J Med Allied Sci 2017; 7 (1): 20-25
www.jmas.in
Print ISSN: 2231 1696 Online ISSN: 2231 170X
Journal of Medical & Allied Sciences

Original article

Antihyperlipidemic effect of crude extract of saffron (*Crocus sativus*) stigma in healthy male rats

Iliass Lahmass[1], Sabir Ouahhoud[1], Assia Sabouni[1], Mohammed Elyoubi[1], Redouane Benabbas[1], Rachid Elmoussaoui[2], Mohammed Choukri[2], Ennouamane Saalaoui[1]

[1]Laboratory of Biochemistry and Biotechnology, Department of Biology, Faculty of Sciences, University Mohamed I[st], Oujda-60000, Morocco.

[2]Unité de Biochimie, Centre Hospitalier Universitaire Mohammed VI, Oujda, Morocco.

Article history

Received 07 November 2016
Revised 14 December 2016
Accepted 14 December 2016
Early online 27 December 2016
Print 31 January 2017

Corresponding author

Iliass Lahmass
Laboratory of Biochemistry and Biotechnology,
Department of Biology,
Faculty of Sciences,
University Mohamed I[st],
Oujda-60000, Morocco.
Phone: +212-629530036
Email: iliass.lahmass@gmail.com

Abstract

In this study, we investigated for the first time the antihyperlipidemic effects of crude extract of stigmas from *Crocus sativus* (saffron) against hyperlipidemia induced by tartrazine (synthetic dye) in normal male rats. Thirty adult male albino rats weighing about 150 - 200 g, were divided into 5 groups (n = 6) and daily treatment was given orally. Clinical biochemistry and metabolic parameters were evaluated at the end of the experiment and after 105 days. (n=6, for all groups). Our data revealed that the metabolic parameters like consumption of food and water, pH and urine volume have not been affected; also the difference between liver, right kidney and heart weight was not significant. The levels of cholesterol and triglyceride were significantly increased in group 2 and group 3 compared to control group. There was no significant difference in the level of cholesterol and triglyceride in group 4. Treatment with saffron alone did not have any significant effects on the level of fat compared to control group. The oral administration of the crude extract of saffron revealed good hypolipidemic effects in adult male albino rats. These results suggest that aqueous saffron extract reduced plasma cholesterol and decreased triglyceride. Therefore, it could conceivably lead to suitable changes in blood lipid profiles.

Key words: Antihyperlipidemic effect, Crocus sativus, Saffron, Tartrazine

DOI: 10.5455/jmas.248536

Hyperlipidemia, raised cholesterol and triglycerides, contribute significantly in the causation of atherosclerosis and coronary artery diseases (CAD). Several factors contribute to the emergence of cardiovascular disease such as age, hereditary, diet, life style and hypertension. Likewise high level of cholesterol in blood particularly low density lipoprotein (LDL) cholesterol is mostly responsible for the commencement of CAD[1]. To reduce the level of cholesterol in blood, numerous medicinal plants were reported to have a potential to decrease fat in the body.

Saffron (*Crocus sativus*) is one of the highest priced and the most used spice around the world as both flavouring and colouring agent[2]. Numerous studies have demonstrated that saffron have antispasmodic, gingival sedative, nerve sedative, carminative, diaphoretic and expectorant[3], antioxidant[4,5] and have beneficial effects in the treatment of neurodegenerative disorders such as Alzheimer's disease[6], also saffron or its active constituents has demonstrated an antinociceptive effect, as well as acute and/or chronic anti-inflammatory activity[7]. Saffron and its constituent, crocin, have

an overall protective effect against hyperlipidemic manifestation in rat[8] likewise aqueous extract of saffron and its constituent showed an aphrodisiac activity in normal male rats[9]. Recently, it was found that saffron extract, exhibited significant decrease of blood cholesterol[10] and glucose level[11,12].

Tartrazine is an orange-coloured, water soluble powder used worldwide as food colouring agent. This food additive is most often responsible for allergic reactions in specific human populations[13,14]. The results of some studies showed that Tartrazine has the carcinogenic and mutagenic effects[15-19]. Tartrazine also increase blood glucose level and plasma creatinine, protein, cholesterol and triglyceride[20].

The aim of this study was to evaluate the antihyperlipidemic effect of crude extract of stigmas from *Crocus sativus linnaeus*.

Materials and methods

Plant materials

Crocus sativus (Saffron) was obtained from Taliouine (Taroudant Province, Souss-Massa-Drâa, Morocco). Three specimens of the plant have been deposited at the plant section of Herbarium University Mohammed Premier, Oujda, Morocco (HUMPOM), under the voucher number (HUMPOM210). The identification of the plant has been done and confirmed by a professional botanist, Professor Fennane Mohammed from Scientific Institute in Rabat, Morocco. Dried milled powder of stigmas of *Crocus sativus* was macerated for 12 hours in distilled water before usage and crude extract was used to treat male rats.

Chemicals

Tartrazine (CAS 1934-21-0, Purity 86.7%), was purchased from Alfa Aesar (Germany), Sigma-Aldrich (Japan) and was dissolved in distilled water 12 hours before use.

Qualitative determination

One hundred µL of extracts samples were injected into a liquid chromatography (HPLC) to determine the chemical compounds of the saffron extract. A Waters Symetry® C18 (4,6µm x 250mm) column. A linear gradient of methanol (10–100%) in water (15% of acetonitrile) was used as a mobile phase with a flow-rate of 1 ml/min for a maximum elution time of 60 min at room temperature. The sample size was 20 µl of the test solution[21]. The analyses were triplicated for each sample.

Animals

Maintenance and handling of rats were in accordance to the international conventional standard guidelines and with the Helsinki declaration for use of laboratory animals. 30 male Wistar rats weighing 150 – 200 g were housed in individual cage under standard laboratory conditions in a 12 h/12 h light/dark cycle and at a temperature of 21 - 25°C (animal house of the Department of Biology, Faculty of Sciences, Oujda, Morocco) and were given free access to water and dry rat pellets feeds (SONABETAIL Society, Oujda, Morocco).

Experimental design

Animals were arbitrarily separated to five groups of equal number and weight (six animals each). All animals were treated by daily oral gavage for 105 days with a volume of 10 ml/kg body weight (b. w.).

Group 1 (Control group): Rats were given distilled water.

Group 2 (Tartrazine-Saffron group): Animals were treated with Tartrazine (10 mg/kg) for 60 days and then administered with saffron (120 mg/kg) until the last day of treatment.

Group 3 (Tartrazine group): Rats were administered only with Tartrazine (10 mg/kg) for all period of treatment.

Group 4 (Saffron-Tartrazine group): Animals were treated with saffron (120 mg/kg) for 60 days and then administered with Tartrazine (10 mg/kg) until the last day of treatment.

Group 5 (Saffron group): Rats were administered only with saffron (120 mg/kg) for all period of treatment.

On the day of necropsy, blood samples were collected via the abdominal aorta for measurements of biochemical parameters. Cholesterol levels in plasma wawes estimated by the method used by Allain et al[22]. Level of triglyceride in plasma was determined by the Trinder method[23] and biochemistry determinations were performed by using ILab 300 (Instrumentation Laboratory Corporate Headquarters, Barcelona, Spain).

Statistical analysis

All data are expressed as means ± SEM. Significant differences among control and experimental groups was determined by one-way analysis of variance (ANOVA) followed by Tukey post-test using Graph Pad Prism 5.

Results

As shown in the table 1, treatment with Tartrazine and Saffron did not affect metabolic parameters like pH and urine volume and the difference was significant on consumption of food and water; also, the difference between liver, right kidney and heart weight is not significant (Table 2).

The levels of cholesterol and triglycerides were significantly increased in all groups treated with 10 mg/kg b. w. of Tartrazine compared to control group. The levels of triglycerides were significantly increased in group treated with 10 mg/kg b. w. of Tartrazine + 120 mg/ kg b. w. of Saffron. There was no significant difference in the level of cholesterol and triglyceride among all groups treated with 120 mg/kg b. w. of saffron + 10 mg/kg b. w. of Tartrazine. Treatment with 120 mg/kg b. w. of Saffron did not have any significant effects on the level of cholesterol and triglycerides (Fig 1 & 2).

Table 1: Metabolic parameters of Wistar rats feeding with Tartrazine and saffron and sacrificed after 105 days of treatment

Metabolic parameters	Control group	Tartrazine (10 mg/kg)	Tartrazine (10mg) + Saffron (120mg)	Saffron (120mg) + Tartrazine (10 mg)	Saffron (120mg/kg)
Water consumption	37.5±1.71	46.00±3.65*	37.5±4.79	36.67±6.15	30.00±5.63
Food consumption	32.21±1.56	20.34±2.04*	25.72±4.91	16.51±2.68*	35.28±3.39
pH	8.71±0.07	8.69±0.09	8.64±0.1	8.44±0.13	8.38±0.27
Urine volume	13.0±1.63	15.16±1.46	12.17±0.87	15.17±3.91	11.33±1.2

Note: values represent the mean ± SEM of six rats; *p<0.05. Significantly different from controls.

Table 2: Organ weight of Wistar rats sacrificed on day 105 of subchronic treatment and feeding with Tartrazine and Saffron

Organ weight	Control group	Tartrazine (10 mg/kg)	Tartrazine (10mg) + Saffron (120mg)	Saffron (120mg) + Tartrazine (10 mg)	Saffron (120mg/kg)
Liver	6.63±0.15	6.68±0.21	6.9±0.18	6.25±0.58	6.51±0.29
Heart	1.00±0.03	0.96±0.04	0.98±0.04	0.85±0.05	0.79±0.03
Right kidney	0.92±0.03	0.92±0.05	0.92±0.04	0.91±0.04	0.91±0.05

Note: values represent the mean ± SEM of six rats

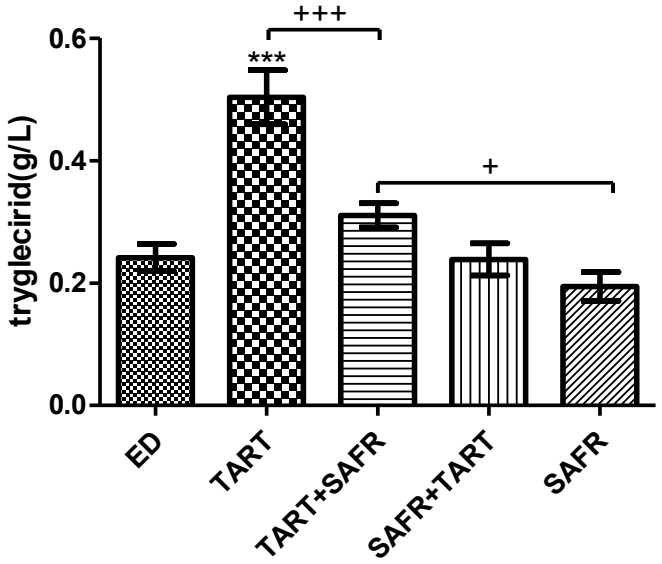

Fig 1. Effects of Tartrazine and Saffron on plasma triglyceride level. **ED:** treated with distilled water; **TART:** treated with 10 mg/kg b. w. of Tartrazine; **TART+SAFR:** treated with 10 mg/kg b. w. of Tartrazine + 120 mg/kg b. w. of Saffron; **SAFR+TART:** treated with 120 mg/kg b. w. of Saffron + 10 mg/kg b. w. of Tartrazine; and **SAFR:** treated with 120 mg/kg b.w of Saffron.

Note: values represent the mean ± SEM of six rats; +++ p<0.001 highly significantly different from group 2. + p<0.05 significantly different from group 4. *** p<0.001 highly significantly different from controls. (+ symbol of comparison with other groups; * symbol of comparison with control group).

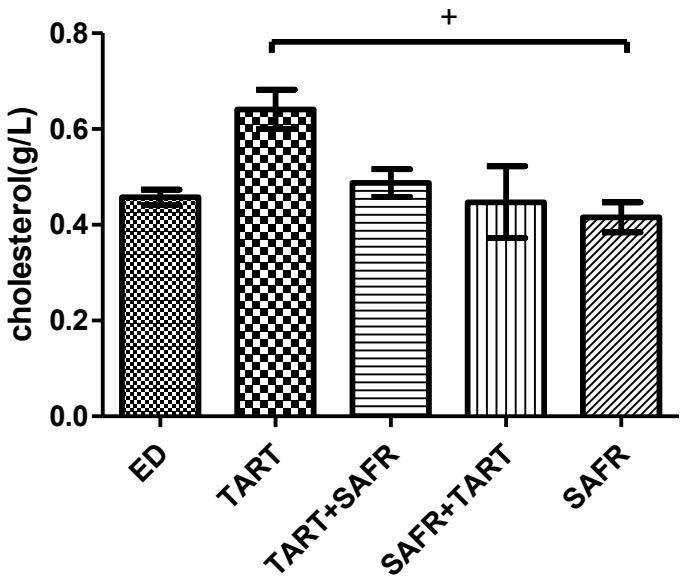

Fig 2. Effects of Tartrazine and Saffron on plasma cholesterol level. **ED:** treated with distilled water; **TART:** treated with 10 mg/kg b. w. of Tartrazine; **TART+SAFR:** treated with 10 mg/kg b. w. of Tartrazine + 120 mg/kg b. w. of Saffron; **SAFR+TART:** treated with 120 mg/kg b. w. of Saffron + 10 mg/kg b. w. of Tartrazine; and **SAFR:** treated with 120 mg/kg b.w of Saffron.

Note: values represent the mean ± SEM of six rats; + $p<0.05$ significantly different to group 4.

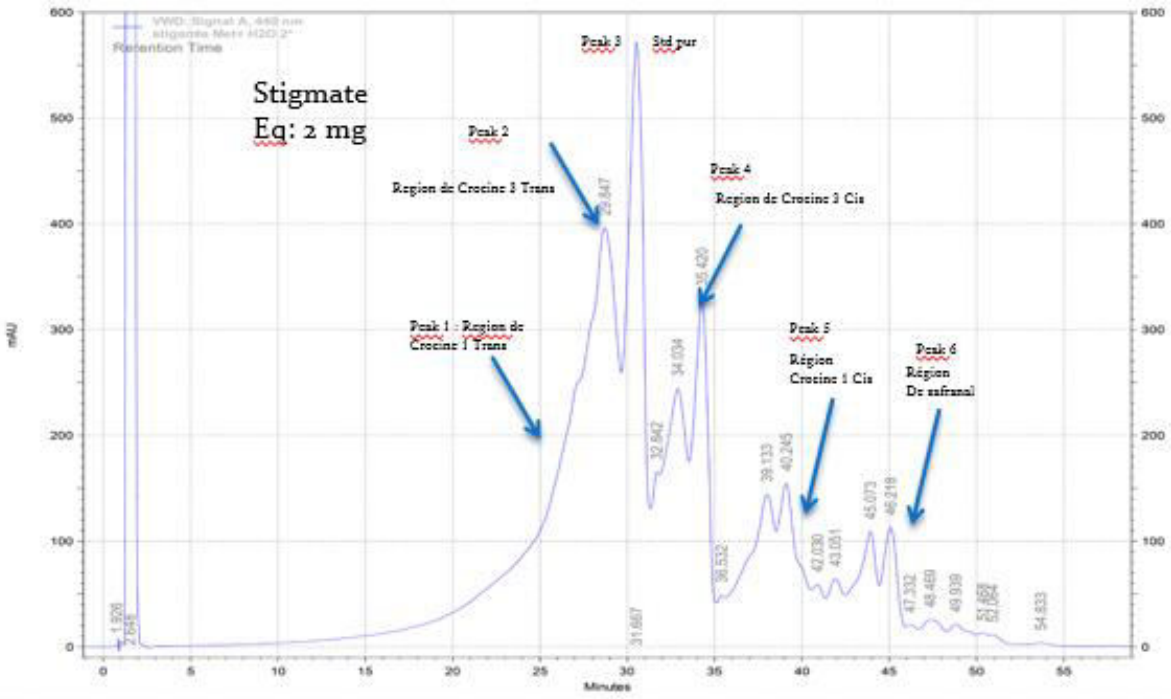

Fig 3. HPLC chromatograms of extract of saffron with different peaks of various components of the stigma. A Waters Symetry® C18 column, a linear gradient of methanol (10–100%) in water (15% of acetonitrile), and a flow rate of 1 ml/min were used for qualitative determinations.

Discussion

One hundred µL of extract samples were injected into a liquid chromatography (HPLC) to determine the chemical compounds of the saffron extract. The carotenoid compounds were identified based on their retention times and quantified according to the respective standard calibration curves (Fig 3). The HPLC chromatogram of the saffron extract indicated crocin as the major compound present in the extract.

The peak identification is as follows: number 1 was crocin 1 trans, peak 2 was crocin 3 trans, peak 3 was standard crocin pure, peak 4 was crocin 3 cis, peak 5 was crocin 1 cis and peak 6 was safranal. According to our analysis, different forms of crocins were detected in our saffron samples.

As shown in figure 1, the day before experiment and until the end of treatment with Tartrazine and saffron extract in the control group, no significant difference in triglyceride plasma was found compared to the group 2, group 3 and group 4 ($p > 0.05$). There was significant difference on plasma triglyceride between control groups compared to group 1 ($p < 0.05$). The level of plasma triglyceride of the group 2 significantly increase compared to group 4 and significantly decrease compared to group 1. No significant difference of the group 3 compared to group 4.

For the plasma cholesterol (Fig 2), there was no significant difference between the control group compared to the group 1, group 2, group 3 and group 4 ($p > 0.05$). There was no statistically significant difference between the group 1, group 2 and group 3 ($p > 0.05$). Significant difference was observed in plasma cholesterol between the group 1 compared to the group 4 ($p < 0.05$).

The differences in mean body weight, organ weights and metabolic parameters between control and groups treated with Tartrazine and saffron were not significant.

For groups treated with Tartrazine, these results are in accordance with the data from Himri and Borzelleca[15,20] who suggested that the decrease of body weight is not toxicological but it was due to decreased caloric intake because of the Tartrazine component of the diet. For groups treated with saffron, our results are in agreement with Elgazer[12] who observed that oral administration of saffron extract caused significant increase in body weight.

Our work showed that the treatment with Tartrazine for 105 days exhibited a significant increase in plasma triglyceride and cholesterol concentration when compared with control rats. This result was in agreement with Himri[20] who observed a significant elevation in serum triglyceride in rats which consumed Tartrazine in different doses. While our results are in a contrast with study of Amin et al which demonstrated that a high dose of Tartrazine decreases serum triglyceride concentration when rats consumed (500 mg/kg b. w.) or low dose of Tartrazine (15 mg/kg b. w.) and with Ashour and Abdelaziz[24] who obtained a significant reduction in serum total cholesterol and triglycerides level when food colour azo dye (fast green) was consumed orally by male albino rats for 35 days.

For group treated with saffron, our study showed that the level of fat (triglyceride, cholesterol) is low compared to control group, these results are in accordance with Elgazer who demonstrated that oral administration of saffron extract caused important differences in body weight, serum levels of blood glucose and insulin and lipid profile as well as the improvement in liver and kidney functions[12]. Mohajeri demonstrated that saffron extract is effective in the reduction of blood sugar and fat amount[25], also Zheng reported that in hyperlipidemic rabbits, crocetin prevented atherosclerosis disease[26]. Sheng suggested that crocin had hypolipidemic properties[27]. Furthermore, He et al[28] indicated that saffron especially crocin inhibit the formation of atherosclerosis in quails, whereas all of his results are in agreement with our study.

The level of plasma triglyceride of the group treated with 10 mg of Tartrazine followed by 120 mg of saffron significantly decreased compared to the group treated with Tartrazine only, this result showed the hypolipidemic effect of consumption of crude extract of saffron for 105 days. This effect could be attributed firstly to the scavenging activity of crocin and safranal and to regenerative properties of the extract.

Conclusion

We can determine from this data that oral administration of crude extract of stigmas from *Crocus sativus linnaeus* has significant beneficial effects. In fact, saffron extract contains crocin and safranal and has shown good antihyperlipidemic effect and could be effective in reducing plasma cholesterol and triglyceride against hyperlipidemia induced by Tartrazine. Further studies are necessary to elucidate in detail the mechanism of action of this medicinal plant. Therefore, saffron may be regarded as a useful therapy for hyperlipidemia.

Acknowledgements: This research is financially sponsored by the ARES “Coopération au développement”. We are very thankful to unit of biochemistry in the hospital of Al Farabi, Oujda, Morocco. We thank also El Mostapha Bedraoui for helping in animal care.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Yokozawa T, Ishida A, Cho EJ, Nakagawa T. The effects of Coptidis Rhizoma extract on a hypercholesterolemic animal model. Phytomedicine. 2003 Jan; 10(1):17-22.

2. Sampathu SR, Shivashankar S, Lewis YS, Wood AB. Saffron (Crocus Sativus Linn.) — Cultivation, processing, chemistry and standardization. C R C Critical Reviews in Food Science and Nutrition. 1984; 20(2):123-57.
3. Ríos JL, Recio MC, Giner RM, Máñez S. An update review of saffron and its active constituents. Phytotherapy Research. 1996; 10(3):189-93.
4. Chen Y, Zhang H, Tian X, Zhao C, Cai L, Liu Y, et al. Antioxidant potential of crocins and ethanol extracts of Gardenia jasminoides ELLIS and Crocus sativus L.: A relationship investigation between antioxidant activity and crocin contents. Food Chemistry. 2008; 109(3):484-92.
5. Kanakis CD, Tarantilis PA, Tajmir-Riahi HA, Polissiou MG. Crocetin, dimethylcrocetin, and safranal bind human serum albumin: stability and antioxidative properties. J Agric Food Chem. 2007 Feb; 55(3):970-7.
6. Naghizadeh B, Mansouri MT, Ghorbanzadeh B, Farbood Y, Sarkaki A. Protective effects of oral crocin against intracerebroventricular streptozotocin-induced spatial memory deficit and oxidative stress in rats. Phytomedicine. 2013;20(6):537-42.
7. Hosseinzadeh H, Younesi HM. Antinociceptive and anti-inflammatory effects of Crocus sativus L. stigma and petal extracts in mice. BMC Pharmacol. 2002 Mar; 2:7.
8. Asdaq SM, Inamdar MN. Potential of Crocus sativus (saffron) and its constituent, crocin, as hypolipidemic and antioxidant in rats. Appl Biochem Biotechnol. 2010 Sep; 162(2):358-72.
9. Hosseinzadeh H, Ziaee T, Sadeghi A. The effect of saffron, Crocus sativus stigma, extract and its constituents, safranal and crocin on sexual behaviors in normal male rats. Phytomedicine. 2008 Jun;15(6-7):491-5.
10. Arasteh A, Aliyev A, Khamnei S, Delazar A, Mesgari M, Mehmannavaz Y, Azar MH. The effects of hydromethanolic extract of saffron (Crocus sativus) on some biochemical parameters of serum blood in constant darkness and light conditions in healthy male rats. Scientific Research and Essays. 2011 Nov ; 6(26) :5595-9.
11. Kianbakht S, Hajiaghaee R. Anti-hyperglycemic effects of saffron and its active constituents, Crocin and Safranal, in alloxan-induced diabetic rats. Journal of Medicinal Plants. 2011 Summer; 10(39):82-9.
12. Elgazar AF, Rezq AA, Bukhari HM. Anti-hyperglycemic effect of saffron extract in alloxan-induced diabetic rats. Eur J Biol Sci. 2013; 5(1):14-22.
13. Neuman I, Elian R, Nahum H, Shaked P, Creter D. The danger of 'yellow dyes' (tartrazine) to allergic subjects. Clin Allergy. 1978 Jan; 8(1):65-8.
14. Devlin J, David TJ. Tartrazine in atopic eczema. Arch Dis Child. 1992 Jun; 67(6):709-11.
15. Borzelleca JF, Hallagan JB. A chronic toxicity/carcinogenicity study of FD & C Yellow No. 5 (tartrazine) in mice. Food Chem Toxicol. 1988 Mar; 26(3):189-94.
16. Collins TF, Black TN, Brown LH, Bulhack P. Study of the teratogenic potential of FD & C Yellow No. 5 when given by gavage to rats. Food and Chem Toxicol. 1990 Dec; 28(12):821-7.
17. Koutsogeorgopoulou L, Maravelias C, Methenitou G, Koutselinis A. Immunological aspects of the common food colorants, amaranth and tartrazine. Vet Hum Toxicol. 1998 Feb; 40(1):1-4.
18. Walton K, Walker R, van de Sandt JJ, Castell JV, Knapp AG, Kozianowski G, Roberfroid M, Schilter B.. The application of in vitro data in the derivation of the acceptable daily intake of food additives. Food Chem Toxicol. 1999 Dec; 37(12):1175-97.
19. Sasaki YF, Kawaguchi S, Kamaya A, Ohshita M, Kabasawa K, Iwama K, Taniguchi K, Tsuda S. The comet assay with 8 mouse organs: results with 39 currently used food additives. Mutat Res. 2002 Aug; 519(1-2):103-19.
20. Himri I, Bellahcen S, Souna F, Belmekki F, Aziz M, Bnouham M, Zoheir J, Berkia Z, Mekhfi H, Saalaoui E. A 90-day oral toxicity study of tartrazine, a synthetic food dye, in wistar rats. International Journal of Pharmacy and Pharmaceutical Sciences 2011; 3(Suppl3):159-69.
21. Caballero-Ortega H, Pereda-Miranda R, Abdullaev FI. HPLC quantification of major active components from 11 different saffron (Crocus sativus L.) sources. Food Chemistry. 2007;100(3):1126-31.
22. Allain DS, Kagan IG. An evaluation of the direct agglutination test for Chagas' disease. J Parasitol. 1974 Feb ; 60(1):179-84.
23. Trinder P. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann Clin Biochem. 1969; 6(1):24-7.
24. Ashour AA, Abdelaziz I. Role of fast green on the blood of rats and the therapeutic action of vitamins C or E. Int J Integr Biol. 2009; 6(1):6-11.
25. Mohajeri D, Mousavi G, Doustar Y. Antihyperglycemic and pancrease-protective effects of Croucus sativus L.(saffron) stigma ethanolic extract on rat with alloxan-induced diabetes. J Biol Sci. 2009; 9(4):302-10.
26. Zheng S, Qian Z, Sheng L, Wen N. Crocetin attenuates atherosclerosis in hyperlipidemic rabbits through inhibition of LDL oxidation. J Cardiovasc Pharmacol. 2006 Jan; 47(1):70-6.
27. Sheng L, Qian Z, Zheng S, Xi L. Mechanism of hypolipidemic effect of crocin in rats: crocin inhibits pancreatic lipase. Eur J Pharmacol. 2006 Aug; 543(1-3):116-22.
28. He SY, Qian ZY, Tang FT, Wen N, Xu GL, Sheng L. Effect of crocin on experimental atherosclerosis in quails and its mechanisms. Life Sci. 2005 Jul; 77(8):907-21.