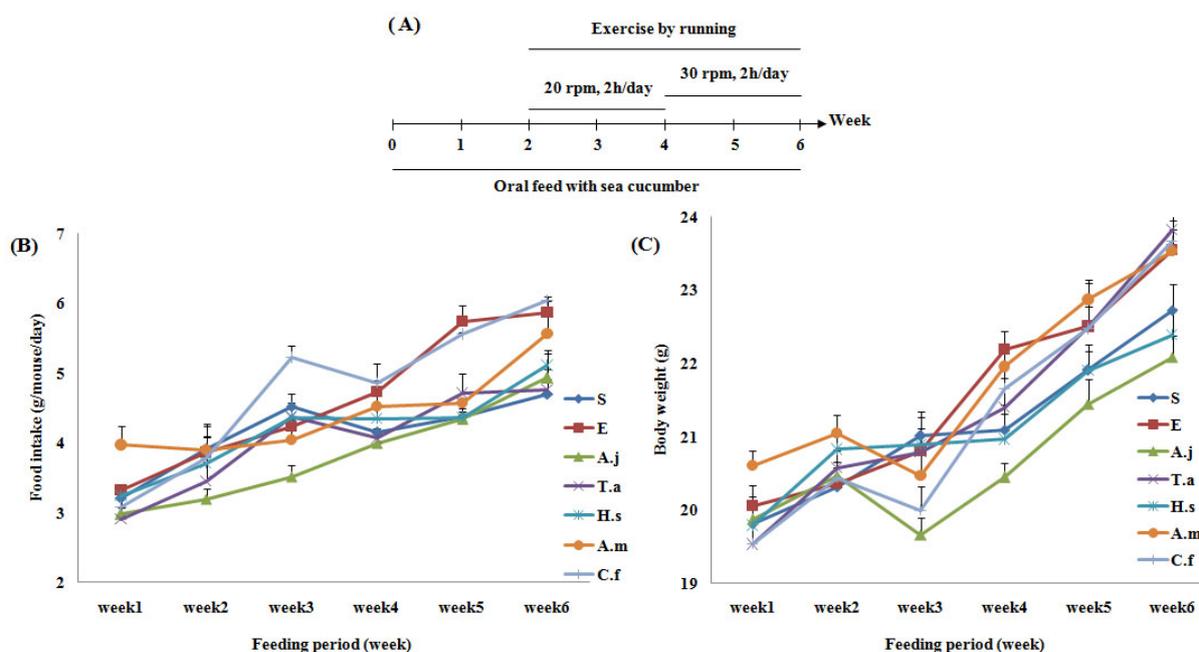


Figure 1. Effect of supplementation with five sea cucumber species on food intake and body weight of mice. (A) A schematic representation of the experimental timeline. (B) Food intake and (C) body weight of mice. Data represent the mean \pm SEM of eight mice in each group. Sedentary control (S); Exercise control (E); *A. japonicas* (A.j); *T. ananas* (T.a); *H. scabra* (H.s); *A. molpadioides* (A.m); *C. frondosa* (C.f) at the dose of 512 mg kg⁻¹ per day

3.2. Effect of Supplementation with Five Sea Cucumber Species on Exercise Performance

Exercise performance is one of the main indicators used to estimate antifatigue activity of compounds. In this study, we used a patented motor-driven wheel-track treadmill, which records the number of times that the animal stops running when it can no longer keep pace on the treadmill. The number of stop times on weeks 2 to week 4 (first 2 weeks) when the mice ran at 20 rpm were lower than on week 4 to week 6 (next 2 weeks) when the mice ran at 30 rpm. There was no significant difference in the stop times between the groups on week 2 to week 4. However, the

stop times of A.j, H.s and A.m groups were significantly lower than those of E group on day 39 ($p < 0.01$, $p < 0.05$ and $p < 0.05$, respectively; Figure 2). These results show that, exercise performance was increased in A.j, H.s and A.m groups, suggesting that supplementation with A.j, H.s and A.m may have a beneficial effect on exercise performance.

3.3. Effect of Supplementation with Five Sea Cucumber Species on Blood LA and CK Levels Related to Fatigue

During anaerobic conditions, blood LA is the product of glycolysis, the main energy source during intense exercise. Thus, a reduction in LA level indicates

antifatigue activity [35,36,37]. After 6 weeks of the experiment, blood LA levels were significantly higher in the E group than those in the S group ($p < 0.01$). The blood LA levels were higher in the E group compared to those in the A.j, T.a groups ($p < 0.01$ for both), as well compared to the H.s and C.f groups ($p < 0.05$ for both, Figure 3A). In fatigued conditions, CK, the enzyme that induces anaerobic metabolism, is released into the serum and is used as an indicator of membrane damage [38]. CK levels were significantly higher in the E group compared to S and C.f groups ($p < 0.01$ for both), as well compared

to A.j and H.s groups ($p < 0.05$ for both, Figure 3B). During intense exercise the body consumes more energy, blood LA level increases causing CK levels to also increase. Increased CK levels occur because the energy derived from aerobic metabolism is not sufficient to support energy needs of the tissue and anaerobic metabolism is initiated, leading to tissue damage and fatigue. A.j, H.s and C.f groups had a significantly lower blood LA and CK levels, suggesting that A.j, H.s and C.f may have antifatigue activity.

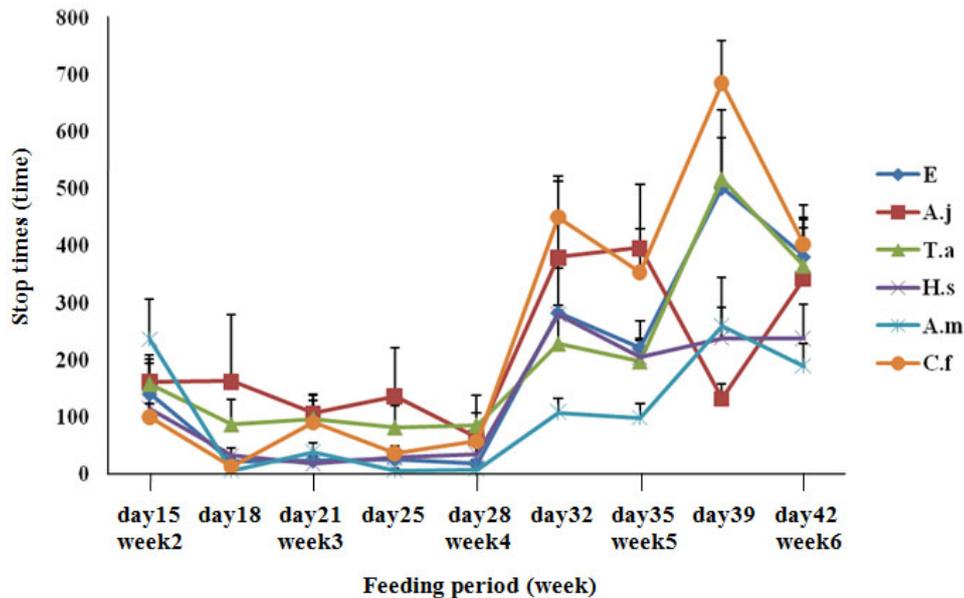


Figure 2. Effect of supplementation with five sea cucumber species on exercise performance. The stop times were recorded when the mice stopped running on wheel-track treadmill test. Data represent the mean \pm SEM of eight mice in each group. Exercise control (E), *A. japonicas* (A.j), *T. ananas* (T.a), *H. scabra* (H.s), *A. molpadioides* (A.m), *C. frondosa* (C.f)

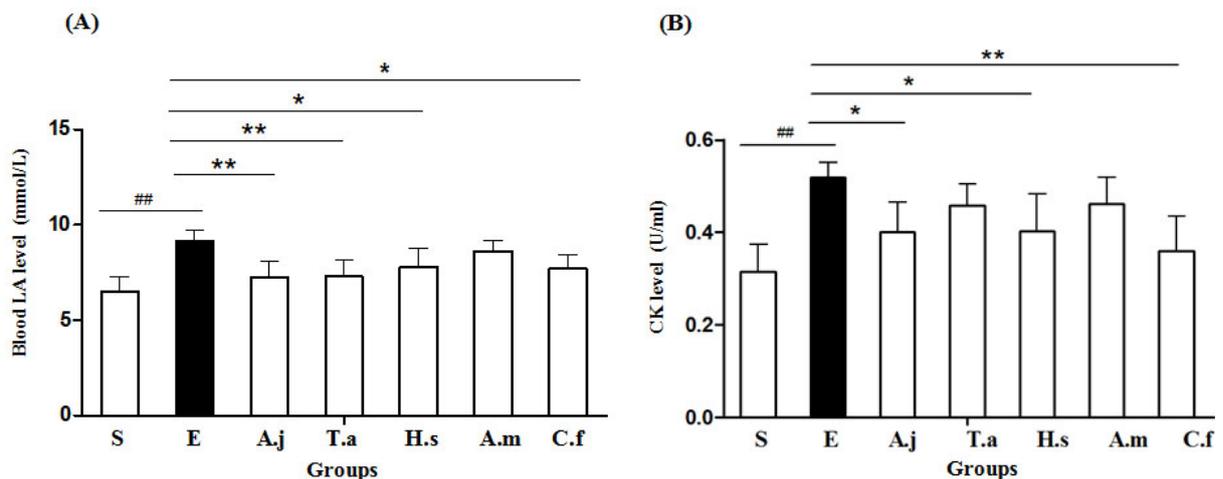


Figure 3. Effect of supplementation with five sea cucumber species on reducing fatigue in mice. (A) Blood lactic acid and (B) creatine kinase activity after 6 weeks of the experiment. Each value represents the mean \pm SEM of eight mice in each group. Sedentary control (S), exercise control (E), *A. japonicas* (A.j), *T. ananas* (T.a), *H. scabra* (H.s), *A. molpadioides* (A.m), *C. frondosa* (C.f). ##Significantly different from sedentary control group, $p < 0.01$. *Significantly different from exercise control group, $p < 0.05$. **Significantly different from exercise control group, $p < 0.01$

3.4. Effect of Supplementation with Five Sea Cucumber Species on Liver and Muscle Glycogen Levels Related to Fatigue

The energy for exercise is derived from the breakdown of glycogen for aerobic or anaerobic ATP production

[37,39], and exercise performance is directly affected by glycogen storage [40]. Therefore, we analysed liver and muscle glycogen levels. The liver glycogen levels were higher in the S group than in the E group ($p < 0.01$). Furthermore, liver glycogen levels were higher in the sea cucumber supplementation groups than in the E group. Specifically, A.j and H.s groups had significantly higher

liver glycogen levels compared to the E group ($p < 0.01$ and $p < 0.05$, respectively; Figure 4A). Muscle glycogen levels were higher in S than in E group ($p < 0.01$). Muscle glycogen levels were higher in the sea cucumber supplementation groups than in E group. Specifically, A.j

and H.s groups had significantly higher muscle glycogen levels than E group ($p < 0.05$) (Figure 4B). These data show that A.j and H.s groups have a significantly higher levels of liver and muscle glycogen, suggesting that A.j and H.s improve exercise performance.

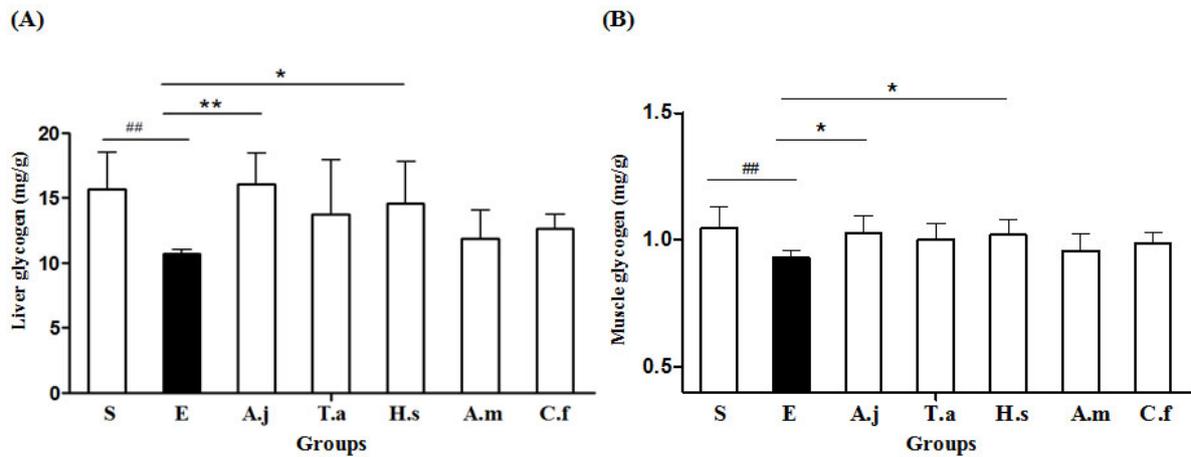


Figure 4. Effect of supplementation with five sea cucumber species on glycogen for energy storage. (A) Liver glycogen levels and (B) muscle glycogen levels at 6 weeks after the start of the experiment. Each value represents the mean \pm SEM of eight mice in each group. Sedentary control (S), exercise control (E), *A. japonicas* (A.j), *T. ananas* (T.a), *H. scabra* (H.s), *A. molpadioides* (A.m), *C. frondosa* (C.f). #Significantly different from sedentary control group, $p < 0.01$. *Significantly different from exercise control group, $p < 0.05$. **Significantly different from exercise control group, $p < 0.01$

3.5. Effect of Supplementation with Five Sea Cucumber Species on MDA and SOD Levels Related to Antioxidant Activity

Intense exercise may cause an imbalance between oxidation and antioxidation systems in the body and result in the increased production of reactive oxide species (ROS), which cause lipid peroxidation and disruption of membrane structure. Lipid peroxidation produces MDA, an indicator of membrane structure damage or oxidative stress [14]. MDA levels were lower in the S group than in the E group ($p < 0.01$) and were lower in the sea cucumber supplementation groups than in the E group. Specifically, A.j and T.a groups had significantly lower MDA levels

compared to E group ($p < 0.01$ and $p < 0.05$, respectively; Figure 5A). SOD levels were higher in S groups than that in E group ($p < 0.01$) and were higher in the sea cucumber supplementation groups than in the E group. Specifically, A.j, H.s, A.m and C.f groups had significantly higher MDA levels than the E group ($p < 0.01$, $p < 0.01$, $p < 0.01$ and $p < 0.05$, respectively; Figure 5B). SOD is an enzyme that is important in protection against oxygen free radical damage [2,41]. Our results show a significant decrease in MDA levels in A.j and T.a groups, while SOD levels were significantly higher in A.j, H.s, A.m and C.f groups. These results suggest that the sea cucumber could increase antioxidant activity during exercise and delay fatigue.

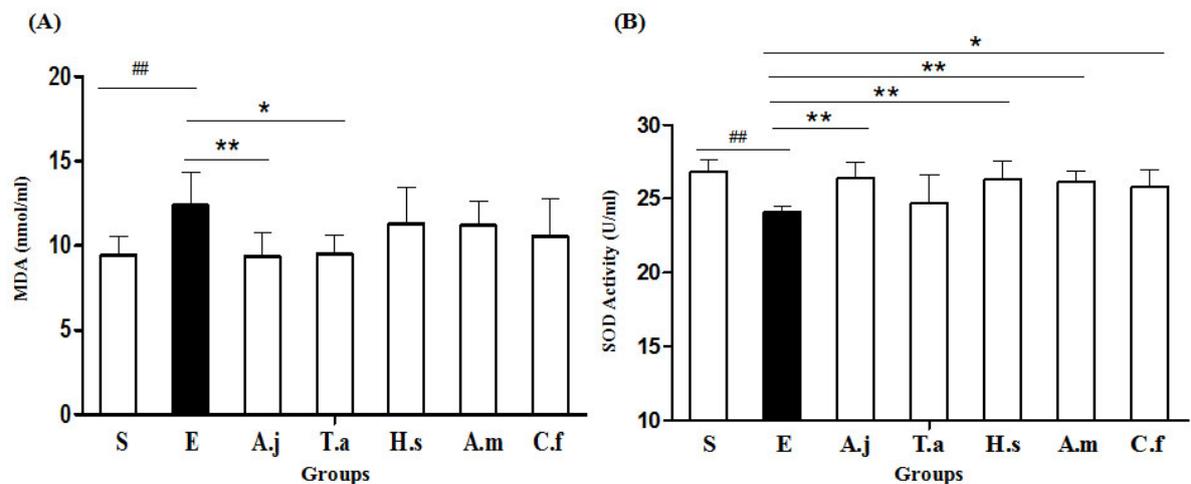


Figure 5. Effect of supplementation with five sea cucumber species on MDA and SOD levels. (A) malondialdehyde (MDA) levels and (B) superoxide dismutase (SOD) levels after 6 weeks of the experiment. Each value represents the mean \pm SEM of eight mice in each group. Sedentary control (S), exercise control (E), *A. japonicas* (A.j), *T. ananas* (T.a), *H. scabra* (H.s), *A. molpadioides* (A.m), *C. frondosa* (C.f). #Significantly different from sedentary control group, $p < 0.01$. *Significantly different from exercise control group, $p < 0.05$. **Significantly different from exercise control group, $p < 0.01$

4. Discussion

Studies have demonstrated that sea cucumbers are rich in valuable nutrients, such as vitamins and numerous bioactive compounds. In our study, the sea cucumber supplementation affects the body weight and food intake in mice. A.j, T.a, H.s and A.m supplementation decreased food intake of mice. It is possible that some valuable nutrients have a direct effect on decreased food intake of mice and due to decreased the body weight of mice, especially *A. japonicus* and *H. scabra* [19,20,21].

Fatigue has broad impacts on human health. It is a symptom of many illnesses and also affects the performance of athletes. In general, fatigue can be divided into physical and mental fatigue. Research on physical fatigue has primarily focused on exhaustion and free radical theories [2,4,42]. Fatigue resulting from medium to high intensity exercise can affect athletic performance. Numerous studies have examined how fatigue limits exercise performance [7] and investigated antifatigue effects of various herbs and functional foods. Sea cucumber is the one of the popular functional foods with antifatigue effects. While most studies investigated antifatigue activity of *A. japonicus*, the current study is the first to compare antifatigue activity of five sea cucumber species in an intense exercise model in mice. In our study, supplementation with A.j and H.s had a stronger antifatigue activity in mice than other three species, according to many studied that A.j supplementation has an obvious antifatigue effect. A.j and H.s supplementation improved exercise performance and displayed antifatigue activity, as detected by biochemical markers such as decreased blood LA and CK levels, increased liver and muscle glycogen levels, as well as antioxidant activity such as decreased MDA and increased SOD levels. These findings are in agreement with previous studies, which have demonstrated that A.j supplementation decreased blood LA and increased liver glycogen levels. Numerous studies have reported that sea cucumber is a source of antioxidants [11,19,43,44] and that supplementation with A.j increases exercise performance [23,24,25,26].

Several animal exercise models have been developed to simulate the physical activities of humans. Rodent models of exercise include treadmill running, wheel running and swimming. However, these models do not control exercise intensity and duration, and include noncontinuous swimming behaviors. In this study, antifatigue activity of five sea cucumber species was evaluated during intense exercise in mice using a patented motor-driven wheel-track treadmill (YLS-10B), which can control the intensity and duration of exercise by setting the parameters. Running duration, distance and the number of stop times were automatically recorded by the system, which is suitable for used in the investigation of animal behaviors in the laboratory.

Previous studies have demonstrated that supplementation with a daily oral liquid of sea cucumber prolonged swimming time of mice [25] and that the sea cucumber polypeptide significantly prolonged the time of weighted swimming and rolling stick in mice [23,24]. Consistent with these reports, Bing *et al.* [26] reported that supplementation with sea cucumber A.j prolonged the

time of exhaustive swimming in mice. Our study showed that A.j, H.s and A.m groups had better exercise performance and significantly fewer stop times compared to the exercise group that did not receive a sea cucumber supplement. The stop times represent fatigue in mice because they indicate the number of times that the mice stop running. Thus, our results suggest that A.j H.s and A.m supplementation may delay fatigue. Additionally, stress of electric shock that mice experienced when they stopped running should be considered.

The composition of sea cucumbers is complex and includes polysaccharides, proteins, lipids and saponins. It is possible that some components have a direct effect on fatigue and that unique contents of each species may affect the dose of sea cucumber required to improve exercise performance. This suggestion is in agreement with a previous report on antifatigue effects of sea cucumber oral liquid in mice, which has shown that a high dose of oral liquid had an effect on blood LA content after swimming [25].

Numerous studies have investigated medical and bioactive compounds of sea cucumber species in China, where sea cucumber industry is highly developed. In contrast, the sea cucumber industry in Thailand is still being developed. We examined *H. scabra*, a sea cucumber species commonly consumed in Thailand, and compared its effects to those of sea cucumber species commonly consumed in China so that our results could be used as a basis for the development of the sea cucumber industry in Thailand in the future. Our results showed that supplementation with three sea cucumber species had antifatigue activity in a mouse model of intense exercise, with *A. japonicas* from China and *H. scabra* from Thailand having a stronger effect than *A. molpadioides*. The stronger effect of *A. japonicas* and *H. scabra* compared to other species may be due to their highly bioactive compounds such as polysaccharides, proteins, lipids and saponins. Because a high dose of *T. ananas*, *C. frondosa* also exhibited antifatigue activity, these sea cucumber species may have different nutrient composition, which should be investigated by future studies.

5. Conclusions

Our data demonstrated that supplementation with sea cucumber species, such as *A. japonicus* and *H. scabra*, had antifatigue activities, which were mediated by a decrease in blood LA and CK levels and an increase in liver and muscle glycogen levels in mice after intense exercise. Furthermore, supplementation with *A. japonicus* and *T. ananas* decreased MDA levels and supplementation with *A. japonicus*, *H. scabra*, *A. molpadioides* and *C. frondosa* increased SOD levels. These results indicate that these sea cucumber species have antioxidant effects in mice. *A. japonicas* and *H. scabra* supplementation can ameliorate biochemical variables related to fatigue and oxidative activities, suggesting that the sea cucumber may be a valuable functional food for people. Further studies are needed to clarify the different effects of the composition in sea cucumber on antifatigue and antioxidant activities.

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Statement of Competing Interests

The authors have no competing interests.

A List of Non-standard Abbreviations

A.j: *Apostichopus japonicas*, T.a: *Thenenota ananas*, H.s: *Holothuria scabra*, A.m: *Acaudina molpadioides*, C.f: *Cucumaria frondosa*, S: sedentary control, E: exercise control, LA: lactic acid, CK: creatine kinase, MDA: malondialdehyde, SOD: superoxide dismutase.

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