Journal of Applied Pharmaceutical Science Vol. 3 (07), pp. 142-146, July, 2013
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2013.3727
ISSN 2231-3354

The effect of 8 weeks endurance training and *Wild Pistachio* supplementation on liver micronutrients in rats

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ARTICLE INFO

Article history:
Received on: 24/04/2013
Revised on: 06/05/2013
Accepted on: 04/06/2013
Available online: 30/07/2013

Key words:
Endurance training, Wild Pistachio extract, micronutrients, female rat.

ABSTRACT

Availability of some minerals in the body is pivotal for human's health. Micronutrients play a supportive role in physiologic performance and physical activities. Authors have shown that exercise may cause the loss of such minerals via a few mechanisms. Therefore, the present paper aimed at determination of the effect of endurance training and wild pistachio supplementation on liver micronutrients in female rats. 28 rats (6-8 years old and 122-180 gr) were randomly divided into four groups, namely Saline-control (SC), Baneh-control (BC), Saline-training (ST), and Baneh-training (BT). Training groups exercised for 8 weeks (5 days a week, 60 min, 25 m/min on treadmill with 0° slope). The rats were orally fed by saline and pistachio extracts for 4 weeks. Tissue biopsy was performed 72 h following the final training session and after 4 h fasting. Statistical analyses were performed by one-way ANOVA at $p \leq 0.05$. A significant difference was detected in amounts of zinc, copper, and calcium between BT group and others (p=0.00). The results obtained from the present study showed that combination of training and pistachio supplementation may improve micronutrients rate after 8 weeks endurance training compared with non-supplemented group.

INTRODUCTION

Until now, many researches have been done on the effectiveness of various kinds of natural products in the improvement of micronutrients. Circa early 19th century, scientists realized that specific amounts of minerals in body are essential for human's health. Minerals are necessary for metabolic and physiologic processes in human body. Some of such elements are required for life, some for growth, and some for cellular functions (Henry *et al.,* 2004). Investigations have been recently performed on the relationship between sports, training, and micronutrients and some found significant variations in micronutrients as a result of different trainings (Ersan *et al.,* 2010; Alan *et al.,* 1998; Ahmet., 2013). On the other hand, heavy exercise has been shown to increase body's mineral demand. Furthermore, training may cause loss of mineral elements (Keen et al.,1987). In addition, it seems that high amounts of minerals are transferred to the circulation system during training presumably from muscles or liver. Some. minerals may be lost by kidneys and excreted through urine (Troy *et al.,* 2008).

In the meanwhile, some minerals may be lost via sweating which is heightened in hot weather. Such excretion may also occur during training through digestive system; however, the mechanism has not been understood yet (Henry *et al.,* 2004; Troy *et al.,* 2008; Koing *et al.,* 1998; Matthew *et al.,* 2011).Authors believe that insufficient uptake of some minerals may cause lower endurance capacity, adverse effect on immune system's performance, and several ailments.

Therefore, training necessitates mineral supplementation (Albion Research., 2008). Use of alternative therapy, especially herbal therapy and nutritional supplements, has increased in order to cure variety of illnesses and improve sport performance. Wild pistachio tree (which is called Iranian turpentine) is from Anacardiaceae. The tree grows is different parts of Iran. This plant can be considered a good substitute for excreted minerals due to inclusion of minerals (Saffarzadeh *et al.,*1999; Davarynejad *et al.,* 2012) and its possible effect on micronutrients amounts. Considering the effect of

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pistachio extract supplementation on micronutrients amounts as a resistance factor against micronutrients loss during endurance trainings, the present paper, thus, aimed at determination of the effect of endurance training and wild pistachio supplementation on liver micronutrients in female rats.

MATERIALS AND METHODS

Pistachio collection

Ripe and fresh naturally-grown fruit were collected in the late summer in Harat City – Yazd Province and they were, then, gently ground by a home grinder in ambient temperature and dry shaddow after acquiring confirmation of botany section of Mazandaran University.

Preparation of pistachio extract

The method proposed by (Hamdan *et al.,* 2004) was adopted for preparing pistachio extract. 10 gr of the fruit powder was boiled with 150 ml water for 45 min. following cooling to room temperature; the solution was filtered by No. 4 filter paper.

Subjects

28 wistar female rats with mean weight 152.25±16.8 gr were acquired from breeding and research center of laboratory animals – Pasteur Institute – Amol.

The subjects were kept in transparent polycarbonate properly-ventilated cages (30×15×15 cm, Razi-Rad Company) at 22±2°C, 12 h darkness, and 50±5% humidity.

The subjects were fed by 10 gr food (Khorak-Dam-Behparvar Co) in 100 gr bd/wt and water was provided in 500 ml bottles ad libitum. After introduction and acquaintance to investigation area and training on treadmill, the subjects were divided into two groups, i.e. control and training. The members of control group did not participate in any training program.

Study Design

Training program was classified into three steps (Fig. 1) as follows: Step 1 (introduction): the subjects walked on treadmill at 5-8 m/min for 5-10 min. Step 2 (overload): the subjects walked on treadmill at 20 m/min for 20 min; duration and intensity of the training increased gradually during 2 weeks and reached the maximum of 25 m/min for 60 min. Step 3 (load preservation): the subjects reached the considered duration and intensity within 2 weeks and continued the training for 6 weeks at fixed duration and intensity.

The animals were further subjected to 5 min warm-up (8 m/min) and 5 min cool-down (8 m/min). Afterwards, the subjects were divided into 4 groups, i.e. Saline-control (SC), Saline-training (ST), Baneh-control (BC), and Baneh-training (BT), each comprised of 7 rats. BC and BT received pistachio extract (75 ml/kg bd.wt) through gavage for 4 weeks; SC and ST, also, received saline in a same manner.

Biopsy

Seventy-two hours after the last training session and 4 h fasting to avoid extreme effects of training, 2 rats from each group were anesthetized with intra-peritoneal administration of a mixture of ketamine (30– 50 mg / kg body weight) and xylazine (3– 5 mg / kg body weight). Liver tissue was immediately frozen by liquid nitrogen and kept at -70°C.

RESULTS

As it is evident in Table 1, a significant difference was detected in liver zinc between BT and other groups (p=0.00) (Fig. 1). Also, a significant difference was seen in liver copper between BT and other groups (p=0.00) (Fig. 2). In addition, liver calcium was significantly different in BT (p=0.00) (Fig. 3). However, no significant difference was seen in liver Fe (p=0.80) and liver phosphorus (p=0.626) in all groups (Figs. 4 and 5).

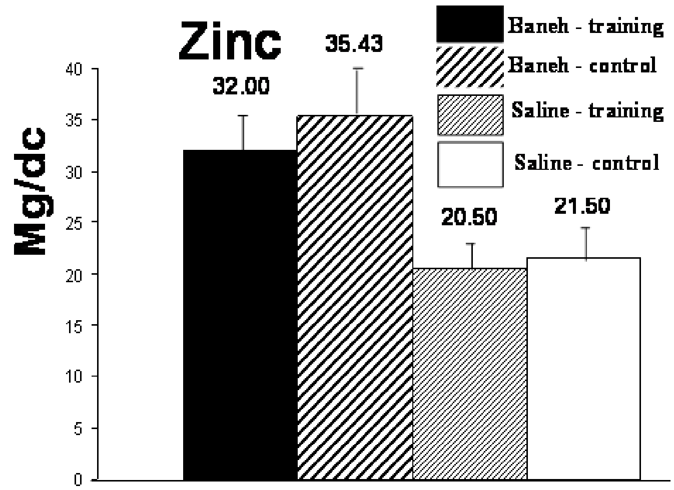

Fig. 1: the mean and SD of liver zinc during exercise with and without pistachio extract for 4 groups (with 7 rats). There were significant differences in liver zinc between BT and ST (p=0.000), SC (p=0.000), ST and BC (p=0.000), SC and BC (p=0.000).

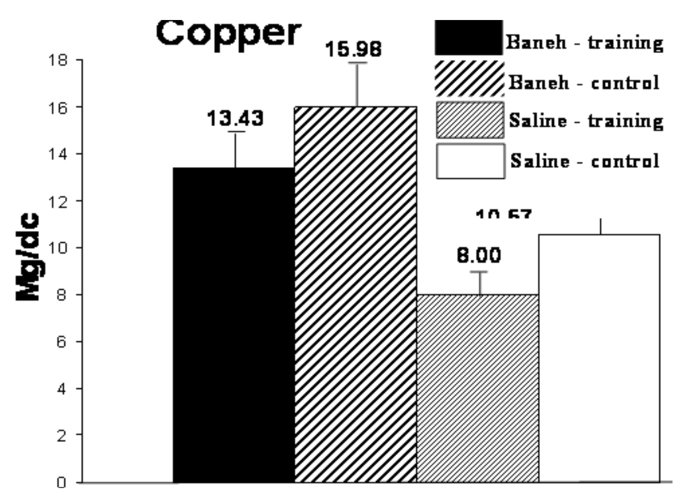

Fig. 2: the mean and SD of liver copper during exercise with and without pistachio extract for 4 groups (with 7 rats). There were significant differences in liver copper between BT and ST (p=0.003), SC and BC (p=0.003), ST and BC (p=0.000).

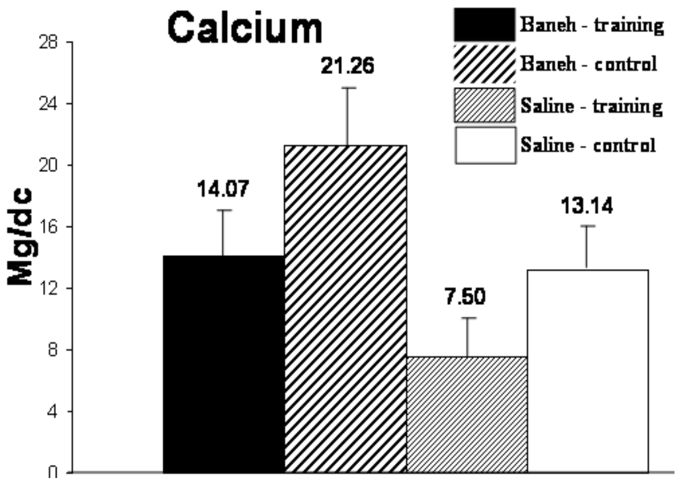

Fig. 3: the mean and SD of liver calcium during exercise with and without pistachio extract for 4 groups (with 7 rats). There were significant differences in liver zinc between BT and ST (p=0.001), BC (p=0.000), ST and SC (p=0.005), BC (p=0.000) and SC and BC (p=0.000).

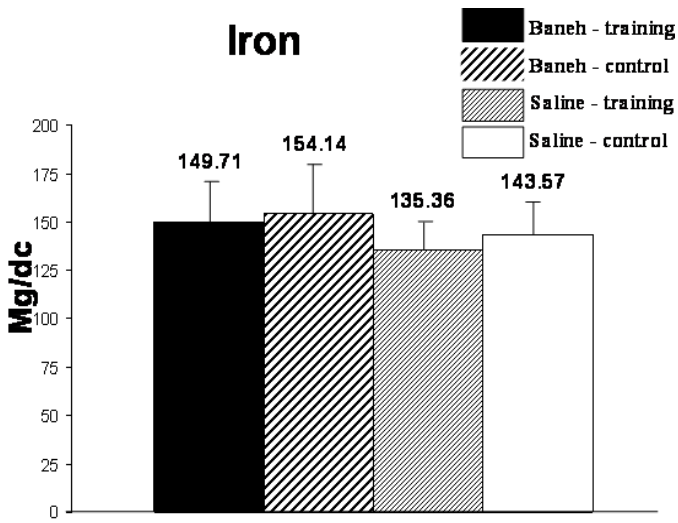

Fig. 4: the mean and SD of liver Iron during exercise with and without pistachio extract for 4 groups (with 7 rats). There were no significant differences in liver iron between the groups (p=0.806).

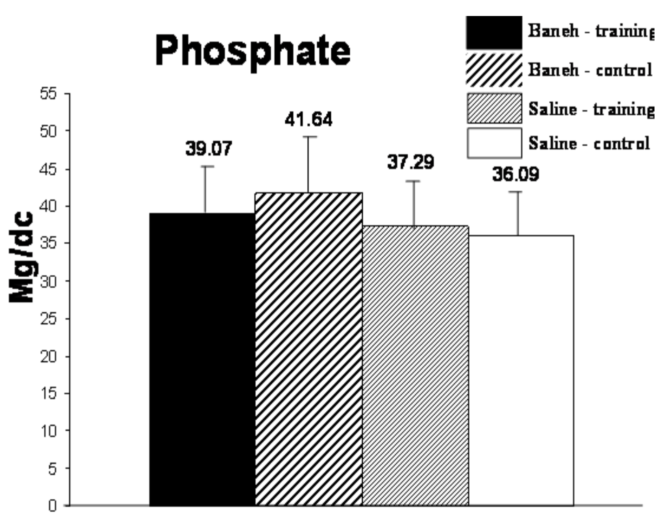

Fig. 5: the mean and SD of liver phosphorus during exercise with and without pistachio extract for 4 groups (with 7 rats). There were no significant differences in liver phosphorus between the groups (p=0.626).

DISCUSSION AND CONCLUSION

The results obtained from the present study showed significant differences in liver calcium, zinc, and copper after 8 weeks endurance training in BT compared with other groups. Furthermore, the results showed that 8 weeks endurance training caused lower liver Fe which is consistent with the results obtained by (Henry *et al.*, 2004; Schumacher *et al.*, 2002). However, the results of this study are not in agreement with those of (Alan *et al.*, 1998) who determined effect of marathon running on concentrations of some minerals in plasma and urine and found that serum Fe concentration decreased considerably after the training. Nevertheless, variations in Fe concentration may response differently in variety of physical activities. In a study, authors investigated the effects of 5-day matches on electrolyte levels in the blood in handball players, blood samples were taken from 12 handball players before and after the match and a statistically insignificant increase was observed in blood iron levels (Koc *et al.*, 2011). In the studies of literature, it was reported that Fe amount in blood decreased with anaerobic exercises while increased with aerobic exercises. In some studies, serum zinc concentration did not change post-race in runners although significant decreases following a 10 mile run and 2 to 4 hours post-stair climbing have been described (Anderson et al. 1984; Van *et al.*, 1986). (Alan *et al.*, 1998) observed no change in urinary zinc losses. It is possible increased urinary zinc losses accounted for the decreased serum zinc concentration observed in the other studies. However, the reported zinc losses appear insufficient to have caused any decline in total body zinc status, which could affect skeletal muscle performance. (Donald *et al.*, 1986) witnessed no significant change in phosphate tablet supplementation and intensive training on treadmill after the training period along with administration of phosphate tablets; this is inconsistent with our results. Increased amount of zinc in BT after 8 weeks endurance training in the present study is in agreement with the results obtained by (College of Physical Education and Sports, Kayseri, Turkey.,2011). They showed that 8 weeks use of zinc supplementation and training had a positive influence on hematocrit parameters of athletes resulting in higher endurance performance. In another investigation (Seco *et al.*, 1998), a significant difference was detected after an intensive training as a result of administration of zinc supplementation in spinal and leg bones in rats. Moreover, (Kaya *et al.*, 2006) detected a significant difference as a result of swimming training. However, Serum calcium concentration did not change, similar to data reported following a 6 mile run by Anderson et al or after the Boston Marathon (Anderson *et al.*, 1984; Rose *et al.*, 1970). However, Olha et al reported a significant increase in plasma calcium concentration following another form of endurance exercise, bicycle ergometer (Olha *et al.*, 1982). These authors were unable to account for the variance. (Alan *et al.*, 1998) found no significant change in plasma copper concentration, consistent with that reported by Anderson et al. in subjects following a 6 mile run (Anderson *et al.*, 1984). On the contrary, both (Ohla *et al.*, 1982;

Ohno *et al.,* 1984) found serum copper concentration increased significantly following bicycle ergometer. The results obtained from the present study about liver calcium of female rats showed that 8 weeks endurance training along with administration of pistachio extract had a significant effect on calcium which is consistent with the results acquired by (Samantha *et al.,* 2003) while it was inconsistent with those of (Ersan *et al.,* 2010). Additionally, some reported that a possible explanation is that almost all nuts are good sources of minerals as magnesium, copper. Magnesium (Mg) level in nuts (pistachio and almonds), provide 8–10% of the DRI for this essential mineral in a 25 g (Allen *et al.,* 1977).

Also, our results showed that liver copper reduced in training group without supplementation after endurance training while it increased in trained-supplemented subjects; this is in agreement with what (Karen e al., 1981) found. On the other hand, (Troy *et al.,* 2008) reported that sweat copper remained fixed after 10 days exercise in heat. (Ahmet *et al.,* 2013) has found when the effects of different training types (interval running, maximum strength, power endurance) were examined, it was determined that acute exercises may lead to increases and decreases in the concentrations of some elements in blood. Such variations may account for the effect of heavy and short-term trainings on reduction of micronutrients.

Effect of pistachio extract on liver micronutrients as a result of training has not been understood very well. However, from edible nuts, pistachio is very popular but less known than others. Nevertheless, pistachios are rich source of energy and contain many health benefiting nutrients, minerals, antioxidants and vitamins that are essential for optimum health (Ferguson *et al.,* 1995). In addition, some authors have shown that nitrogen, phosphorus, potassium, and calcium content of examined pistachio cultivars which were rich sources of different essential elements and their consumption in human dietary is beneficial to human health and can provide necessary dose of elements needed by body (Davarynejad *et al.,* 2012).

In general, pistachio can be considered one of suitable supplements in order to increase micronutrients in body. In this regard, the results obtained from the present study indicate that training along with pistachio supplementation may prevent from reduction of such micronutrients as calcium, zinc, copper, and phosphate and hinder fatigue and inefficiency caused by heavy and long-term trainings.

It can be concluded that administration of pistachio extract during training period, including endurance training, as an herbal supplement may act as a dam against reduction of liver micronutrients.

ACKNOWLEDGMENT

The authors would like to thank the chairman of Payam Noor university of Ilam, Mr Seyed Mohammad Taheri Moghaddam and also Dr Abbas Sohrabzade, chairman of university of central Payam Noor , Ilam, Iran.

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How to cite this article:

Mehdi Said Yousefi., Parvin Farzanegi., Seyed Javad Mirghani., The effect of 8 weeks endurance training and *Wild Pistachio* supplementation on liver micronutrients in rats. J App Pharm Sci, 2013; 3 (07): 142-146.