Effects of Endurance Training in Presence of Crude Magnolia Officinalis Extract on Catalase and MDA of Plasma, Liver and Hypothalamus Tissues in Male Rats

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Abstract
Aims: The antioxidant effects of Magnolia officinalis along with endurance training have not been studied so far. In the present study, the antioxidant effects of crude extract of Magnolia officinalis along with endurance training have been investigated on the antioxidant marker levels of brain and liver tissues in male rats.

Methods: Twenty eight male Wistar rats were randomly divided into four groups as control (saline), training, Magnolia, and Magnolia-training. Training groups underwent an endurance training program for 8 weeks. Rats in training and saline groups received 2 ml crude Magnolia extract orally for 4 weeks. 72 hours after the last training session, plasma samples and tissues were collected for the catalase and MDA measurements. Data were analyzed using t test and Mann-Whitney test at α≤0.05.

Results: Plasma catalase activity in Magnolia group was shown to be significantly higher (p≤0.002) than saline group. Training as well as Magnolia extract results in significant differences (p<0.05 and p≤ 0.007) in the activity of liver catalase. However, liver MDA changes were only significant in Magnolia group compared to saline group (p≤0.013).

Conclusion: The findings suggest that the behavior of plasma and tissue catalase and MDA are different in response to Magnolia and training. It is also shown that catalase activity is intensified by Magnolia extract. Magnolia extract seems to be more effective on liver in comparison with plasma and hypothalamus.

Keywords: Magnolia Officinalis, Plasma, Liver, Hypothalamus
**Introduction**

Today, regular physical activities are known to be a good tool for improving one’s cardio-vascular health and antioxidant defense system. As such, many researchers in cell damage studies especially in the more vital organs such as heart, brain, liver, muscles, and blood cells, have been attending this subject more recently. Free radicals are among the risk factors in cell damage in normal physiological conditions, which need to be fought back by the antioxidant defense system [1,2].

In normal functioning, the major source of producing oxidant free radicals is the electron transport chain which results in H₂O₂. Neutrophilia and viruses do also produce free radicals like Mitochondria. In addition, the production of Hydrogen peroxide can considerably increase as a result of the decomposition of fats in beta-oxidation [1]. It is estimated that normally a cell produces 2*10⁻¹⁰ O₂ and H₂O₂ every day which equals 3.3*10⁻¹⁰ mol per day. The body should decompose this amount and fight it using enzymatic and non-enzymatic antioxidants of catalase and glutathione [2]. It is believed that long and intense physical exercises can act like a double-edged sword. While physical activities can improve the antioxidant defense system, they can increase the generation of free radicals damaging DNA [2,3].

As a result of the improvements in human knowledge and the better functioning of tools and methods, the use of natural and herbal elements effective in sports functioning, in comparison with the more artificial products, has been the focus of attention. However, there has been a limited number of studies carried out on the role of such natural and nutritional complements with Polyphenols, Flavonoids, Isoflavonoids, and other antioxidant elements [4-6].

The athletes and their couches have shown interest in a number of such healing plants and herbs, the most famous of which were Ginseng [7], Berberis [8], Silybum Marianum [9], Fenugreek [10], Teucrim Polium [11], Tribulus Terrestris [12], and Magnolia Officinalis.

Chinese medical herb experts and doctors have achieved a lot of experience in weight control using medical herbs especially Magnolia [13], which is also used for treating a number of diseases and disorders such as depression, anxiety, stomach and intestinal disorders, neurological disorders, asthma, headache, muscular pains, and fever [14-18]. Two of the main and active phenol compounds in magnolia are Magnolol and Honokiol which have strong antibiotic, antioxidant and anti-lipid effect on biological systems. The antioxidant activity of magnonol in heart mitochondria is 1,000 times stronger than Vitamin E in rats. It is also 340 times stronger than Vitamin E in the case of liver mitochondria [21].

In response to endurance trainings, the oxygen consumption increases systematically by 10 to 20 times [22]. It is much higher in the muscles and can reach 100 to 200 times more than resting time [23]. Since 1 to 3 percent of the consumed oxygen changes to free radicals, an increase in the amount of oxygen consumed can result in an increase in electron transfer via respiratory chain and the generation of more free radicals accordingly [24].
Oxygen Species (ROS) that leak from mitochondria during exercising are the main sources of oxidative stress [23]. A number of research studies indicate that oxidative stress is involved in the pathogens of many diseases such as Atherosclerosis, blood pressure, heart ischemia, diabetes, cancer, Rheumatoid arthritis, degenerative neurological system disorders, and the aging process [25-31]. Maintaining the oxidative and anti-oxidative balance in organisms is the obvious survival strategy in all living creatures. The daily physical activities and the stressful conditions can distort such a balance. As such, it is necessary that we avoid the consequences of such an imbalance by the use of natural or artificial nutritional complements containing antioxidants. Consequently, athletes and their couches can prevent dysfunctioning in their performance as a result of a fall in the antioxidant defense system by using antioxidant complements such as vitamin E and C and Carotens or benefiting from the rich nutrients or natural complements in their daily eating programs [32].

The present study was carried out with the aim of reducing the destructive effects of free radicals at the time of exercising by studying the use of complements obtained from magnolia. Considering the lack of information on the effect of using magnolia during endurance training, the present study was an attempt to investigate the effect of using crude magnolia extract and the antioxidant effects of endurance training.

Methods
In this study, the antioxidant effect of endurance training and magnolia was examined using 28 rats divided into four groups: control, training, magnolia, and training-magnolia. The control group did not receive any exercising. The rats in the training group ran on rat treadmills. The other two groups received magnolia orally.

Magnolia Extraction: In order to prepare the magnolia extract, Sohn et al.’s [33] method was used for extraction from the trunk and bark of the Magnolia plant. Every 100 grams of magnolia powder was mixed with 600 ml 80% ethanol and was kept for 72 hours at room temperature. The solution then passed through Whatman no. 1 filter paper and was then rotary dried. After being dried in vacuum and very low temperature, every gram of the obtained material contained 41.15% magnolol which was then mixed with 10 ml Saline.

Animals: There were 28 male Wistar rats with an age range of 8-10 weeks and a weight ranging from 125-135 grams. They were kept in 22±2 C° with a 12:12 light-dark cycle and a humidity of 45-50 percent with an appropriate air conditioning. For each 100 grams in weight, 10 grams food and water were provided for them.

Magnolia Extract Dose: Two hours after the training was over, the magnolia and training-magnolia groups for each 1 kg in weight received 100 mg of crude magnolia extract. This continued for four weeks, six days a week [33].

Endurance Training: The rats in the training groups were made familiar with working with the treadmill for a few days before the training program began. The running was done with an intensity of 25 meters per minute in a zero degree slope for
60 minutes each session, five days a week for 8 weeks as follow:

1) In the first stage, the rats were made familiar with the process by walking on the treadmill for 5 sessions with a speed of 5-8 meters per minute at a zero degree slope for 5-10 minutes each session.

2) In the next stage, the rats ran for 20 minutes with a speed of 20 meters per minute. During 2 weeks, the length and intensity of the exercising gradually increased to reach the experimental condition.

3) In the stability stage, the rats had to run at 25 meters per minute for 60 minutes. This stage continued for 4 weeks, five days a week.

72 hours after the last exercise, the rats were killed. The plasma was prepared from their blood, and the liver and hypothalamus tissues were taken and were washed using sterile physiology serum. They were then frozen immediately using liquid nitrogen and kept in -80°C for measuring Catalase and MDA. For measuring Catalase, the laboratory method was used (Catalase assay kit, Nanjing Jiancheng Bioengineering, Institute, Nanjing, Chaina, Intraassay CV%: 4.1, Sensitivity: 0.55 U/mL). For measuring MDA, the following method was used (TBARS, Chemical colorimetric, Cayman, MI, USA, Intraassay CV%: 6.4 Sensitivity: 0.08 µM).

**Data Analysis:** Both descriptive and inferential statistics were used. In order to check the normality of data, Kolmogorov–Smirnov test was used. In order to check each group with the control group either independent samples t test or Mann-Whitney test was used according to the nature of the normality of the data.

**Results**

As evident in Table 1, the results showed that the catalase in plasma was significantly higher in the magnolia group than the control group. The training-magnolia showed a significantly higher level of liver catalase among the four groups in comparison with the control group. However, the changes in liver MDA were significant only in the case of magnolia group in comparison with the control group.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Group</th>
<th>MDA Mean (S.D)</th>
<th>Catalase Mean (S.D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>Control</td>
<td>4.00(±0.67)</td>
<td>18/45(±0.96)</td>
</tr>
<tr>
<td></td>
<td>Training</td>
<td>4.14(±0.74)</td>
<td>19.32(±1.21)</td>
</tr>
<tr>
<td></td>
<td>Magnolia</td>
<td>4.67(±0.54)</td>
<td>21.30(±1.59)</td>
</tr>
<tr>
<td></td>
<td>Magnolia-training</td>
<td>4.26(±0.46)</td>
<td>20.01(±1.12)</td>
</tr>
<tr>
<td>Liver</td>
<td>Control</td>
<td>4.27(±1.32)</td>
<td>79.58(±38.78)</td>
</tr>
<tr>
<td></td>
<td>Training</td>
<td>3.59(0.49)</td>
<td>82.57(±25.83)</td>
</tr>
<tr>
<td></td>
<td>Magnolia</td>
<td>4.45(±0.96)**</td>
<td>94.14(±24.90)</td>
</tr>
<tr>
<td></td>
<td>Magnolia-training</td>
<td>6.23(±2.27)</td>
<td>133.87(±25.24)</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>Control</td>
<td>3.63(±0.92)</td>
<td>78.14(±29.12)</td>
</tr>
<tr>
<td></td>
<td>Training</td>
<td>3.70(±1.44)</td>
<td>72/29(±35.34)</td>
</tr>
<tr>
<td></td>
<td>Magnolia</td>
<td>4.13(±1.15)</td>
<td>92/57(±23/69)</td>
</tr>
<tr>
<td></td>
<td>Magnolia-training</td>
<td>3.59(±1.51)</td>
<td>69.29(±26.32)</td>
</tr>
</tbody>
</table>
Although the amount of MDA in plasma showed an increase in the magnolia group, it was not found significantly different. Magnolia could significantly increase MDA in liver, but training and magnolia together could not make any significant change in MDA level in Hypothalamus.

While magnolia could significantly increase the catalase level in plasma, training-magnolia group could show a significant increase in catalase level in liver, but none could result in any significant change in catalase level in hypothalamus.
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Discussion

There have been numerous studies on the use of herbal and nutritional complements with antioxidant effects for fighting oxidative effects, but there has been no study on the antioxidant effect of magnolia in conjunction with endurance training. As such, in the present study magnolia extract and endurance training were used for the evaluation of the activity of catalase and MDA in male rats’ plasma and liver and brain tissues.

Physical activities can both help improve the antioxidant defense system and damage DNA by releasing free radicals. It is possible to use antioxidant complements to change the destructive effects of exercising into positive one. The results of the present study showed that the catalase enzyme in plasma was significantly higher in the magnolia group than the control group. However, the training group could not show such a difference. MDA did not change with magnolia or training. However, both magnolia and training-magnolia could significantly increase catalase in liver. Liver MDA changes, however, was significant only in magnolia group.

In the present study, magnolia could increase plasma catalase and MDA. There is little information about the effect of magnolia and training together on plasma, liver, and hypothalamus MDA and catalase. The majority of the studies on the antioxidant effect of magnolia have been focused on the effect of two major elements of this plant, namely, magnolol and Honokiol containing phenol compounds [15, 33, 36]. The previous research shows that these two elements have strong antioxidant effects and can increase antioxidant enzymes such as catalase [37-39]. Magnolol has been shown to decrease catalase activity in heart muscles [39], which contradicts our results. This could be due to the type of organ the enzyme was extracted from. The research indicates that endurance training works like an antioxidant by increasing the antioxidant enzymes [39-45]. The effect of exercising with different intensity in increasing the oxidative indices in both human and animal subjects has been shown in the previous research, though the results of these studies are quite contradictory, which could be due to differences in the subjects, studied tissues, and the length and intensity of the trainings. For example, it is stated that exhaustive swimming could not affect the level of TBARS reactions in heart tissues and plasma in rats but had a significant effect in liver [46]. As such, the type of tissue being studied can be very important. In addition, it was found that as the length of exercising
increases, the lipid peroxidation increases [47].

Differences in the effect of exercising on different antioxidant enzymes can be a reflection of the type of special cellular tissues and the oxidative capacity in different tissues. Endurance training can support an individual against oxidative stress by stimulating her antioxidative capacity [48]. Exercising can affect the oxidative stress process differently. Although continuous exposure to the pressures resulting from endurance training can help decrease oxidative stress by enzyme compatibilities, if the length and intensity of such pressures exceeds a certain amount, it can lead to oxidative disorders, lipid peroxidation, and even cell death [49-51]. The lack of any increase in the antioxidative indices could be due to the effect of regular exercising on the body antioxidant system which slows or even stops lipid peroxidation.

Based on the findings of the present study, it seems that magnolia can strengthen the effect of exercising on the liver catalase. Although the combined effect of magnolia and exercising had not been examined, other studies examining the effect of other antioxidant complements with exercising did not find any significant change in MDA level [52,53]. However, Chen et al. [54] reported that endurance training together with a nutritional complement could increase MDA level in liver, muscles, and plasma.

An increase in the antioxidant enzyme activity reflects the adaptation of the cells or tissues with the created stress [55]. There are many studies which indicate that the low level or lack of lipid peroxidation reflects the protective effects of antioxidant enzymes. Moreover, the type of change in antioxidant enzymes may differ according to the combination, the studied tissue, dose, and length of time [56].

It seems that the best tissue for investigating catalase level is liver. Exercising as a physical stress could not greatly affect the antioxidant activities in brain tissues. It is suggested that future research consider the antioxidative effects of magnolia during exercising on muscle tissues.

**Conclusion**

The results of the present study indicate that Catalase and MDA enzymes respond differently to endurance training and magnolia in plasma and body tissues. In addition, it was found that the Catalase activity level is increased as a result of crude magnolia extract, and it seems that the effect of magnolia extract is more in liver than plasma or hypothalamus.

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