

Crocus Sativus extract effects on serum biochemical profile in dog

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Journal of Livestock Science (ISSN online 2277-6214) 8: 150-154
Received on 3/4/2017; Accepted on 28/6/2017

Abstract

Crocus sativus is a grassy, perennial, stem less and bulbous plant which belongs to iridaceae family. The saffron has aphrodisiacs effects and decrease fat and cholesterol. The aim of the current study is to evaluate the serum biochemical changes due to saffron use in dog. For this purpose, 14 male and 14 female mature dogs selected randomly, and kept in separate cages with standard dietary and desired water. The treatment groups were treated with 100 mg/kg and 200 mg/kg oral dosage of saffron daily, for 2 weeks, and in control group placebo was used. The AST, ALT, BUN, creatinine, cholesterol, Na and K levels was evaluated. In treatment groups, there were significant increase in AST, ALT, BUN, Creatinine and significant decrease in Cholesterol and Na and K levels. Saffron extract has significant effects on biochemical profile of dogs, although, more studies needed to indicate its effects on heart, sport, and stress.

Keywords: Serum biochemical profile, Dog, Saffron, *Crocus sativus*, Tabriz

Introduction

Saffron (*Crocus Sativus* L.) from Iridaceae family is a herbaceous, perennial growing from corms. Bold type saffron onions and nearly spherical shape with a diameter of 3 to 5 cm and has a brown cover is placed below the soil. Each onion produces 6 to 9 narrow leaves, grass like weed leaves. Saffron roots of the onions and the printers that grow on the base circle. Flower of Saffron is the first limbs that appear in early fall. In the first year of cultivation, due to the weakness and lack of complete settlement of the soil and deep planted flower of crocus buds had not enough ability for growth even in the first leaves appear later than usual (Lust, 2014; Moghadam *et al.*, 2013).

Saffron reducing fat and cholesterol, and increased penetration of oxygen in plasma in laboratory mice (Asdaq and Inamdar, 2010). In commons medicine around the world, saffron is used as a sedative, antispasmodic, appetizer and tonic stomach (Charles, 2012). In Germany, saffron used as a sedative, to treat stomach and abdominal pain, and asthma. Because saffron has the color of happiness, it is found in foods that cause human endeavor. Saffron for its flavor, odor and its special yellow color are particularly abundant in foods (especially along with rice), confectionary industry, pharmaceutical and other industries is spent (Sampathu *et al.*, 1984). The main ingredients in saffron includes yellow colored compounds well solute in the water (crocin derivatives), Bitter compounds including picrocrocin that are particularly nourishing the stomach, aromatic substances (essential oil) is the main composition Safranal sometimes constitutes up to 1% of saffron, fixed oil content of up to 10 percent moisture content of about 13% and inorganic compounds about 5% (Escribano *et al.*, 1996; Van Calsteren *et al.*, 1997; Winterhalter and Straubinger, 2000).

Saffron considered as stomach ailment recover and an antispasmodic, which has been help digestion and increases appetite. It also relieves renal colic, reduces stomachaches and relieves tension. In Persian tradition medicine, it is used for depression. Recent studies indicate its potential as an anticancer agent and memory enhancer (Akhondzadeh *et al.*, 2004; Akhondzadeh *et al.*, 2005; Noorbala *et al.*, 2005).

Iranian saffron has 4 types crocin and 3 type picrocrocin that is different from the rest of the world (Bolhasani *et al.*, 2005). Chemical analysis of the water extract of saffron stigma showed two main chemical compositions. Carotenoid containing crocin analogues, which were divided into four types. This material are crocetin sugary derivatives of crocetin. Crocetin and menu derivatives and di-methylated of them, found to be lower in saffron. Main crocin is crocin type 1 or digentiobioside crocetin (Hadizadeh *et al.*, 2010). Responsible for the color of saffron are crocin of it that unlike other natural carotenoids, are water soluble (Moghaddasi, 2010).

Monoterpene aldehyde that picrocrocin (this factor under the influence of the temperature and environment is converted to Safranal) responsible for the bitter taste of saffron and its sugar-free derivatives and as well Safranal, and the fragrant scent of saffron. So three factors of color: Crocin, taste: picrocrocin and fragrance of saffron: Safranal is in dried stigmas. It should be noted that other chemical compounds, including anthocyanin, flavonoids, vitamins, amino acids, proteins, starches, minerals and resins to a much lesser extent than the above two categories are present in the saffron stigmas (Hosseinzadeh and Sadeghnia, 2005; Hosseinzadeh *et al.*, 2008; Lozano *et al.*, 2000). The aim of the present study was to evaluate the effects of saffron use on serum biochemical factors.

Materials and Methods

In this study (experimental -interventional) of 28 dogs (14 male dogs and 14 female dogs) selected randomly. All animals under cycle of light-dark for 12 hours and a temperature of 21 ± 2 °C and placed on a bed of straw and had free access to water and special standard food for dogs.

Animal Selection

Before starting the research, animals were clinically examined. Heart of every single animal of any congenital or acquired problems in terms of heart audible murmur was examined, After obtaining the ECG and observing normal ECG and examining biochemical profiling (Basic Biochemical Profile) of serum and after rejecting of any disturbances in other organs were added to the tested collection.

Saffron extract

Saffron obtained from Novin saffron company of Mashhad. First stigma of the saffron with mechanical grinding for becoming fine powder fully. Then the process of extraction was performed with methanol 70% by maceration method. Extraction procedure five times, and every time for 12 hours was repeated. After the extracts that were collected with Rotary evaporator under vacuum and dried at temperatures below 45°C and kept in the refrigerator at temperatures below zero degrees Celsius until use (Modaghegh *et al.*, 2008; Mohajeri *et al.*, 2007). When prescribing, first the dry material which was obtained, weighed, and 5 grams of dry matter solute per 100 cc distilled water and a solution of five percent (50mg/ml) were prepared so from each of 2 days, so new drug made entirely taking and the next day fresh solution should be prepared (Li, 2002).

Drug administration

All dogs before drug administration weighed and recorded on sheets of animal file to calculate exact dose for each case. Dogs aged between 1.5 to 2 years was considered that have between 13 to 18 kg of weight to prevent the possible confounding effects of age, high or low of the biochemical profile. Then all the animals were fed with standard diet for 2 weeks, to ward off parasitic infection medication Mebendazole 20mg/kg and Ivermectin 0.004 mg per kg of body weight of the animal was used (Ettinger and Feldman, 2009). And several times without any examination and any special tests were taken to the place where the examination have been said to take place and with dried precooked food, tasty food fed that used to the new environment and people so during the experiments any confounding factors due to stress were deleted. From the perspective of cognitive behavior, each of the test cases that during the test shows aggressive behavior, agitation, excessive stress, heavy trembling went out from experiment. Two days before starting the research the hair shaved according to the cephalic vein to facilitate blood sampling path and irritation from shaving the hair and to be eliminated by skin abrasion.

Serum biochemical parameters

Before drug administration, blood samples were obtained from all cases before breakfast for amount of 7cc and biochemical profiles of all dogs was determined that to be considered as the time zero. In general, animals of examination divided into three groups according to dose: A-control group, B-normal dose and C- double dose (each group consisting of seven dogs – control group is that of a zero-time). All animals after confirming the overall health and the measurement basis of serum biochemical parameters treated by various doses of saffron. In the first group, each dog received *Crocus sativus* extract orally at a dose of 100 mg per kg of body weight per day for 7 consecutive days was administered and In the second group with dose 200 mg per kg of body weight per day for 7 consecutive days was administered (Van Asseldonk and Beijer, 2006). On the seventh day after the completion of drug administration again took blood from the cephalic vein in the fasting baseline serum biochemical parameters measured and changes between groups, particularly during peak drug effect were studied (4 hours after drugs consumption) in each group and between the two groups.

This research Animal right was according to emphasize research ethics oversight committee on animal rights, Pharmaceutical Research Center, Tabriz University of Medical Sciences. During research were not entered any damage to animals (physical and mental) and all keeping condition and the treatment with the animals was like the human and that carried out during the entire period of investigation by thesis supervisor help and have been supervision and confirmation.

Results

All quantitative data were presented as mean \pm standard error (SE) and differences between groups by ANOVA test (one-way analysis of variance) were determined at a significance level of $p < 0.05$ by the SPSS version 22 software package (Table 1 & 2). The AST levels was increased significantly in 200 mg/Kg saffron treatment group ($p < 0.01$), but 100 mg/Kg treatment and control group was not significantly different ($p > 0.01$). The ALT levels was increased significantly in 200 mg/Kg saffron treatment group ($p < 0.01$), but 100 mg/Kg treatment and control group was not significantly different ($p > 0.01$).

The BUN levels was increased significantly in 100 and 200 mg/Kg saffron treatment group comparing to control ones ($p < 0.01$). The creatinine levels was increased significantly in 100 and 200 mg/Kg saffron treatment group comparing to control ones ($p < 0.01$). The glucose levels was decreased significantly in 100 and 200 mg/Kg saffron treatment group comparing to control ones ($p < 0.01$). The cholesterol levels was decreased significantly in 100 and 200 mg/Kg saffron treatment group comparing to control ones ($p < 0.01$). The total protein levels was decreased insignificantly in 100 and 200 mg/Kg saffron treatment group comparing to control ones ($p > 0.05$). The serum Na levels was decreased significantly in 100 and 200 mg/Kg saffron treatment group comparing to control ones ($p < 0.05$). The serum K levels was decreased significantly in 100 and 200 mg/Kg saffron treatment group comparing to control ones ($p < 0.05$).

Table 1: Biochemical factors levels in different groups (mean \pm SE)

| | AST(U/L) | ALT(U/L) | BUN(mg/dl) | Creatinine (mg/dl) | Glucose (mg/dl) |
|---------|-------------------------------|-------------------------------|-------------------------------|------------------------------|--------------------------------|
| NC | 55.21 \pm 3.03 ^a | 62.42 \pm 5.06 ^a | 12.89 \pm 2.19 ^a | 1.05 \pm 0.02 ^a | 77.61 \pm 10.21 ^c |
| TRE.1 | 56.94 \pm 4.63 ^a | 71.04 \pm 5.83 ^b | 14.15 \pm 3.01 ^b | 1.21 \pm 0.03 ^b | 71.06 \pm 9.23 ^b |
| TRE.2 | 73.19 \pm 6.01 ^b | 82.61 \pm 8.20 ^c | 18.23 \pm 4.16 ^c | 1.48 \pm 0.06 ^c | 62.51 \pm 10.16 ^a |
| P value | 0.002* | 0.009 | 0.001 | 0.001 | 0.001 |

* $p < 0.05$ considered as significant difference

Table 2: Protein, cholesterol and electrolytes of serum in different groups (mean \pm SE)

| | Cholesterol(mg/dl) | Total protein(g/dl) | Serum Na (mEq /L) | Serum K (mEq /L) |
|---------|---------------------------------|---------------------|---------------------------------|------------------------------|
| NC | 258.63 \pm 21.14 ^c | 6.86 \pm 0.01 | 141.05 \pm 12.35 ^a | 5.01 \pm 0.06 ^a |
| TRE.1 | 241.36 \pm 25.81 ^b | 6.75 \pm 0.03 | 137.82 \pm 21.15 ^b | 4.81 \pm 0.05 ^b |
| TRE.2 | 226.17 \pm 19.51 ^a | 6.71 \pm 0.12 | 130.01 \pm 18.61 ^c | 4.70 \pm 0.03 ^c |
| P value | 0.006* | 0.459 | 0.041 | 0.024 |

*p<0.05 considered as significant difference

Discussion

Researchers, in a study on laboratory rats administered saffron intra-peritoneally found that blood glucose levels of the experimentally diabetic rats were significantly decreased and were able to apply the hypoglycemic effects (Katzung *et al.*, 2001; Mostafa *et al.*, 2011; Nelson and Couto, 2008).

The saffron has affected the cholesterol in humans, which is in consistent with our results. Although it is not seem to be done mediated by insulin because studies show that saffron has no significant effect on insulin, this seems lowering of blood sugar is because of vegetable Glucokinin makes adding the result of reduction in urinary glucose reabsorption in the kidney (proximal tubule) (Katzung *et al.*, 2001; Koono *et al.*, 2010; Li, 2002; Zaman, 1989). It was also observed that lowering effect of cholesterol of this extract in humans that however in the long run can affect the increase in blood pressure indirectly that also will require time (Arasteh *et al.*, 2011; Stephen, 2006; Zaman, 1989). Previous research have been identified that saffron always lower the blood pressure, however, the present study also shows significant decrease of sodium and potassium of the blood can justify further reduction in blood pressure as well (Katzung *et al.*, 2001).

It was reported that intraperitoneal administration of saffron causes significantly reducing on amount of cholesterol and blood sugar (Katzung *et al.*, 2001), that the above results are in consistent with the results of the present study. It was identified that the saffron lowering cholesterol after consumed dissolved in drinking water for four weeks fed in mice with high cholesterol that the results were corresponded with the results of the present study (Thomson *et al.*, 2009).

Researchers, investigated of dietary saffron impact on some blood parameters and found that sodium and BUN had increased non-pathological and normal ranges (Modaghegh *et al.*, 2008), Which is consistent with the results of the current study, likely due to adverse effects of high doses of saffron in the renal tubules. However, this increase in the present study were in the normal range but in a study of other researchers found that intraperitoneal injection of saffron extract at high doses (5gr/kg) caused significant increases in BUN and creatinine and liver enzymes including SGOT and SGPT and have been shown to have toxic effects. Saffron toxic effects have shown at high doses on renal tubules that again similarly can justify increased blood creatinine in current study (Mohajeri *et al.*, 2007). Finally, we can conclude that the results of investigations and studies by other investigators and investigations of current study have not bad effect in usual doses, but have multiple good effects on body.

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