

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 on the 6th and the 14th days of the experiment. Also, no significant differences were identified in the concentrations of glucose, blood urea nitrogen, creatinine, uric acid, total cholesterol, triglyceride, albumin, globulin, total protein, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, gamma glutamyl transferase, total bilirubin, direct and indirect bilirubin between control and treatment groups in male lambs during this study. Recorded vital signs and body weight in control and treatment groups showed no significant differences. The present results suggest that the hydroalcoholic extract of saffron petals has antioxidant activity in sheep.

Introduction

Crocus sativus L., commonly known as saf-

fron, is a bulbous perennial stem less plant of the *Iridaceae* family. Saffron, widely cultivated in Iran, is well adapted to arid and semi-arid lands and produces stigmas annually. It is also adaptable to temperate and sub-tropical climates. Saffron has been used in folk medicine for more than 3000 years. In common, the harvested stigma is used as a food spice or as an herb with medicinal properties. Saffron petal is a by-product of saffron and is usually discarded as a waste.¹ In some areas the remaining by-products of saffron or saffron petals is fed to flocks of sheep and goats. A number of studies indicate that ethanol extract of saffron petals possesses antidepressant,²⁻⁵ anti-inflammatory, antinociceptive,⁶⁻⁸ antihypertensive,⁹ anti-cancer and antitumor activities.^{10,11} Phenolic compounds, among which are flavonoids and anthocyanins,¹² are likely the biologically active components of the saffron petal and have been associated with the health benefits in human and animals.¹³⁻¹⁵ The antioxidant and antimicrobial properties of saffron have been noticed in recent years.¹⁶ The beneficial effects derived from phenolic compounds have been attributed to their antioxidant activity.¹⁷ The present study was undertaken firstly to investigate the oxidant/ antioxidant status in male lambs receiving the petal extract and secondly to evaluate whether the hydroalcoholic extract of saffron petal has any adverse effect on the health of the male lambs.

To our knowledge this is the first study of effects of saffron petals on serum biochemical parameters of sheep.

Materials and Methods

Animals

The study was conducted on fourteen 4-6 month-old Baluchi male lambs, weighing 38.40±1.04 kg, during November and December, 2011. In this study, 12 lambs were divided randomly into 3 treatment groups (n=4 each). Two lambs were considered to be the control group; they received normal saline as placebo. The treatment groups I, II and III, respectively, received 500, 1000 and 1500 mg/kg saffron petal extract by gavage once daily at days 1 and 3 of the experiment. The animals were treated against internal and external parasites with Albendazole (Damloran Company, Borujerd, Iran) 10 mg/kg, and Ivermectin (Razak company, Tehran, Iran) 0.22 mg/kg, 30 days prior the beginning of the study.

Preparing the petal 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,

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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 University, Iran. To prepare the petal extracts, the 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 was 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.*¹⁸ MDA is formed as an end product of lipid peroxidation, which reacts with the TBA (thiobarbituric acid) reagent under



Figure 1. The white arrow indicates saffron petal, which is used in the experiment.

Table 1. The concentration of some blood biochemical variables in control and treatment groups in healthy male lambs (mean ± S.E.M).

Variable	Unit	D	Control	Treatment I	Treatment II	Treatment III	Sig.
MDA	μmol/L	6	2.29±0.27	2.67±0.23	2.53±0.21	2.61±0.17	N.S
		14	2.92±0.35	2.02±0.29	2.15±0.11	2.46±0.16	N.S
T-SH	μmol/L	6	19.99±2.79	24.25±1.12	25.46±1.51	21.69±3.26	N.S
		14	34.63±5.96	32.44±3.24	43.24±5.58	34.10±4.48	N.S
TAC	μmol/L	6	321.07±6.14 ^a	400.55±22.76 ^b	376.84±15.78 ^b	419.87±18.41 ^b	*
		14	322.83±78.15	345.22±26.71	427.33±20.02	427.33±37.77	N.S
Glucose	mg/dL	6	87.00±8.00	92.00±13.78	81.50±4.21	83.50±2.60	NS
		14	77.500±1.500	84.00±6.94	74.25±2.71	71.00±3.31	NS
BUN	mg/dL	6	7.50±3.33	11.11±1.60	11.66±1.67	10.00±5.06	NS
		14	6.04±1.87	10.97±0.77	6.97±1.10	9.06±0.74	NS
Urea	mg/dL	6	18.00±8.00	23.75±3.99	28.00±4.02	24.00±3.72	NS
		14	14.500±4.500	24.00±2.68	16.75±2.65	21.75±1.80	NS
Creatinin	mg/dL	6	1.10±0.20	0.93±0.21	0.85± 0.13	0.90± 0.11	NS
		14	1.05±0.25	0.82±0.13	0.90± 0.20	0.82±0.04	NS
Uric acid	mg/dL	6	1.05±0.15	1.07±0.44	1.07±0.28	0.68± 0.19	NS
		14	1.70±0.60	0.70±0.07	2.70±1.48	1.02±0.34	NS
Total cholesterol	mg/dL	6	56.00±8.00	52.75±2.87	53.75±4.13	46.00±2.04	NS
		14	30.50±5.50	35.75±1.65	38.00±4.14	36.75±6.94	NS
Triglyceride	mg/dL	6	32.00±2.00	38.50±5.33	37.00± 2.38	30.75± 6.87	NS
		14	19.00±1.00	33.25±5.28	44.00±14.54	30.00±2.94	NS
Albumin	g/dL	6	3.80±0.10	3.73±0.05	4.00± 0.09	3.65± 0.05	NS
		14	3.35±0.05	3.52±0.25	3.62±0.17	3.60±0.07	NS
Globulin	g/dL	6	4.75±0.55	4.40±0.10	4.97±0.27	5.05±0.48	NS
		14	4.80±0.20	4.10±0.12	4.50±0.24	4.22±0.23	NS
A:G ratio	%	6	0.81±0.11	0.84±0.02	0.81±0.04	0.74±0.06	NS
		14	0.69±0.03	0.86±0.03	0.81±0.07	0.85±0.04	NS
Total protein	g/dL	6	8.55±0.45	8.13±0.09	8.98±0.31	8.70±0.48	NS
		14	8.15±0.15	7.62±0.10	8.12±0.023	7.82±0.26	NS
AST ¹	IU/L	6	102.00±20.00	115.75±8.75	178.50±43.57	109.75±8.70	NS
		14	90.00±6.00	107.75±15.17	127.75±4.50	95.00±17.71	NS
ALT ²	IU/L	6	12.50±0.50	23.75±4.80	45.00±14.29	31.25±3.57	NS
		14	27.50±4.26	21.50±5.78	24.75±5.40	25.00±4.70	NS
ALP ³	IU/L	6	899.00±408.00	724.00±41.67	727.00±36.92	723.75±132.65	NS
		14	863.50±428.50	644.50±57.12	526.75±53.28	659.75±114.33	NS
γ-GT ⁴	IU/L	6	57.90±2.60	85.30±12.36	72.43±5.12	74.50±7.08	NS
		14	49.20±18.70	77.92±11.71	57.40± 6.18	67.45±4.82	NS
Direct Bilirubin	mg/dL	6	0.10±0.00	0.10±0.00	0.10±0.00	0.10±0.00	NS
		14	0.10±0.00	0.10±0.00	0.10± 0.00	0.10± 0.00	NS
Indirect Bilirubin	mg/dL	6	0.25±0.05	0.20±0.04	0.12±0.02	0.12±0.02	NS
		14	0.55±0.25	0.20±0.04	0.30±0.16	0.15±0.05	NS
Total Bilirubin	mg/dL	6	0.35±0.05	0.30±0.04	0.22±0.02	0.22±0.02	NS
		14	0.65± 0.25	0.30±0.04	0.40± 0.16	0.25± 0.05	NS

^{a,b}Different superscripts letters indicate significant differences; ¹Aspartate aminotransferase; ²Alanine aminotransferase; ³Alkaline phosphatase; ⁴γ-Glutamyltransferase. *P≤0.05; NS, P>0.05. D=day.

Table 2. Recorded vital signs and body weight during the experiment in control and treatment groups in healthy male lambs (mean ± S.E.M).

Variable	D	Control	Treatment I	Treatment II	Treatment III	Sig.
Heart rates (Beats/minute)	2	114.00±6.00	120.00±5.88	94.00±6.21	99.00±3.78	NS
	4	120.00±0.00	121.00±1.00	120.00±6.32	129.00±8.73	NS
	7	104.00±4.00	96.00±3.65	98.00±7.39	95.00±3.00	NS
	9	116.00±4.00 ^a	109.00±4.12 ^b	114.00±8.71 ^a	108.00±6.92 ^b	*
	11	100.00±4.00	106.00±2.00	97.00±3.78	103.00±3.00	NS
	13	150.00±22.00	132.00±8.48	124.00±8.48	122.00±7.74	NS
Respiratory rates (Beats/minute)	2	36.00±4.00	41.00±2.51	42.00±3.83	35.00±2.51	NS
	4	42.00±6.00	41.00±2.51	38.00±2.00	38.00±2.58	NS
	7	38.00±6.00	39.00±1.91	35.00±1.91	35.00±3.41	NS
	9	34.00±2.00	39.00±3.00	39.00±1.91	33.00±3.00	NS
	11	38.00±2.00	36.00±1.63	38.00±2.00	38.00±2.58	NS
	13	30.00±2.00	30.00±3.46	28.00±2.82	32.00±3.65	NS
Rectal temperatures (C°)	2	39.70±0.50	40.02±0.10	39.47±0.22	39.52±0.33	NS
	4	39.85±0.35	39.67±0.11	39.40±0.17	39.50±0.12	NS
	7	39.80±0.40	39.90±0.20	39.40±0.14	39.45±0.27	NS
	9	40.05±0.25	40.07±0.27	39.80±0.22	39.62±0.13	NS
	11	39.50±0.10	39.35±0.08	39.32±0.30	39.40±0.18	NS
	13	39.75±0.05	39.72±0.02	39.60±0.10	39.25±0.27	NS
Body weight (kg)	0	38.60±1.90	38.87±2.35	39.37±2.50	36.85±1.71	NS
	7	37.20±1.80	36.50±2.21	36.72±2.33	34.50±1.55	NS
	13	39.40±2.10	38.52±2.16	38.82±2.31	37.22±1.47	NS

^a^bDifferent superscripts letters indicate significant differences. *P≤0.05; NS, P>0.05. D, day.

acidic conditions to generate a pink-colored product. Total thiol level was measured according to the method of Sedlak and Lindsay.¹⁹ Antioxidant status was evaluated using ferric reducing antioxidant power (FRAP) assay.²⁰ 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 glutamyl transferase (GGT) were measured by an autoanalyser apparatus (Prestige 24i, Japan).²¹ 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<0.05.²²

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.²³ 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 not 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.^{1,24} 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.²⁴ Kaempferol, isolated from the fresh flower petals of saffron has also been mentioned as the important component.^{8,25} Kaempferol was effective in scavenging free radicals.²⁶ 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.

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