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Original Article

Anti-fatigue and Antioxidant Effects of Aqueous Extract of *Echium amoenum* on a Rat Model of Acute Fatigue

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ABSTRACT

Echium amoenum Fisch. & C.A.Mey. has been used for the management of the common cold, inflammation, depression, anxiety, and fatigue. This study aimed to explore the anti-fatigue and antioxidant effects of aqueous extract of *E. amoenum* (AEEA) on a rat model of acute fatigue. After preparing for AEEA, 30 Wistar rats were divided into five groups of six animals. Group 1 received distilled water; Group 2 was treated with distilled water and underwent a forced swimming test (FST); Groups 3-5 rats were administered AEEA (250, 500, and 1000 mg/kg), and subsequently underwent FST. Then, the levels of some biochemical parameters and oxidative stress markers were measured in the serum and liver tissues of rats. AEEA treatment significantly augmented the swimming time of rats compared to the control group. AEEA-treated animals had increased serum glucose and decreased serum urea levels. AEEA diminished serum activities of creatine phosphokinase (CPK) and lactate dehydrogenase (LDH). Treating the rats with AEEA activated superoxide dismutase (SOD) and catalase (CAT) and decreased total oxidant status (TOS) in serum. AEEA increased the levels of thiols and glutathione (GSH) as well as the activity of glutathione peroxidase (GPx) and CAT in the liver tissues. In the liver tissue, AEEA also induced the activity of SOD and reduced TOS. These results may suggest AEEA as a potential anti-fatigue natural product to ameliorate the adverse effects of acute fatigue on the body.

INTRODUCTION

Fatigue is a complication with a variety of symptoms such as tiredness, energy depletion, decreased endurance during exercise, and different forms of physical and mental discomfort [1]. This type of complication may have a detrimental effect on the quality of life of individuals by direct action on energy metabolism in their bodies [2]. A variety of diseases have been shown to be associated with fatigue, such as inflammatory disorders, cancer, autoimmune diseases, hypertension, diabetes mellitus, coronary heart disease, Parkinson, and depression conditions [3-5]. According to recent reports, more than 0.2% of people in the United Kingdom and 800000 individuals in the United States suffer from fatigue [6]. Most individuals with fatigue side effects receive no effective therapy due to the lack of exact knowledge about the pathophysiology and etiology of this discomfort [7]. Therefore, new research projects need to be conducted to find effective and safe anti-fatigue remedies to combat fatigue complications.

A number of mechanisms are shown to be involved in the pathophysiology of fatigue including alterations in the immune system, overwhelming oxidative stress, alterations in some metabolic pathways, genetic predisposition, and hormone imbalances [8]. Among them, oxidative stress has been reported as a key role player in the pathophysiology of fatigue [9]. Oxidative stress is

defined as a condition with an overwhelming amount of free radicals such as reactive oxygen/nitrogen species (ROS/RNS) in the body [10, 11]. This condition generally arises from an imbalance between free radicals and antioxidant systems which can result in augmented levels of free radicals [12, 13]. Interestingly, the evaluation of oxidative stress levels and anti-oxidative activity in patients with chronic fatigue syndrome has been revealed as useful biomarkers for identifying acute, subacute, and resting fatigue in these patients compared to the healthy controls [14].

Natural products may play a role as potential antioxidants with the capacity to protect the body from oxidative stress-induced damage to body organs leading to the prevention of the initiation and progression of many diseases [15, 16]. Plant-derived natural products have been identified to prevent fatigue and oxidative stress-induced organ damage by activating different anti-oxidative mechanisms in the body [17]. In Iranian traditional medicine (ITM), *Echium amoenum* Fisch. & C.A.Mey. has been used as an herbal remedy for the management of the common cold, bronchitis, inflammation, depression, anxiety, and fatigue [18, 19]. This plant has many phytochemical compounds with antioxidant activities [20]. This data show that *E. amoenum* can be used as an antioxidant with anti-fatigue activity. Therefore, the present study was conducted to explore the effect of the aqueous extract of *E. amoenum* on a rat model of fatigue by using a forced swimming test and determining some oxidative stress and biochemical parameters in the serum and liver tissue of the rats.

MATERIALS AND METHODS

Preparation of Aqueous Extract of *Echium amoenum*

Echium amoenum Fisch. & C.A. Mey. flower was purchased from the herbal market of Tehran and was identified by a botanist at Traditional Medicine and Materia Medica Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran (No: HMS-567). The aqueous extract of *E. amoenum* (AEEA) was prepared by boiling in water for 20 min (Plant: Solvent 1:20). Then, the extract was filtered and dried using a freeze-dryer. Total anthocyanins as cyaniding 3-O-glucoside chloride content was determined in the extract. Forty-five ml of methanol was added to 2.5 g aqueous dry extract of *Echium* and stirred for 30 min. The mixture was filtered and diluted to 50 ml. A 10-fold dilution of this solution was prepared in 0.1% HCl in methanol and the absorption of the solution was measured at 528 nm using 0.1% HCl in methanol as compensation liquid. The content of anthocyanins was calculated using specific absorbance of cyanidin 3-O-glucoside chloride (718) [21]. The amount of plant used in traditional medicine is equal to 5 g/day. Therefore, the animal dose of AEEA was calculated as 500 mg/kg of body weight/day. Accordingly, three doses of 250, 500, and 1000 mg/kg of AEEA were used to treat the animals in the present study.

Animals

A total of thirty 10-12 weeks old male Wistar rats weighing between 180 and 200 g were purchased from Pasteur Institute, Tehran, Iran. All rats were housed in a temperature-controlled room (22 ± 2 ° C) with a 12 h light/dark cycle and relative humidity of 50 ± 10%. The animal room had an allergen-pathogen-free condition. The weights of animals were measured and those with the same weight were kept in each cage one week before the experiments. The rats had free access to food and water during the experiments and were handled according to the national institute of health (NIH) guidelines for the care and use of laboratory animals [22]. All experiments were approved by the ethics committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran (Code: IR.SBMU.AEC.1401.045 and IR.SBMU.AEC.1401.046).

Experimental Design

Thirty Wistar rats were randomly divided into five groups of six each. Group 1, as a sham, received distilled water by gavage; the rats in group 2 (control) received distilled water orally and underwent forced swimming test; groups 3-5 were treated with 250, 500, 1000 mg/kg AEEA for 28 days by gavage, respectively, and subsequently underwent forced swimming test.

Forced Swimming Test

The forced swimming test was carried out according to a method described by Yadollahi, et al with some modifications [23]. The test was conducted about one hour after the last treatment. Initially, a lead fish sinker, which was equal to 10% of body weight, was attached to the tail of each rat. Then, the rats were placed in a glass cylinder with a radius of 20 cm and

a length of 65 cm. The cylinder was 40 cm deep with water at a temperature of 25 ± 1 °C. Any failure to harmonic motions and return to the water surface within 10 seconds was considered exhaustion or fatigue sign, and this was recorded as the swimming time.

Measurement of Biochemical Markers of Serum

The effects of AEEA on levels of glucose and urea as well as the activity of lactate dehydrogenase (LDH) and creatine phosphokinase (CPK) in serum samples were measured following the forced swimming test. Briefly, the blood samples were immediately collected in the glass tubes and centrifuged at 3000 rpm for 10 minutes at 4 °C to prepare the serum. All parameters were measured by the spectrophotometric method using an auto-analyzer. CPK and LDH were measured using Pars Azmoon Kits (Pars Azmoon Co., Iran).

Measurement of Oxidative Stress Markers of Serum and Liver Tissue

After separation of the serum and liver tissue supernatants, the level of total thiols, reduced glutathione (GSH), and total oxidant status (TOS) as well as the activity of anti-oxidant enzymes glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT) were spectrophotometrically determined. All these parameters were measured using corresponding commercial kits according to the manufacturer's instructions (Kiazist Co. Hamadan, Iran)

Statistical Analysis

All data reported in the present study are expressed as mean ± SD. To compare the data between the treated and control groups, one-way analysis of variance (ANOVA) followed by Tukey test was used. The results were analyzed using GraphPad PRISM software version 8. A p-value of < 0.05 was considered a statistically significant level.

RESULTS

Extraction and Standardization

The yield of the extract was 27%. The content of total anthocyanins as cyanidin 3-O-glucoside chloride was found as 0.078% ± 0.0016 of the extract.

Effect of AEEA Treatment on Swimming Time

Swimming time for each rat was recorded to evaluate the effect of AEEA consumption on the endurance of animals during exercise. Figure 1 depicts that the treatment of rats with 500 mg/kg AEEA for 28 continuous days significantly ($p < 0.05$) expanded the time of swimming compared to the control group. However, the rats receiving AEEA at doses of 250 and 1000 mg/kg showed no significant change in the swimming times in comparison to the control animals. This may suggest that very low or high doses of AEEA didn't recover the exhaustion of the animal during swimming, but the medium dose of AEEA, which is a common human dose in ITM, could reinforce the exhausted animal.

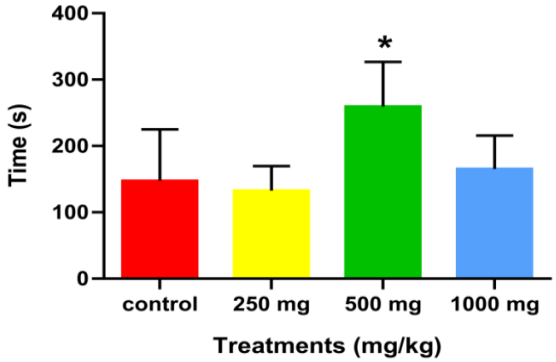


Fig. 1 Effect of different doses of AEEA on the swimming time of rats. A medium dose of AEEA (500 mg/kg) significantly increased the swimming time. All data are presented as mean ± S.D. *p< 0.05 was considered a significant level.

Effect of AEEA Treatment on Biochemical Parameters of Serum

As illustrated in Figure 2 A and B, treating the rats with different doses of AEEA (250, 500, and 1000 mg/kg) significantly ($p< 0.01$, $p< 0.05$) reduced the activity of phosphocreatine kinase (CPK) as compared with the control group. However, only 1000 mg/kg AEEA could significantly ($p< 0.05$) decrease lactate dehydrogenase (LDH) activity when compared with the control group. Of note, the activities of both CPK and LDH in the control group were non-significantly higher than those of the sham group. Figure 2 C demonstrates that the control group showed lower glucose levels than those of the sham group ($p< 0.05$). Besides, the rats receiving 500 and 1000 mg/kg AEEA had significantly decreased glucose levels ($p< 0.05$). Serum levels of urea in control rats were considerably higher than

those of the sham group ($p< 0.01$). However, AEEA at a dose of 500 mg/kg remarkably diminished urea levels in comparison to the control group ($p< 0.01$).

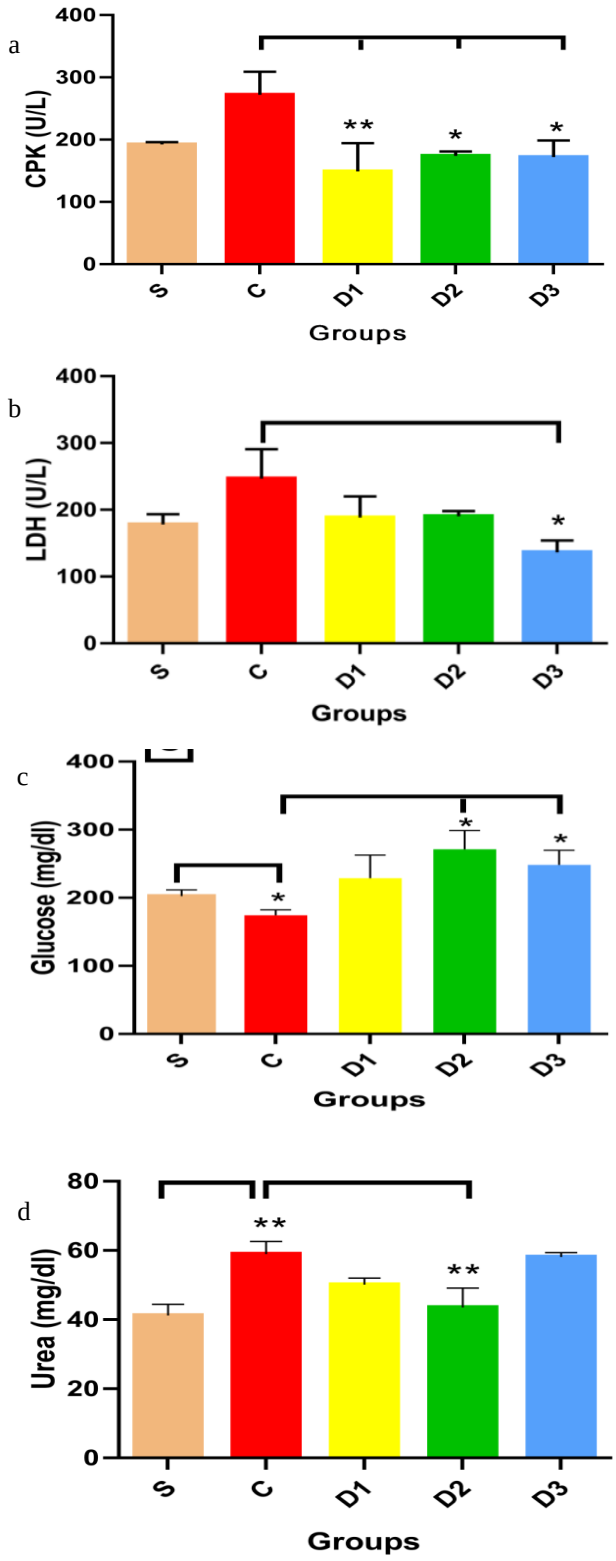


Fig. 2 Effect of different doses of AEEA on the serum activities of creatine phosphokinase (CPK, a) and lactate dehydrogenase (LDH, b) as well as serum levels of glucose (c) and urea (d). The results are shown as mean ± S.D. *$p< 0.05$ and **$p< 0.01$ was considered the significant levels compared to the control. S, Sham; C, Control; D1, 250 mg/kg; D2, 500 mg/kg; D3, 1000 mg/kg.

Effect of AEEA Treatment on Oxidative Stress Markers of Serum

Figure 3 (A-C) represents the effect of AEEA treatment and forced swimming alone on the activity of antioxidant enzymes SOD and CAT as well as TOS in serum samples of rats. Forced swimming in the control group significantly diminished the activity of CAT enzyme in comparison to the sham group ($p< 0.05$). However, AEEA at the doses of 500 and 1000 mg/kg markedly elevated CAT activity compared with the control rats ($p< 0.05$).

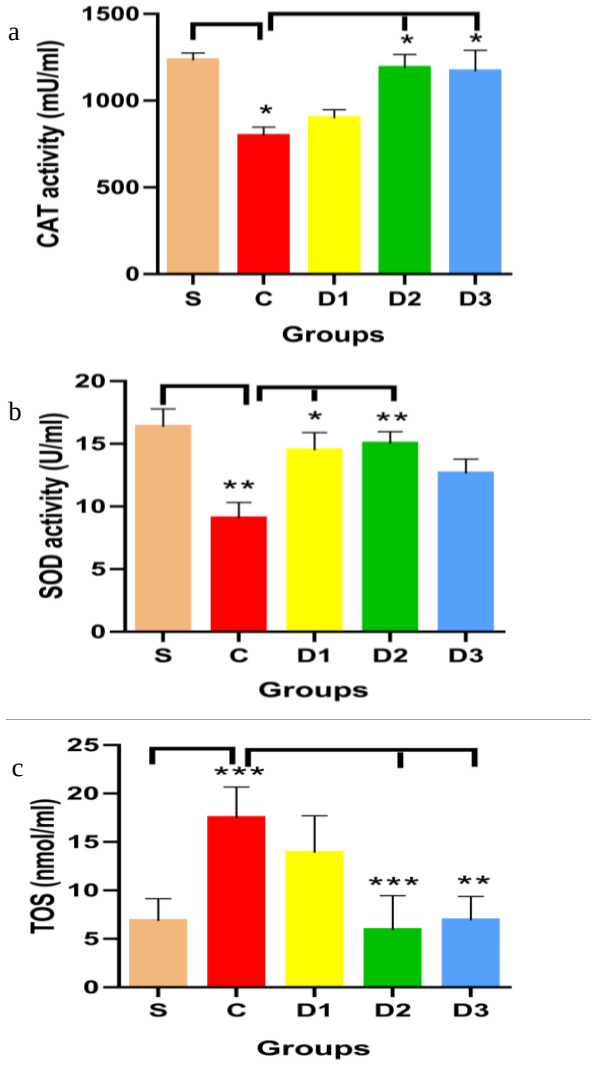
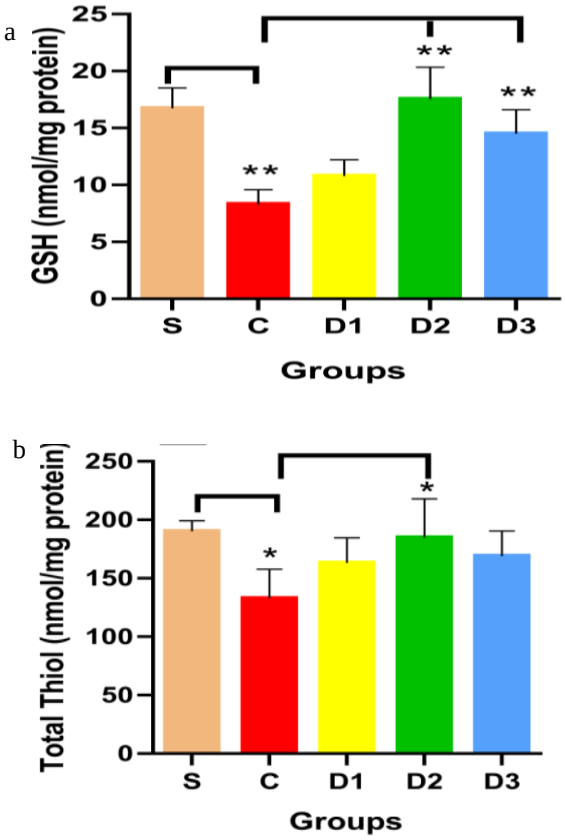


Fig. 3 Effect of different doses of AEEA on the activities of catalase (CAT, a) and superoxide dismutase (SOD, b), and total oxidant status (TOS, c) in serum samples. The results represent mean ± S.D. *$p< 0.05$ and **$p< 0.01$ was considered the significant levels. S, Sham; C, Control; D1, 250 mg/kg; D2, 500 mg/kg; D3, 1000 mg/kg.

SOD activity was also decreased due to forced swimming in control rats compared with the sham group ($p< 0.01$). Nonetheless, 250 ($p< 0.05$) and 500 mg/kg ($p < 0.01$) AEEA significantly augmented the activity of SOD in comparison to the controls. TOS was elevated in the control group when compared with the sham group ($p< 0.001$), but AEEA at the doses of 500 ($p< 0.001$) and 1000 mg/kg ($p< 0.01$) remarkably reduced it in comparison to control rats.

Effect of AEEA Treatment on Oxidative Stress Markers of Liver

The effect of AEEA consumption and forced swimming alone on the levels of GSH, total thiols, and TOS as well as the activity of antioxidant enzymes GPx, SOD, and CAT in liver tissues of experimental rats are shown in Figure 4. Control rats had significantly lower levels of GSH than the sham group ($p< 0.01$). AEEA at the doses of 500 and 1000 mg/kg reversed the levels of GSH compared with controls ($p< 0.01$). Total thiols in control rats were significantly decreased in comparison to the sham group ($p< 0.05$).



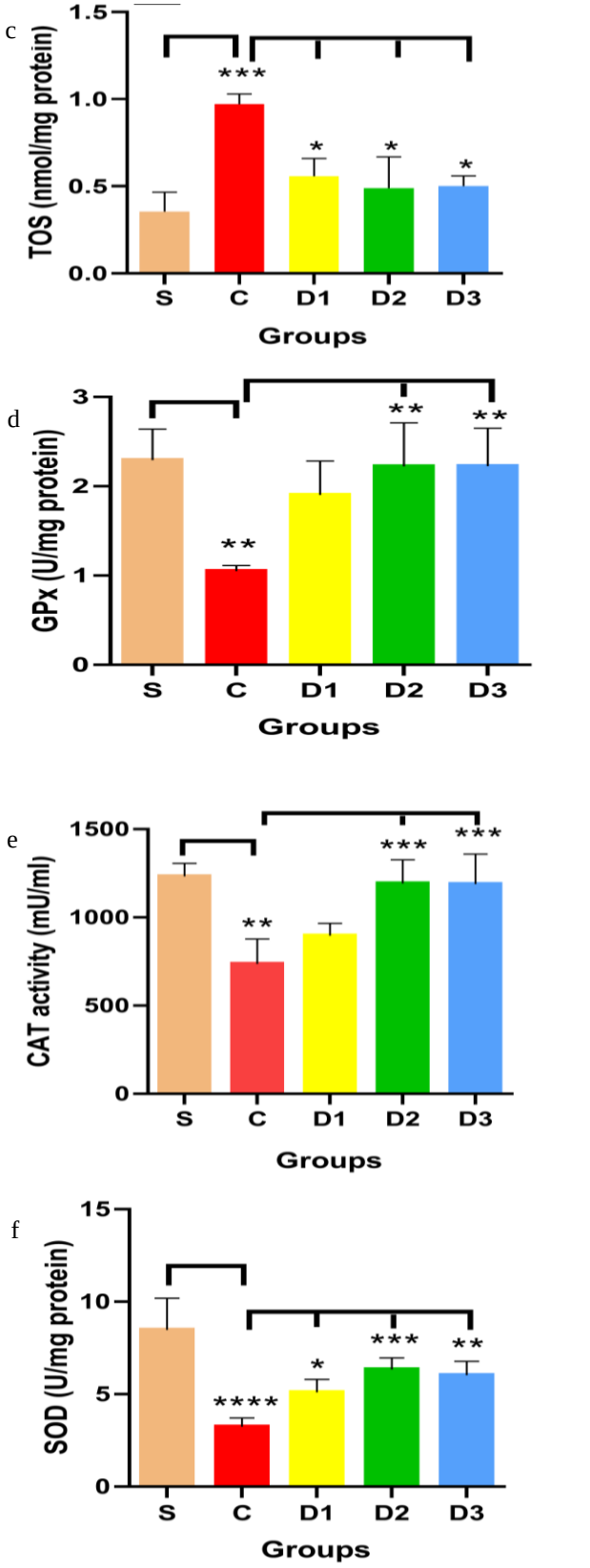


Fig. 4 Effect of different doses of AEEA on the levels of glutathione (GSH, a), total thiol (b), and total oxidant status (TOS, c) as well as the activities of glutathione peroxidase (GPx, d), catalase (CAT, e), and superoxide dismutase (SOD, f) in liver. The results are presented as mean ± S.D. *$p< 0.05$ and **$p< 0.01$ was considered the significant levels. S, Sham; C, Control; D1, 250 mg/kg; D2, 500 mg/kg; D3, 1000 mg/kg.

However, AEEA at a dose of 500 mg/kg raised thiol levels compared with the control animals ($p < 0.05$). Forced swimming in control rats meaningfully amplified TOS compared with the sham group ($p < 0.001$).

However, all three doses of AEEA decreased TOS as compared with the control rats ($p< 0.05$). GPx and CAT activities were significantly lowered in the control group ($p< 0.01$), but AEEA treatment (500 and 1000 mg/kg) reversed their activities to the levels equal to the sham groups ($p< 0.01$, $p< 0.001$). Three doses of AEEA markedly incremented the activity of SOD in comparison to the control group ($p< .05$, $p< 0.01$, $p< 0.001$).

DISCUSSION

According to the present data, rats receiving 500 mg/kg AEEA for 28 days had a significantly incremented swimming time in comparison to the control group. However, AEEA treatment at doses of 250 and 1000 mg/kg had no significant effect on the swimming time of animals. These data suggest that a medium dose of AEEA, which is the common dose in ITM, can increase the endurance of animals against acute fatigue and may be considered a potential anti-fatigue natural drug. To assess the effect of forced swimming and AEEA treatment on energy metabolism, the serum levels of glucose and urea, as two important metabolites in carbohydrate and protein metabolism, were measured in the present study. Forced swimming in the control group significantly decreased the serum levels of glucose and increased urea compared with the sham group. However, AEEA treatment considerably augmented glucose (at 500 and 1000 mg/kg) and reduced urea (at 500 mg/kg). Naviaux, et al showed that individuals with chronic fatigue syndrome are hypometabolic because a variety of metabolic mediators such as amino acids, purines, and lipid derivatives are decreased in the serum of these cases [24]. Decreased levels of serum glucose in the present study may arise from the hypometabolic state that occurs in fatigue or due to the entrance of blood glucose into muscles to be used as metabolic fuel. However, AEEA treatment has increased serum glucose possibly by acting on carbohydrates catabolic pathways. Increased levels of urea in the serum of the rats with fatigue in this study may be suggestive of the catabolic degradation of protein resources in the rat body organs, especially in muscles, and their entrance into the urea cycle. The effect of AEEA on the reduction of serum urea may arise from its effect on protein metabolism either by the inhibition of protein catabolism or its entrance into the urea cycle.

In this study, CPK activity in serum samples was markedly decreased in rats receiving all three doses of AEEA. Forced swimming didn't change the activities of both CPK and LDH in serum specimens of the control group. The plasma activity of the CPK enzyme is one of the important fatigue indices, and thus the elevated activity of this enzyme in serum may suggest muscle damage after unaccustomed eccentric exercise [25]. Besides, persistent fatigue and muscle aches are related to an increased activity of CPK in serum [26]. Therefore, decreased activity of serum CPK following AEEA treatment may show the protective effect of AEEA on fatigue-induced muscle damage in rats treated with this natural product. LDH is an enzyme that catalyzes the reversible reaction of pyruvate to lactate. The activity of LDH in serum can be indicative of released enzymes from damaged organs and tissues. Thus, the serum activity of LDH is a marker for detecting cell injuries or damaged tissues [27]. In the present investigation, a high dose of AEEA (1000 mg/kg) diminished the serum activity of LDH, which may suggest the protective role of this plant product against tissue damage induced by fatigue.

In the final step of the present study, oxidative and anti-oxidative induces of serum and liver were estimated. Forced swimming significantly declined the activity of CAT and SOD enzymes but elevated TOS in serum samples of the control group compared with the sham group. AEEA at doses more than 500 mg/kg markedly elevated CAT activity and diminished TOS levels in serum samples in comparison to the control rats. Serum SOD activity was also increased due to AEEA treatment at doses 250 and 500 mg/kg. Forced swimming reduced total thiols, GSH level, and activity of GPx, CAT, and SOD and elevated TOS in the liver tissue of control rats compared with the sham group. However, treating the rats with 500 mg/kg AEEA augmented the levels of thiols and GSH as well as the activity of GPx and CAT in the liver tissues compared with the control animals. In addition, AEEA treatment, at doses more than 250 mg/kg increased the activity of SOD and decreased

the level of TOS in the liver tissues of rats. Although the present study failed to detect some anti-oxidative indices in serum samples due to technical problems, looking at the levels of other detectable markers in serum and liver tissue may substantially propose an anti-oxidative effect of AEEA, at least in some doses, on acute fatigue-induced stress in the present rat model. The anti-oxidative activity of *E. amoenum* has been reported in several previous studies. Sadeghi, et al provided evidence to highlight that oral administration of AEEA remarkably decreased the levels of ROS and malondialdehyde (MDA) in the hippocampus tissue of a rat model of Alzheimer's disease (AD) [28]. Rabiei and colleagues reported that treatment of AD model rats with hydroalcoholic extract of *E. amoenum* flowers reversed memory impairment and reduced oxidative stress in the brain tissues of animals by decreasing MDA and increasing total anti-oxidant capacity [29]. In another study on a rat model of Parkinson's disease, the results revealed that oral gavage of *E. amoenum* extract exerted neuroprotective effects on hippocampus tissue by attenuating oxidative stress and apoptosis in this tissue [30]. Therefore, it seems that the neuroprotective effects of *E. amoenum* may act by the amelioration of oxidative stress and inflammation as well as the prohibition of apoptosis in neurons [31]. Hence, the anti-fatigue effect of AEEA in the present investigation may arise from its anti-oxidant activity in different organs of the body.

CONCLUSION

The present outcomes provided evidence to underscore the anti-fatigue and anti-oxidant effects of AEEA. According to our data, AEEA significantly expanded the swimming time of rats. In addition, it reduced some fatigue-induced damage markers of body organs. AEEA's effects on some oxidative and anti-oxidative indexes of serum and liver highlighted its anti-oxidant activity in this rat model of acute fatigue. Overall, these results suggest that this drug may act as a potential natural plant-derived anti-fatigue product by a mechanism that boosts endogenous anti-oxidant system to ameliorate the adverse effects of acute fatigue on the body.

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Conflict of Interest

The authors have no conflict of interest to declare.

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