A preliminary study on antioxidant activities of saffron petal extracts in lambs

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Abstract

This study assessed the effects of hydroalcoholic extract of saffron (Crocus sativus L) petals on male lambs and was aimed at evaluating the antioxidant activity of this extract during a 15-day period. Fourteen male lambs were divided randomly into three treatment groups (n=4 each) and a control group (normal saline; n=2). Saffron petal extract at 500, 1000 and 1500 mg/kg was administered by gavage once daily on days 1 and 3 of the experiment for treatment groups. Blood samples were obtained on days 6 and 14 of the study. Serum and plasma were stored at −21°C for further analysis. Heart rate, respiratory rate, rectal temperatures and body weight of each lamb were recorded in the distinctive intervals from day 0 to the last day of the study. Total antioxidant capacity levels were increased significantly with any dose of the extract on the first day of sampling. There was no statistical difference in the levels of malondialdehyde and total thiol between the treatment and the control group son the 6th and the 14th days of the experiment. Also, no significant difference in the levels of malondialdehyde and total thiol between the treatment groups. Blood samples were obtained on days 6 and 14 of the study. Serum and plasma were stored at −21°C for further analysis. Heart rate, respiratory rate, rectal temperatures and body weight of each lamb were recorded in the distinctive intervals from day 0 to the last day of the study. Total antioxidant capacity levels were increased significantly with any dose of the extract on the first day of sampling. There was no statistical difference in the levels of malondialdehyde and total thiol between the treatment and the control group son the 6th and the 14th days of the experiment. Also, no significant difference in the levels of malondialdehyde and total thiol between the treatment groups.

Preparation of the extract

Crocus sativus L. petals were collected from Torbat-Heidariye district (35.27° north latitude, 59.22° east longitude, about 1330 meters above sea level) in Khorasan-Razavi province, North-east of Iran, during autumn 2011 (Figure 1). The samples were identified by the Agricultural Faculty of Birjand University, Iran. The voucher number specimen (No. 74/1525) was deposited in the herbarium of Birjand Agricultural Faculty, Shiraz University, Iran. To prepare the petal extracts, samples were dried in shadow and then pulverized with a grinder (Hamilton Beach brand, Canada). Hydroalcoholic extract was prepared by using 50 g of the dried powder in 1000 mL of 80% v/v ethanol and shaking for 24 h. Then, the mixture was filtered through No. 1 Whatman filter paper and oven dried at 40°C for 24 hours. The final powdered extract was then weighed to calculate the yield. The yield (w/w) of the ethanolic petal extract was 30%.

Clinical study

The safety of the extract was assessed in the animals by monitoring the vital signs and clinical findings as well as performing laboratory tests. The lambs were closely observed twice a day for any sign of illness. Heart and respiratory rates, rectal temperatures and live weights of the lambs were recorded for 15 days (10:00 AM) from day 0 of the study onwards. Blood samples were obtained by jugular venepuncture into plain and EDTA tubes at days 6 and 14.
of the experiment between 8:00 and 9:00 am. The experiment was approved by the animal welfare committee of the Agricultural Faculty of Birjand University, Iran.

**Laboratory analysis**

Serum and plasma were separated by centrifugation of the samples at 3000×g for 15 min at room temperature, and were stored at -21°C for further analysis. Serum oxidant status was evaluated by measuring malondialdehyde (MDA) and serum antioxidant status was assessed by measuring total thiol (T-SH) levels and total antioxidant capacity (TAC). MDA was determined according to the method described by Ohkawa et al. MDA is formed as an end product of lipid peroxidation, which reacts with the TBA (thiobarbituric acid) reagent under...
Table 2. Recorded vital signs and body weight during the experiment in control and treatment groups in healthy male lambs (mean ± S.E.M.).

<table>
<thead>
<tr>
<th>Variable</th>
<th>D</th>
<th>Control</th>
<th>Treatment I</th>
<th>Treatment II</th>
<th>Treatment III</th>
<th>Sig.</th>
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<td>94.0±6.21</td>
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<td>109.0±4.12²</td>
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<td>108.0±6.92²</td>
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<td>38.82±2.31</td>
<td>37.2±1.47</td>
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</table>

¹,²Different superscripts letters indicate significant differences. *P<0.05; NS, P>0.05. D, day.

Acidic conditions were used to generate a pink-colored product. Total thiol level was measured according to the method of Sedlak and Lindsay. Antioxidant status was evaluated using ferric reducing antioxidant power (FRAP) assay. The concentrations of glucose, blood urea nitrogen (BUN), creatinine, uric acid, total cholesterol, triglyceride, albumin, total protein, total and direct bilirubin as well as the activities of aspartateaminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma glutamyle transferase (GGT) were measured by an autoanalyser apparatus (Prestige 24i, Japan). Serum globulin was calculated by subtracting the serum albumin from serum total protein. Serum albumin: globulin ratio (A:G ratio) was calculated by dividing the values of serum albumin by serum globulin. Also serum indirect bilirubin was calculated by subtracting the serum direct bilirubin from serum total bilirubin.

**Statistical analysis**

All data were analyzed using SPSS 16/PC software. Statistical analysis was performed using one-way ANOVA followed by Tukey-Kramer multiple range test for multiple comparisons. Statistical significance was set at P<0.05.

**Results**

The results are shown in Tables 1 and 2. The plasma levels of TAC were significantly higher on the 6th day of the experiment for lambs received saffron petal (P=0.01) compared to those of the control lambs (Table 1). There were no differences in the MDA levels between the control and treatment groups at days 6 and 14 of the experiment (P>0.05). There was no difference in the T-SH levels between the control and treatment groups on the 6th and the 14th days (P>0.05). No significant differences were identified in the concentration of glucose, BUN, creatinine, uric acid, total cholesterol, triglyceride, albumin, globulin, total protein, AST, ALT, ALP, GGT, total bilirubin, direct and indirect bilirubin between the control and treatment groups in male lambs during this study (Table 1). The vital signs and body weights showed no significant differences during the experiment in control and treatment groups (Table 2).

**Discussion**

Processes or reactions involving in production of reactive oxygen and nitrogen species (ROS and RNS) can potentially make harmful effects on weight. Compounds with antioxidant capacity are capable of protecting biological systems against oxidative stress. In the present study, total antioxidant capacity levels were increased significantly with either dose of the saffron petal extract at the first day of sampling. In the present study there were no differences in the MDA levels between the treatment and the control groups. MDA is a three carbon, low molecular weight aldehyde that can be produced from free radical attack on polyunsaturated fatty acids of biological membranes. Therefore, it is a frequently measured biomarker of oxidative stress. The results of this investigation showed no difference in the total thiol levels. The major part of thiol in plasma is derived from proteins, especially albumin, and they are susceptible to oxidation. According to the results of this study, significantly higher serum TAC concentration in the treatment groups compared to that of control sheep reflects a higher total antioxidant capacity in lambs that received saffron petals. In this study there were no any condition of disease or disorders based on clinical and laboratory findings and the extract of saffron petal, up to 1500 mg/kg body weight, did not cause any adverse effect on health status of male lambs. It can be concluded that the saffron petal extract could have an antioxidant effect in healthy sheep. So it can be assumed that saffron petal extract can help sheep to combat oxidative stress in many pathological conditions. Saffron stigma is the world’s most costly spices but saffron petal costs little. Saffron petals are the main by-product of saffron processing which is produced in large amounts annually in Iran and is usually discarded. The main overall finding from this study is that petal of *Crocus sativus* may be useful for its antioxidant benefit in lambs. The extracts of saffron petals may possess antioxidant activities in sheep. In some cases, the
remaining saffron farms are eaten by sheep. Due to antioxidant effects, it is recommended that saffron petals in the remaining fields be used to feed livestock. However, further studies are needed to assess the antioxidant activity of saffron petals in various diseases and poisonings of sheep. Phenolic compounds are likely to be the biologically active components of the petals. Kaempferol, isolated from the fresh flower petals of saffron has also been mentioned as the important component. Kaempferol was effective in scavenging free radicals. Materials rich in phenols can retard oxidative degradation of lipids and improve the quality and nutritional value of food. Livestock feeds are subjected to oxidative spoilage. Further studies on improving the quality and nutritional value of the feed mixed with saffron petals is recommended.

References