Effects of an Intensive Resistant Training Sessions and Green Tea Supplementation on Malondialdehyde and Total Thiol in Non-Athlete Women

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Introduction

Increased lipids and proteins oxidation are the harmful effects of free radical attacking into cellular organelles during intense physical activities [1-3]. Hydroxyl radicals by attacking to cellular infrastructure caused sulphhydryl or total thiol groups exposed to oxidation. Disulfide oxidation of the thiols combine with other amino acids and make changes in function and structure of the membrane proteins and lipids and therefore oxidative stress is produced [4-9].

It is believed that intensive exercises may induce oxidative stress [3-4], however natural antioxidant supplements can improve the antioxidant system of the body. Among beverages rich in flavonoids, green tea is one of the most common [10-12]. Green tea contains compounds epigallocatechin -3-gallate, epigallocatechin, epicatechin gallate and epicatechin [13]. Epigallocatechin -3-gallate has the most power so that it is 100 and 25 times higher than the antioxidant power of vitamin C and vitamin E respectively [14]. Panza et al. have confirmed the protective effects of green tea (600 ml per day) in reducing of lipid peroxidation during heavy resistance training [12]. Jowko et al. have been reported that green tea can increase capacity of antioxidant system and attenuate the oxidative stress induced by vigorous strength training at 60% of one repetition maximum (1RM) [13]. However, results of Morillas et al. regarding study of sub-maximal aerobic and resistance trainings along with polyphenol supplements, revealed an increase in both malondialdehyde (MDA) and protein oxidation [15]. On the other hand, Dehghan et al. have shown that cinnamon may respectively decreased and increased MDA and total thiols in rats after an exhaustive exercise session [16].

The resistance training is usually an inevitable part of the exercise programs; and the contradictory results make needs to take essential strategies for minimizing the oxidative stress damages induced by acute or heavy physical exercises. Most researches have focused on industry antioxidant supplements, however it is reported that such supplements may accompanied by long-term complications [17, 18]. In order to preserve and enhance the health of athletes and access to newer and cheaper supplements, the green tea have been considered; but it is necessary to pay more attention to the intensity and duration of training for determine the efficient dose. Effect of green tea on a 14-days period is reviewed [12,13], and the present study will evaluate the effect of intensive resistance exercise along with 14 days green tea...
consumption on the total thiols and MDA in non-athletes women. Since the level of physical fitness and exercise experience are effective in response to oxidative stress, non-athletes women were examined in this study.

**Materials and Methods**

The present study is a quasi-experimental research. Through announcement at Birjand University, it is recognized interested students by the researcher. It is asked to volunteers come to gather in certain day. After providing details about the process of study, we explained its benefits and probably potential harms and then written consent was obtained from volunteers. 40 individuals with mean age of 20.95±1.64 years and body mass index of 19.88±0.80 kg/m² divided randomly into 4 equal groups (each one 10 persons) including green tea supplementation (GT), green tea supplementation plus resistance training (GT-RT), resistance training (RT), and control groups. In order to matching selected groups, some characteristics such as age; body mass index; lack of cardiovascular, respiratory, renal, or metabolic diseases; lack of consumption of drugs and narcotics, were checked using by a health survey questionnaire (validity 0.65, reliability 0.90) [19, 20]. Indeed, status of antioxidant supplements and diet were evaluated using by a questionnaire 24-hour food records (validity 0.70 and reliability 0.87); and the physical activity experience was controlled using by the Beach habitual physical activity questionnaire (validity 0.76 and reliability 0.89) [21]. All subjects were students living in the dormitory of the university and therefore they have a same diet; however we asked them to avoid from black tea, coffee, beer, juices and any tablet or complementary medicine during study. Subjects prohibited from any physical activity about 48 hours before intervention. It is worth noting that whole ethical principles including informed consent, confidentiality, non-infringement of privacy, protective subject against stresses, threats, physical and psychological injuries and results feedback have been considered in the present study. Also, exercise protocol was approved in the ethical committee of the physical education and sport sciences faculty. Duration of the designed protocol were 14 days. GT and GT-RT groups consumed 200 ml green tea (2 g of dried green tea leaf powder per 200 ml water, 80-100°C) three times per day (morning, noon and night) at the same times of day before each meal, while the control and RT groups were only taken water at the same times.

Intensive resistance training session consisted a circuit training at 75-85 percent of 1RM, 3 sets and each set included 9 stations (bench press, leg press, seated boat, overhead press, knee/arm extension, knee/arm flexion and heel lifting). Work out time at each station were 30 seconds along with 120 seconds of rest between stations. Total time were about 50-55 minutes including warm-up (15-20 min), weight training program (30 min) and cool-down (10 min) [22]. Before starting of the protocol, subjects were taken instructions about circuit resistance training for 2 sessions and then it is calculated the 1RM of them in 10 movements.

Calorie intake of the last meal of subjects was designed as 500 calories including 50-55 percent carbohydrates, 25-30 percent fat and 15-20 percent protein.

Blood sampling were taken in three phases including at beginning, 14 days after the supplementation and immediately before and after training session. It is collected 10 ml of fasting blood (12 h) from the brachial vein in identical conditions (7-9 am, temperature 26-28°C and humidity 50%).

Blood samples conducted into tubes containing anticoagulant blood (EDTA) and centrifuged (2000 rpm for 10 min) immediately. Then plasma was used for MDA and total thiol measurements. Plasma MDA level was determined based on the thiobarbituric acid reaction using by spectrophotometry system at 532 nm [23]. Spectrophotometer system and 2 & 2 dithiobis nitro benzoic acid (DTNB), as Elman’s reagent, were used for total thiol determination [24]. In order to elimination of temporary effects of exercise training on plasma volume and blood variables, plasma volume changes were controlled using by the Dill and Castile equation [25].

After confirming of normal distribution of data using by the Kolmogorov-Smirnov test (Table 1), it is used the SPSS-16 and ANOVA repeated measure and LSD tests for data analysis, and it is considered significant level if \( p < 0.05 \).

**Table 1. Results of Kolmogorov-Smirnov test for examine of normal distribution of data**

<table>
<thead>
<tr>
<th>Variables</th>
<th>( Z )</th>
<th>( p )-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>1.06</td>
<td>0.21</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>0.48</td>
<td>0.98</td>
</tr>
<tr>
<td>total thiol (mmol/lit)</td>
<td>0.55</td>
<td>0.92</td>
</tr>
<tr>
<td>MDA (umol/lit)</td>
<td>0.49</td>
<td>0.49</td>
</tr>
</tbody>
</table>

**Table 2. Results of ANOVA repeated measure test about comparing MDA and total thiol between different phases of intervention (within comparison)**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Groups</th>
<th>Times</th>
<th>Baseline</th>
<th>Before exercise protocol</th>
<th>After exercise Protocol</th>
<th>( F )</th>
<th>( p )-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total thiol (mmol/lit)</td>
<td>GT-RT</td>
<td>25.69±7.58</td>
<td>29.91±6.25</td>
<td>29.26±5.84</td>
<td>2.27 *</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GT</td>
<td>25.49±2.43</td>
<td>29.80±1.47</td>
<td>29.80±1.47</td>
<td>3.76 *</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>RT</td>
<td>25.19±5.33</td>
<td>24.03±3.63</td>
<td>23.09±7.74</td>
<td>1.21</td>
<td>0.42</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Con</td>
<td>25.19±2.37</td>
<td>24.09±4.84</td>
<td>24.09±4.84</td>
<td>0.70</td>
<td>0.42</td>
<td></td>
</tr>
<tr>
<td>MDA (umol/lit)</td>
<td>GT-RT</td>
<td>5.97±2.37</td>
<td>3.25±2.04</td>
<td>4.68±2.15</td>
<td>3.60 *</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GT</td>
<td>5.88±1.19</td>
<td>3.72±0.69</td>
<td>3.72±0.69</td>
<td>5.23 *</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td>RT</td>
<td>5.63±2.15</td>
<td>6.04±1.91</td>
<td>8.63±1.88</td>
<td>0.65 *</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Con</td>
<td>5.36±1.49</td>
<td>6.07±0.98</td>
<td>6.07±0.98</td>
<td>0.90</td>
<td>0.10</td>
<td></td>
</tr>
</tbody>
</table>

\* \( p<0.05 \). GT-RT=green tea+resistance training; GT= green tea; RT= resistance training; Con= control
Results

According to table 2, the total thiol increased significantly ($p=0.01$) after 14 days consumption of green tea in the GT-RT and GT groups; however it did not change significantly in the RT group. In addition, it is observed that 14 days green tea consumption significantly decreased MDA in the GT-RT ($p=0.01$) and GT ($p=0.03$) groups; although this variable increased significantly ($p=0.01$) in the RT group (Table 2).

Comparison of between groups (Table 3) revealed that total thiol in the GT-RT group is significantly higher than the RT group ($p=0.01$). Other findings indicated that MDA of the GT-RT group is significantly lower than the RT group ($p=0.003$) and this means that green tea attenuated MDA production after an intensive resistance training session.

Discussion

Results showed that after 14 days of green tea consumption, total thiol increased and MDA decreased significantly and these findings suggest that body's antioxidant capacity improved after a session of intensive resistance training.

Many researches obtained increased MDA and protein oxidation in lack of antioxidant supplements after intensive physical activities [26, 28]; however, some of studies established different results [29, 30]. Results of the present study are similar to several other studies in which the natural antioxidant supplements have been used for modulate oxidative stress. McNulty et al. have been reported that foods and beverages containing polyphenols, particularly the catechin, can attenuate exercise-induced oxidative stress [31]. It is also reported that consumption of 2 cups of green tea per day for 42 days, induced a significant increases in total antioxidant capacity and conversely a significant decreases in protein oxidation compared to control group [32]. Furthermore, Shariatzaehdeh et al. have been reported that consumpption of polyphenol increased total thiol groups and thereby, reduced the protein oxidation [33].

Results of Panza et al. in study of the effect of one week green tea consumption on oxidative stress in weightlifting men, revealed a reduction in MDA and protein oxidation [12]. Jowko et al. stated that consumpption of green tea extract for 4 weeks, will reduce oxidative damages in athletes [13]. Based on the mentioned results, it seems that antioxidant supplementation, especially polyphenolic compounds containing green tea, can improve body's antioxidant capacity. Despite these reports, other findings are not consistent with the results of the present study. Freese et al. did not observe any significant changes in total antioxidant capacity and protein oxidation markers in healthy women after 4 weeks consumption of green tea extract [34]. Henning et al. did not obtain any significant changes in oxidative stress markers after consumption of green tea [35]. Molayi have been reported that total thiol reduced after Ziziphora drinking and exhaustive exercise [17].

Discrepancies in the results may due to differences in the type and intensity of exercises, methods of stress oxidative determination or dose of supplementation [36]. For example, in the Freese et al. study, dose of green tea was 6 g per day for 4 weeks; while our subjects have used 200 ml green tea 3 times per day for 14 days. On the other hand, in the study of Henning et al., oxidative stress indices were measured 8 hours after consumption of green tea, while in the present study they measured it immediately after an intensive exercise. Since the level of oxidative stress is determined by the type and intensity of physical exercises, they have very important role in this case. Molayi performed Bruce maximal aerobic test and this protocol may create more oxidative stress. In addition, antioxidant supplementation period was 48 hours before the exercise test; while resistance training session was applied at 75-85 % of 1RM and subjects consumed antioxidant supplement for 14 days in the present study.

These facts suggest that intensive exercises may induce oxidative stress and increase oxidative characteristics, but their levels depends on type (aerobic, anaerobic, resistance, etc.) and intensity (severe, moderately severe, light) of performed exercises. Antioxidant supplements can inhibit the degenerative of exercise-induced free radicals; if dosage and time of antioxidant supplements assigned correctly.

More intensity of physical activity, more oxidative stress and lower antioxidant defense systems will be occured. In other words, heavy exercise may induce cellular damages, and its derived inflammatory responses will associated with overproduction of reactive oxygen species due to neutrophil activation. Non-athletes have no enough adaptation to physical exercise and this will place them to greater and harder oxidative damages [37]. It seems that correct antioxidant supplementation along with...
higher antioxidant defense system, will efficiently attenuate oxidative damages in non-athletes. Green tea, as a powerful antioxidant, plays an effective role in neutralizing of free radicals and reduction of lipid or protein oxidation [38]. It is believed that during intensive resistance training ischemia-reperfusion and mechanical stresses on the soft tissues are responsible more free radicals production [9]. Blood shunt to the skin and active muscles during high intensive exercises will induce transient hypoxia and lack of supply oxygen for active tissues. Following re-oxygenation of tissues, stopping exercise, or reduction in its intensity, it is expected a more free radicals generation. Free radicals, especially hydroxyl radicals, cause sulfidryl or total thiol groups exposed to oxidation through attacking to the cell infrastructural parts [6]. Total thiol include two proteins (such as papain, Niasin, etc.) and non-proteins (such as glutathion, cysteine, etc.) categories. Thiol oxidation-derived disulfide may combine with the glutathione, cysteine, other low mass amino acids, and even actin and myosin myofibrils, and thereby changes in the composition and structure of proteins and production of carbonyl compounds are occurred [37]. On the other hand, reduction of intracellular antioxidants such as glutathione, make possibility for free radicals to attack to cell membranes and alter membrane structural or permeability, and lipid peroxidation [36]. Available catechins in green tea through increasing in intracellular antioxidants such as glutathione, uric acid and bilirubin; improvement in intracellular antioxidant capacity such as glutathione reductase, glutathione peroxide and catalase; may protect cells against glutathione depletion and enhance the capacity of the antioxidant system [39].

In general, since these compounds have hydroxyl groups, they are enable to neutralize free radicals and can act as an electron or hydrogen reduction. Catechins found in green tea, especially epi gallo catechin -3-gallate, may inhibit lipid peroxidation and protein oxidation processes by both free radicals elimination (mainly due to having the dihydroxy phenol) and restructuring of tocopherol (α-tocopherol radical convert to α-tocopherol). Catechins significantly prevent a plasma α-tocopherol reduction and attenuate protein and lipid oxidation through attachment of iron and zinc to lipoproteins [40].

The present results suggest that green tea supplementation may inhibits lipid and protein oxidation by promoting the antioxidant defence capacity after an intensive resistance training session. Hence, it is recommended that non-athletes consume green tea (600 ml per day minimally for 14 days) in order to lowering oxidative stress and exercise-induced tissue damages.

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Conflict of Interest
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References