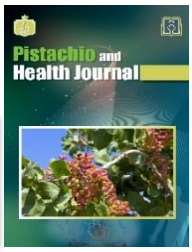
PHJ, Vol. 1, No. 2, Spring 2018, 17-21

**Pistachio and Health Journal**

Journal homepage: http://phj.rums.ac.ir




**ORIGINAL ARTICLE**

# The anti-fatigue effects of the hydro-alcoholic extract of *Pistacia vera* seeds (pistachios) on male Wistar rats

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*Background:* Oxidative stress and free radicals play a crucial role in muscle fatigue. *Pistacia vera* (*P. vera*) contains many antioxidant substances such as coenzyme Q-10, vitamin E and beta-carotene. The current study has been designed to evaluate the effects of *P. vera* seeds (pistachios) on skeletal muscle fatigue in male Wistar rats.

*Materials and methods:* 50 Male Wistar rats were randomly divided into five groups, including the normal, vehicle and treated groups of pistachio extracts (10, 100, and 1000 mg/kg). Muscle fatigue was induced by treadmills. Animals would run at the speed of 20 m/min on a treadmill until they showed fatigue signs. This protocol was repeated for 10 days. The hydro-alcoholic extracts of pistachios were gavaged 30 minutes before the induction of muscle fatigue every day. On the 11$^{th}$ day, rats were sacrificed and biochemical parameters such as creatine phospho-kinase (CPK), lactate dehydrogenase (LDH) and aspartate aminotransferase (AST) levels were measured in the plasma.

*Results:* The different doses of the pistachio extract led to an increase in the fatigue time on days 2 (100 mg/kg) ($p<0.05$), 4 (10 and 100 mg/kg) (all $p<0.05$), 5 (10 and 1000 mg/kg) (all $p<0.05$), 7 (10 and 1000 mg/kg) (all $p<0.05$) and 9 (100 mg/kg) ($p<0.05$). The induction of muscle fatigue led to an increase in the serum activity of CPK, LDH and AST (all $p<0.01$). The pistachio extract (10 and 100 mg/kg) decreased the serum activities of LDH and AST (all $p<0.05$).

*Conclusion:* According to the results of this study, it was concluded that pistachios could decrease skeletal muscle fatigue in male Wistar rats.

**Keywords**: *Pistacia vera*; Muscle fatigue; Antioxidant; Rat

## 1. Introduction

Long and severe physical activities cause muscle fatigue. It is an extremely complex physiological phenomenon that leads to the decrease in the quality of physical activities (1). One of the most established mechanisms of muscle fatigue is the oxidative stress (2, 3). The overproduction of free radicals during exercise can reduce the production of energy in muscle cells and can also damage these cells (4). The intensity and length of the exercise are closely associated with the production of free radicals (5). Some serum biomarkers such as creatine phosphokinase (CPK) (6), lactate dehydrogenase (LDH) and aspartate aminotransferase (AST) are associated with muscle fatigue (3). Free radicals play a significant role in the pathophysiology of muscle fatigue. It has been reported that exposure to the high levels of ROS can lead to an impaired cellular function, a macromolecule damage and apoptosis in skeletal muscle cells (7). Therefore, the administration of compounds such as troxerutin with antioxidant properties protects skeletal muscle cells from possible injuries and induces ameliorative effects (8).

*Pistacia vera* (*P. vera*) belongs to the family of Anacardiaceae. *P. vera* is a plant native to Iran. Many researchers focus on this plant because of the various properties of its leaves, seeds (pistachios) and resins. *P. vera* is a rich source of phenolic compounds with antioxidant properties (9, 10). Pistachios have anti-oxidant, anti-inflammatory (10), hepatoprotective (11), neuroprotective (12), anti-microbial, antiviral (13) and anti-parasitic (14) properties. In addition, raw and non-roasted pistachios contain many minerals and organic substances, including vitamins C and E, beta-carotene as well as tocopherol and mineral elements, including iron, copper, selenium and coenzyme Q-10 (Q10) (9). In previous studies done by the current researches, it was demonstrated that the hydro-alcoholic extract of *P. vera* has antiepileptic, hepatoprotective and nephroprotective effects (15-17).

It seems that oxidative stress plays an essential role in skeletal muscle fatigue, and as pistachios have antioxidant properties, the current study was designed to investigate the effects of the hydro-alcoholic extract of pistachios on the skeletal muscle fatigue time in male Wistar rats.

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DOI: 10.22123/PHJ.2018.121282.1006
Received: 21 April 2018
Accepted: 18 May 2018

## 2. Materials and Methods

### 2.1. Animals

Fifty male Wistar rats (200-250 gr) were purchased from Rafsanjan University of Medical Sciences. The animals were housed under 12-hour dark/light cycles, the temperature was set at 25°C and all animals were fed *ad libitum* until the end of the study. Animal treatments and interventions were approved by the Animal Ethics Committee of Rafsanjan University of Medical Sciences.

### 2.2. The preparation of the pistachio extract

Dried pistachios from *Akbari* species with the genetic code of *M30* were purchased from an herbal pharmacy in Rafsanjan, Iran. The plant originality was verified by Hamid Alipour, an expert teacher in the Pistachio Research Institute of Iran. Pistachios were ground into a fine powder, and 300 grams of the powder were mixed with 900 ml ethanol (80%), as the solvent. The mixed material was macerated and incubated for 12 hours at 50°C. The extraction process was repeated three more times using the slag from the previous stage. The vehicle of the extract was evaporated using the rotary evaporator (Rotavap, England). The extract was frozen and stored at -20°C. The extract was dissolved in dimethyl sulfoxide 2.5% (DMSO, Sigma-Aldrich, Germany), and it was further diluted with DW at the 1:9 ratio, and then administered to the animals proposed through gavage [(16)].

### 2.3. Animal grouping and treatments

The animals were randomly divided into 5 groups (with each group containing 10 rats). Group 1 (normal) received no treatment and intervention. Group 2 (vehicle) received the pistachio extract vehicle (2.5% DMSO, p.o.) half an hour before the induction of muscle fatigue. Groups 3–5 (PE10-1000) received three doses of 10, 100 and 1000 mg/kg/day (p.o.) of the pistachio extract for 10 days, respectively.

### 2.4. The muscular fatigue method

To determine the time of muscle fatigue, a rat treadmill (800-seriesITC-Life science, Spain) was used. One week before the experiment began, the rats were trained for half an hour to run on the treadmill (the speed was set at 15 meters per minute). To induce muscle fatigue, the device was rotated at the speed of 20 meters per minute, and the animals ran everyday on the treadmill (for 10 consecutive days). When fatigue symptoms appeared (the inability to maintain balance on the treadmill), the rats were removed from the treadmill [(18)].

### 2.5. Sampling and serum biomarkers

Twenty four hours after the last treatment, blood samples were taken from retro-orbital veins [(19)]. The blood samples were centrifuged at 3000 rpm for 15 minutes to separate the serum. AST, CPK and LDH levels were determined in serum samples using Pars Azmoon kits (Tehran, Iran) and a BT3000 Auto Analyst (BT3000, Technicon Company, France) [(20)].

### 2.6. Statistical Analysis

SPSS version 18 statistical software was used for the statistical analysis. Results were expressed as mean ± SEM. To compare the fatigue time in different days in each group, the repeated measure ANOVA (RMA) was used followed by the Tukey post-hoc test. Differences among the groups were determined using the one-way analysis of variance (ANOVA) followed by the Tukey post-hoc test. P values less than 0.05 were considered statistically significant

## 3. Results

### 3.1. The effects of the pistachio hydroalcoholic extract on the running time on the treadmill

Fig. 1 shows the duration of running on the treadmill in different experimental groups. The duration of running on the treadmill in animals that received the vehicle group increased gradually during the experiment (from day one to day 10) (RMA followed by Tukey, $p<0.05$). Different doses of the pistachio extract increased the treadmill running time on different days of the experiment, namely day 2 (the dose of 100 mg/kg), day 4 (the doses of 10 and 100 mg/kg), day 5 (the doses of 10 and 1000 mg/kg), day 7 (the doses of 10 and 1000 mg/kg) and day 9 (the dose of 100 mg/kg), in comparison with the vehicle group (RMA followed by Tukey, all $p<0.05$).

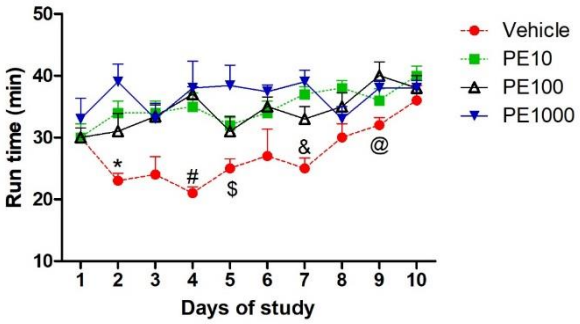


**Fig. 1:** The effect of pistachio extract on the duration of running on treadmill in different experimental groups. *: Significant difference between vehicle and pistachio extract (1000mg/kg) in day 2 ($p<0.05$). #: Significant difference between vehicle and pistachio extract (10 and 100 mg/kg) in the day 4 ($p<0.05$). $: Significant difference between vehicle and pistachio extract (10and 1000 mg/kg) in day 5 ($p<0.05$). &: Significant difference between vehicle and pistachio extract (10 and 1000 mg/kg) in day 7 ($p<0.05$). @: Significant difference between vehicle and pistachio extract (100 mg/kg) in day 10 ($p<0.05$). PE10, PE100 and PE1000: animals that received three doses of 10, 100 and 1000 mg/kg/day (p.o.) pistachio extract for 10 days.

### 3.2. The effects of the hydroalcoholic extract of pistachios on the serum biomarkers

Running for 10 days on the treadmill increased the serum levels of AST, LDH and CPK in the vehicle group compared with the normal group (ANOVA followed by Tukey, all $p<0.05$) (Figs. 2, 3 and 4).

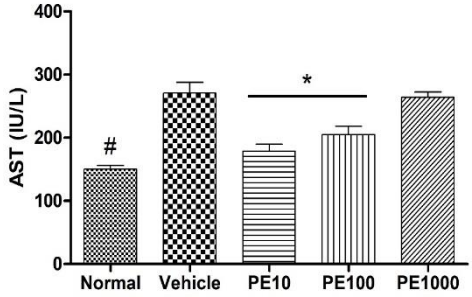


**Fig. 2:** The effect of pistachio extract on the serum levels of AST in different experimental groups. #: Significant difference between normal and vehicle group ($p<0.01$). *: Significant difference between vehicle and pistachio extract (10 and 100 mg/kg) ($p<0.05$). PE10, PE100 and PE1000: animals that received three doses of 10, 100 and 1000 mg/kg/day (p.o.) pistachio extract for 10 days.

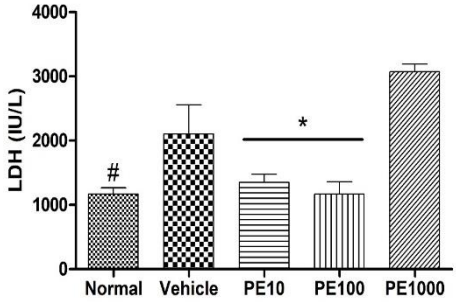


**Fig. 3:** The effect of pistachio extract on the serum levels of LDH in different experimental groups. #: Significant difference between normal and vehicle group ($p<0.01$]. *: Significant difference between vehicle and pistachio extract (10 and 100 mg/kg] ($p<0.05$]. PE10, PE100 and PE1000: animals that received three doses of 10, 100 and 1000 mg/kg/day (p.o.] pistachio extract for 10 days.

The pistachio extract (the doses of 10 and 100 mg/kg) decreased the serum levels of AST (ANOVA followed by Tukey, all $p<0.05$) and LDH (ANOVA followed by Tukey, all $p<0.05$) compared with the vehicle group. The pistachio extract at the doses of 10, 100 and 1000 mg/kg increased the serum level of CPK compared with the vehicle group (ANOVA followed by Tukey, all $p<0.05$).

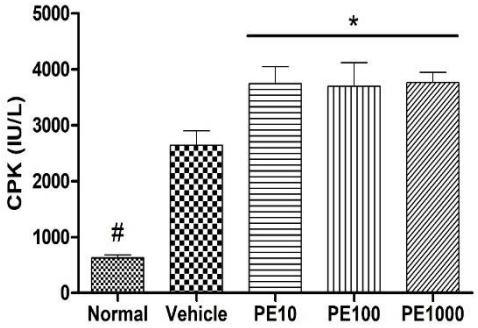


**Fig. 4:** The effect of pistachio extract on the serum levels of CPK in different experimental groups. #: Significant difference between normal and vehicle group ($p<0.01$). *: Significant difference between vehicle and pistachio extract (10, 100 and 1000 mg/kg) (all $p<0.05$). PE10, PE100 and PE1000: animals that received three doses of 10, 100 and 1000 mg/kg/day (p.o.) pistachio extract for 10 days.

## 4. Discussion

Regular exercise and physical activities have many benefits in improving health. However, prolonged and intensive physical activities lead to muscle fatigue [(1).

Muscle fatigue is defined as the decrease or incapability of maintaining the muscle strength that affects the athletic performance in the long term [(1). During exercise, the production of reactive oxygen species (ROS) in the skeletal muscle mitochondria increases, and the oxidative stress damages muscle cells. The current study was focused on the role of antioxidants in delaying skeletal muscle fatigue [(5). Plants and plant-derived extracts are the major sources of antioxidants [(21). In fact, the consumption of plant-based foods can help our body combat free radicals more efficiently. *P. vera* is a valuable plant that grows in Iran and its leaves, seeds and resins contain many antioxidative and anti-inflammatory components [(22). In the current study, the effects of the hydroalcoholic extract of pistachios on skeletal muscle fatigue were investigated. The results of the study showed that the pistachio extract (at the doses of 10 and 100 mg/kg] increases the fatigue time and reduces the serum levels of LDH and AST. However, the pistachio extract increases the serum level of CPK at all doses.

Previous studies have reported numerous pharmacological effects for *P. vera*, including antioxidant [(23), anticancer [(21), anti-inflammatory [(24), anti-microbial [(13) and neuroprotective properties [(12). Phytochemical studies have shown that pistachios have many valuable compounds and are from among the 50 foods with the most antioxidant properties (25). Martorana et al. demonstrated that pistachios have potent antioxidant and photoprotective effects (23). In addition, it has been shown that the pistachio extract can reduce ROS in a dose-dependent manner (24). Tomaino and colleagues studied the phenolic compounds of pistachios and showed that the phenolic compounds of pistachios, including gallic acid, cyanidin-3-

O-galactoside and epicatechin have the highest rates of antioxidant properties (25). One of the most important mechanisms involved in muscle fatigue is the accumulation of free radicals such as ROS and reactive nitrogen species (RNS) (2, 3). Therefore, during exercise, the production of free radicals increases and the production of energy in the skeletal muscles reduces (4). This phenomenon leads to muscle injuries associated with skeletal muscle fatigue (26). As a result, it is probable that the pistachio extract reduces muscular fatigue by antioxidant effects.

In this study, the hydroalcoholic extract of pistachios (10 and 100 mg/kg) reduced the LDH and AST levels in rats. Casoet et al. found that Q10 reduces the muscular pain induced by exercise in patients treated with statin (27). Accordingly, the results achieved might be attributed to Q10 in the pistachio extract.

Also, the results of the current study demonstrated that the hydroalcoholic extract of pistachios increases the level of the CPK activity. However, the study done by Kon et al. showed that although the treatment with Q10 in rats reduces exhaustive exercise-induced muscular injuries, yet it has no effects on plasma CPK (28).

## 5. Conclusions

In conclusion, according to the results of the current study, the hydroalcoholic extract of pistachios can increase the fatigue time and decrease LDH and AST enzymes. In addition, the results of different days demonstrated that the dose of 100 mg/kg of the pistachio extract has effective effects on the body strength and leads to reducing fatigue.

## Conflict of interest

The authors declare no conflicts of interest.

## Acknowledgements

This study was funded by the Research Deputy of Rafsanjan University of Medical Sciences, Rafsanjan, Iran (Grant Number 9/3581).

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