

Original Article

Anti-diabetic effect of aqueous extract *Crocus sativus* L. in tartrazine induced diabetic male rats

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Abstract

Introduction: In this study, we evaluated the antidiabetic and antidiabetogenic effect of saffron against diabetes induced by artificial dye tartrazine on normal male rats.

Methods: Dried saffron was macerated for 12 hours in distilled water before usage and crude extract was used to treat male rats. Rats were divided into 5 groups consisted of 6 rats and treatment was performed daily and orally. Group-1 treated with distilled water, group-2 with tartrazine 10mg/kg followed by saffron 120mg/kg until the last day of treatment, group-3 administered with tartrazine, group-4 treated with saffron for 60 days and administered with tartrazine, group-5 with saffron for 105 days. Levels of blood glucose and body weight have been evaluated every 10 days and clinical demonstrations and metabolic parameters were evaluated at the end of the experiment.

Results: Levels of blood glucose and body weight have been evaluated every 10 days and clinical demonstrations and metabolic parameters were evaluated at the end of the experiment. Results showed that treatment with tartrazine and saffron did not affect body weights, metabolic parameters but changed the blood glucose levels after 105 days of administration. The levels of glucose and creatinine were significantly increased in group-2 and group-3 compared to control group ($P < 0.05$). There was no significant difference in the level of glucose, creatinine in group 4 ($P > 0.05$). Treatment with saffron decreases creatinine level.

Conclusion: The outcomes suggest that saffron has curative (antidiabetic) and protective (antidiabetogenic) effect against diabetes induced by tartrazine via reducing blood glucose level and creatinine. Therefore, it should be considered in future therapeutic researches.

Keywords:

Crocus sativus L.;
Antidiabetic effect;
Antidiabetogenic effect;
Tartrazine;
Saffron

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Introduction

Diabetes mellitus is a metabolic disorder characterized by a persistent hyperglycaemia and

considered as a major health risk in the world. If it is not duly treated, it will lead to serious complications such as atherosclerosis, retinopathy and nephropathy which are the main causes of morbidities and mortalities (Chan et al., 2001; Liu et al., 2013).

Diabetes influences the quality of life of the patients as well as forcing them to undergo lifestyle changes such as regular monitoring of their blood glucose levels (Smyth and Heron, 2006). As a public health problem, Diabetes mellitus type 2 is an important endocrine and metabolic disorder, and the incidence is on growth all over the world, especially in China (Seino et al., 2010). Treatment by chemical medicines such as acarbose or metformin is extensively used to treat this disease, but there have many negative effects can cause great damage to the health of patients. Several studies showed that elements extracted from plants do well in treatment of diabetes mellitus type 2 such as *Crocus sativus* (Mohajeri et al., 2008; Elgazar et al., 2013), *Allium sativum* (Eidi et al., 2006) and *Trigonella foenum-graecum* (Subramanian and Prasath, 2014).

Tartrazine is an orange-coloured, water soluble powder used worldwide as food additives to colour several foods, drugs and cosmetics. It has been added in cooking with a principal aim to give a colour to a foodstuff. Tartrazine has been widely used as a food additive for the yellow colour and is often responsible for allergic reactions in humans (Neuman et al., 1978; Devlin and David, 1992). The results of some studies showed that tartrazine had the carcinogenetic and mutagenetic effects (Borzelleca and Hallagan, 1988; Collins et al., 1990; Koutsogeorgopoulou et al., 1998; Walton et al., 1999; Sasaki et al., 2002). Tartrazine also increase blood glucose level and plasma creatinine, cholesterol and triglyceride (Himri et al., 2011).

Saffron is a spice derived from the flower of *Crocus sativus* Linn. it's a genus in the family Iridaceae. Regarding therapeutic characteristics, saffron is beneficial for curing nervous pains, asthma, rheumatism, cough and gastric disorders (Sampathu et al., 1984). To treat diseases traditionally, a crude extract of pistils of *Crocus sativus* in water was administered orally alone or with other medicinal plants (Srivastava et al., 2010). For a long time, the use of the plant of *Crocus sativus* was interested only in the part of the red stigmata. Egyptians used saffron by mixing with tea or associated with food in the kitchen for its stimulating and euphoric effects (Winterhalter and Straubinger, 2000). The ancient Romans had hoped to benefit from its reputed ability to prevent hangovers by scraping stigma into their wine (Teuscher et al., 2005).

As a medicinal plant, saffron is used in traditional Persian medicine for throat problems, depression, menstrual disorders and inflammation (Akhondzadeh et al., 2004). In Ayurvedic medicine, saffron was used to treat asthma, arthritis, colds and as an aphrodisiac, adaptogenic, antispasmodic, carminative, expectorant and sedative (Ríos et al., 1996). It has been used in the remedy against eye diseases, scarlet fever, asthma, smallpox, colds and heart disease (Abdullaev and Espinosa-Aguirre, 2004; Bhandari, 2015). Numerous studies have demonstrated that saffron have anti-oxidant (Kanakis et al., 2007; Chen et al., 2008), and its crocin have beneficial effects in the treatment of neurodegenerative disorders such as Alzheimer's disease (Naghizadeh et al., 2013). Also saffron or its active constituents has demonstrated an antinociceptive effect, as well as acute and/or chronic anti-inflammatory activity (Hosseinzadeh and Younesi, 2002). Aqueous extract of saffron and its constituent showed an aphrodisiac activity in normal male rats (Hosseinzadeh et al., 2008). Recently, it was found that saffron extract, significantly decrease blood glucose level, cholesterol (Arasteh et al., 2010) and has anti-hyperglycemic effects (Kianbakht and Hajiaghae, 2011; Elgazar et al., 2013).

Many people use traditionally the macerate of saffron in water (crude extract) to treat diseases and the present study aims to assess the curative and protective effects of crude extract of saffron on tartrazine induced diabetic rats. For the first time we investigated the model of diabetes induced by tartrazine in healthy male rats.

Materials and methods

Plant materials

Crocus sativus L. or saffron was obtained from Taliouine (Taroudant Province, Souss-Massa-Drâa, Morocco), local name: zaâfran. 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. The extraction was carried following this protocol:

Dried milled powder of stigmas of *Crocus sativus* L.

was macerated for 12 hours in distilled water at 4 °C before usage and crude extract (the whole of stigmas and distilled water) was used to treat male rats.

Chemicals

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

HPLC analysis of crude extract of stigma from *Crocus sativus*

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 (Caballero-Ortega et al., 2007). The analyses were triplicated for each sample.

Animals

Maintenance and handling of rats were in accordance to the internationally conventional standard guidelines and with the Helsinki declaration for use of laboratory animals. The 30 male Wistar rats weighting 150 – 200 g were housed in individual cages 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.

Group-1 (normal 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 and group-5 (saffron group): rats were administered only with saffron (120 mg/kg) for all period of treatment.

Determination of blood glucose levels during experimental period (105 days) was done once every 10 days by using a One Touch Ultra 2Glucometer based on glucose oxidase. Blood samples were collected from tail veins of the rats after the animals had been fasted for 12 hours. Body weight was evaluated once every 10 days during experimental period.

On the day of necropsy, blood samples were collected via the abdominal aorta for measurements of biochemical parameters. Glucose concentration and creatinine levels in plasma were estimated by enzymatic colorimetric method according to Trinder and Fabiny successively (Trinder, 1969; Fabiny and Ertingshausen, 1971). Plasma concentrations of alanine transaminase (ALT) and aspartate transaminase (AST) were determined by the method of Morris and Bubenik (1983), biochemistry determinations were performed using ILab 300 (Instrumentation Laboratory Corporate Headquarters, Barcelona, Spain).

Statistical analysis

All data were expressed as mean±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

Compared to water control group, treatment with tartrazine and saffron did not affect body weights (Fig. 1), but it influenced the blood glucose levels after 105 days of administration (Fig. 2).

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 glucose and creatinine were significantly increased in all groups treated with 10 mg/kg of tartrazine compared to control group. The level of creatinine was significantly increased in group treated with 10 mg/kg of tartrazine + 120 mg/kg of

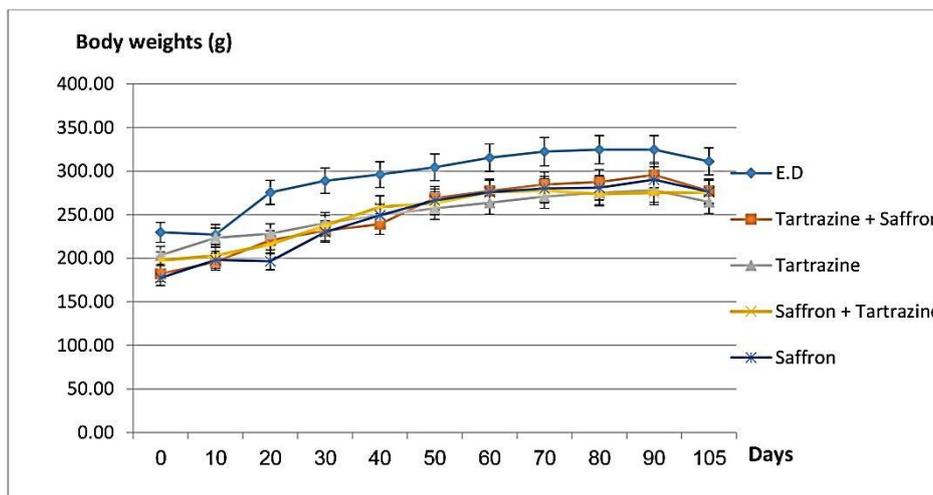


Fig.1. Body weights of Wistar rats for 105 days treated orally with tartrazine and saffron. ED: treated with distilled water, Tartrazine: treated with 10 mg/kg b.w of tartrazine, Tartrazine + Saffron: treated with 10 mg/kg b.w of tartrazine + 120 mg/kg b.w of saffron, Saffron +Tartrazine: treated with 120 mg/kg b.w of saffron + 10 mg/kg b.w of tartrazine and Saffron: treated with 120 mg/kg b.w of saffron.

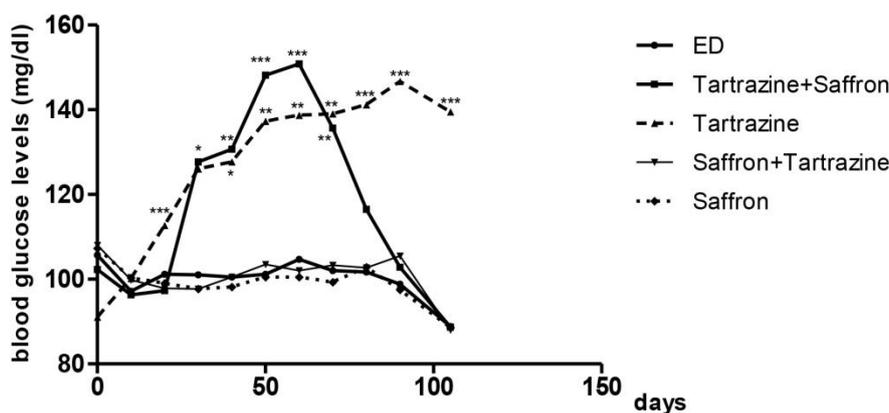


Fig.2. Variation of blood glucose levels during experimental period (105 days). ED: treated with distilled water, Tartrazine: treated with 10 mg/kg b.w of tartrazine, Tartrazine + Saffron: treated with 10 mg/kg b.w of tartrazine + 120 mg/kg b.w of saffron, Saffron + Tartrazine: treated with 120 mg/kg b.w of saffron + 10 mg/kg b.w of tartrazine and Saffron: 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 controls. ** $P < 0.01$ highly significantly different from controls. * $P < 0.05$ significantly different to group control.

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

Metabolic parameters	Control group	Tartrazine (10mg/kg)	Tartrazine (10mg)+ Saffron	Saffron (120mg) +Tartrazine(10mg)	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.

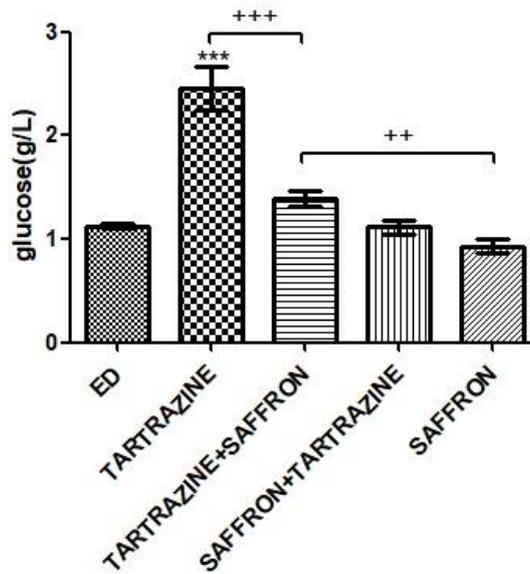


Fig.3. Effects of tartrazine and saffron on plasma glucose level. ED: rats treated with distilled water, Tartrazine: rats treated at the dose of 10 mg/kg tartrazine, Tartrazine + Saffron: rats treated at the dose of 10 mg/kg tartrazine + 120 mg/kg saffron, Saffron + Tartrazine: rats treated at the dose of 120 mg/kg saffron + 10 mg/kg tartrazine and Saffron: rats treated at the dose of 120 mg/kg saffron.
 Note: values represent the mean ± SEM of six rats; +++ $P < 0.001$ highly significantly different from group 2. ++ $P < 0.01$ highly significantly different from controls. *** $P < 0.001$ highly significantly different from controls. (+ symbol of comparison with other groups; * symbol of comparison with control group)

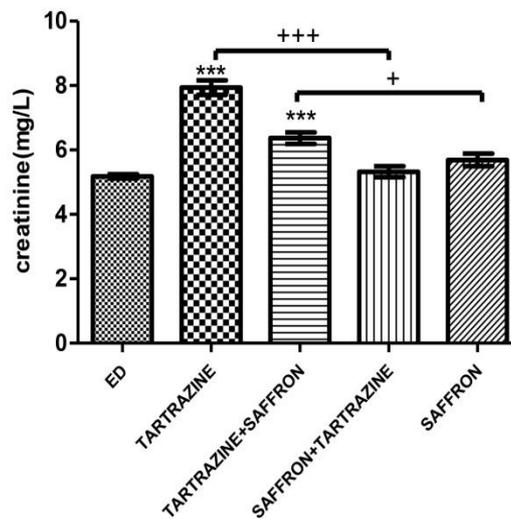


Fig.4. Effects of tartrazine and saffron on plasma creatinine level. ED: rats treated with distilled water, Tartrazine: rats treated at the dose of 10 mg/kg tartrazine, Tartrazine + Saffron: rats treated at the dose of 10 mg/kg tartrazine + 120 mg/kg saffron, Saffron + Tartrazine: rats treated at the dose of 120 mg/kg saffron + 10 mg/kg tartrazine and Saffron: rats treated at the dose of 120 mg/kg saffron.
 Note: values represent the mean ± SEM of six rats; *** $P < 0.001$ highly significantly different to group 3; + $P < 0.05$ significantly different from controls.*** $P < 0.001$ highly significantly different from controls. (+ symbol of comparison with other groups; * symbol of comparison with control group).

saffron. There was no significant difference in the level of glucose and creatinine, among all groups treated with 120 mg/kg of saffron + 10 mg/kg of tartrazine. Treatment with 120 mg/kg of saffron did

not have any significant effects on the level of glucose, but it influenced on creatinine levels. After 105 days of treatment with tartrazine and saffron, significant difference was observed on

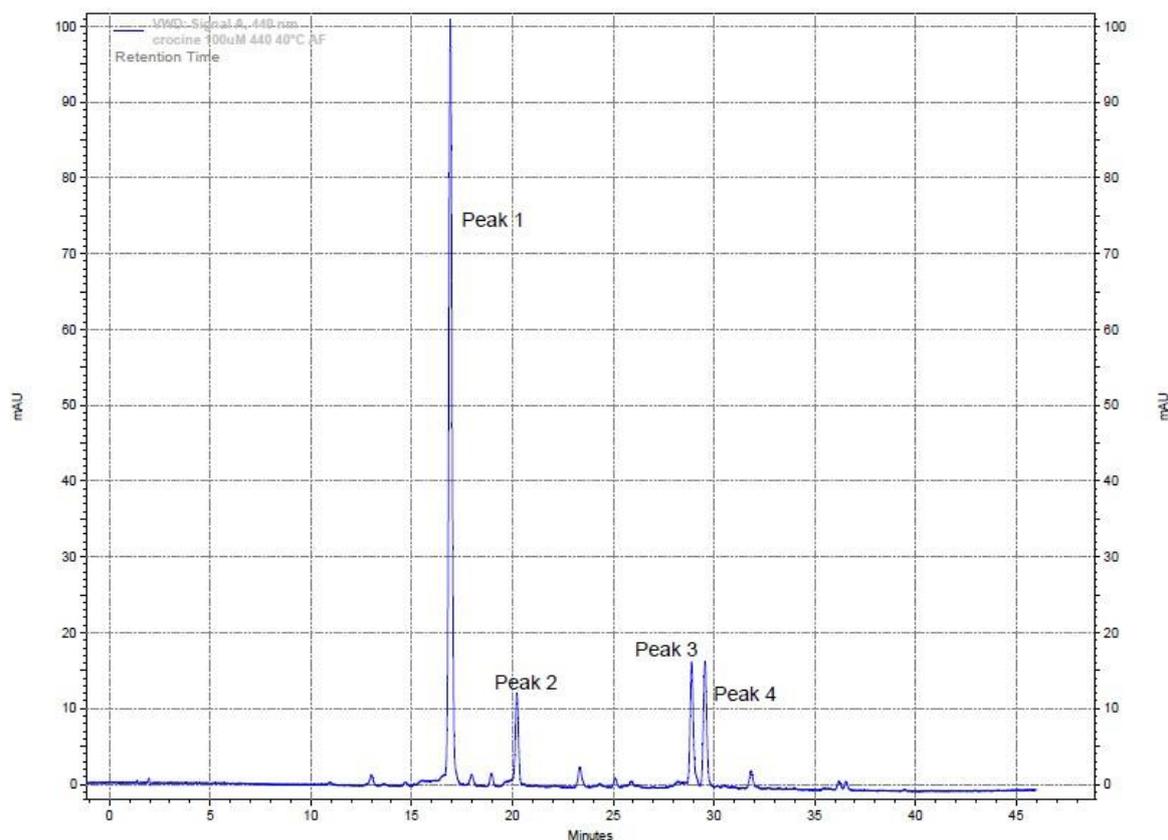


Fig.5. HPLC chromatogram of extract of saffron with different peaks of various components of the stigma at 440 nm. A Waters Symetry® C18 column, a linear gradient of methanol (10–100%) in water (15% of acetonitrile), and a flow rate of 1 ml/min were used for qualitative determinations.

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 (10mg/kg)	Tartrazine (10mg)+ Saffron	saffron (120mg)+ Tartrazine(10mg)	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;

Table 3: Effects of tartrazine and saffron on plasma AST and ALT. Control group treated with distilled water, Group-1 treated with 10 mg/kg b.w of tartrazine, Group-2 treated with 10 mg/kg b.w of tartrazine + 120 mg/kg b.w of saffron, Group-3 treated with 120 mg/kg b.w of saffron + 10 mg/kg b.w of tartrazine and Group-4 treated with 120 mg/kg b.w of saffron.

Parameters	Control group	Tartrazine (10mg/kg)	Tartrazine (10mg)+Saffron	saffron (120mg)+ Tartrazine(10mg)	saffron (120mg/kg)
ALT (U/L)	32,833 ±1.75	40,75 ±1.02*	36,5 ±4.35	42,667 ±3.40*	37.833 ±7.36
AST (U/L)	82,333 ±3.76	85,5 ±1.22	113,167±21.15*	115,5 ±5.17*	107.167±11.9

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

plasma glucose (Fig. 3) between control group compared to group treated with 10 mg of tartrazine (group-1) and there was no significant difference between groups treated with distilled water (control group), treated with 10 mg of tartrazine + 120 mg of saffron (group-2), treated with 120 mg of saffron + 10 mg of tartrazine (group-3) and group-4 treated with 120 mg of saffron. Significant difference in the level of plasma glucose was observed between group-1 compared to group-2 and group-2 compared to group-4. In contrast, the difference between group-3 and group-4 was not statistically significant.

The results presented in Figure 4 revealed that level of plasma creatinine was significantly increased ($P < 0.05$) in group-1 and group-2 as compared to control group. Oral administration of saffron did not cause any significant difference on plasma creatinine of group-3 and 4 compared to untreated group. The level of plasma creatinine on the group-1 significantly decreased compared to group-3 and there was significant difference observed between the group-2 and group-4 ($P < 0.05$).

Plasma concentrations of AST and ALT as indicator of liver functions are recorded in Table 3. Data revealed that significant difference was observed on ALT between control group, groups treated with tartrazine only and saffron followed by tartrazine and on AST between groups treated with tartrazine followed by saffron and saffron followed by tartrazine compared to group treated with distilled water.

The chemical composition of crude extract of stigma was determined using HPLC analysis. The chromatographic conditions employed permitted the identification of major components in saffron sample. The compound was identified by comparison of its retention time as previously described in the literature (Caballero-Ortega et al., 2007). The Figure 5 depicts the HPLC chromatogram of the saffron extract. We identified six peaks of carotenoids: peak 1: crocin 1 trans with a retention time of 27,56 and 3.6 ± 0.001 mg/g dry extract; peak 2: crocin 3 trans with a retention time of 29,84 and $92,33 \pm 0.12$ mg/g dry extract; peak 3: standard crocin pure with a retention time of 31,66 and $110,68 \pm 0.16$ mg/g dry extract; Peak 4: crocin 3 cis with a retention time of 35,42 and $78,04 \pm 0.09$ mg/g dry extract; peak 5: crocin 1 cis with a retention time of 40,24 and $32,13 \pm 0.02$ mg/g dry extract and peak 6: safranal with a retention time of 46,21 and $23,44 \pm 0.14$ mg/g dry extract.

Discussion

The differences in mean body weight, organ weights and metabolic parameters like pH and urine volume between control and groups treated with tartrazine and saffron were not significant. The difference was significant on consumption of food between control group and groups treated with only tartrazine and saffron + tartrazine (group-3). For the water consumption, the difference between control group and group treated only by tartrazine.

The present study showed that the daily administration of tartrazine for 105 days induce a significant increase in serum glucose concentration when compared with control rats. These results were alike to Himri's study (Himri et al., 2011) who observed a significant increase in the serum glucose in rats treated with tartrazine.

The treatment with saffron showed no significant difference in serum glucose concentration with few diminutions compared to control group, this results was in accordance with Mohajeri et al. (2009) who suggest that oral administration and intraperitoneal injection of saffron at different doses reduce the blood glucose levels in healthy rats.

Our work revealed that rats which consumed 10 mg/kg of tartrazine and followed by 120 mg/kg of saffron and the other way around showed no significant difference in serum glucose concentration when compared to control rats. This outcome prove that consummation of saffron can protect the body from elevation of blood glucose levels and keep it stable. Furthermore orally administration of crude extract of saffron decrease the concentration of glucose in blood because this extract contains carotenoids which has antioxidant effect especially crocine which is responsible for these protective effects. Mohajeri and colleagues (2008) exhibited that the ethanolic extract of saffron has significantly decreased blood glucose levels and increased serum insulin in diabetic rats. Also Arasteh et al. (2010) indicated that the saffron extract and its active constituent significantly decreased serum glucose. The active constituent of *Crocus sativus* L. has antioxidants properties which may be very helpful to reduce defects in insulin secretion hence it prevents diabetes complications (Evans, 2007). In a recent study, Mohajeri showed that saffron extract augmented insulin secretion in diabetic rats (Mohajeri

et al., 2009). This data was in accordance with Hemmati et al. (2015) who demonstrated that hydroalcoholic extracts of saffron increased adiponectin levels therefore the decrease of diabetes by carotenoids crocin, the active ingredients of saffron.

Our data indicate that oral administration of saffron caused no significant difference on plasma creatinine of group treated with saffron compared to untreated group. This result was similar to Kianbakht and Hajiaghaee (2011) study who showed that extract of saffron did not have any significant effects on the blood creatinine levels in the diabetic rats after 6 weeks of administration.

This study suggests that the level of plasma creatinine was significantly increased in the group treated with tartrazine and the group of tartrazine followed by saffron as compared to the control group. Moreover, these results are in accordance with study reported by Himri who observed a significant rise of serum creatinine in rats treated with tartrazine orally for 90 days and with Ashour who concluded that creatinine level of rats treated by gavage with fast green for 35 days had a significant rise (Ashour and Abdelaziz, 2009; Himri et al., 2011). Furthermore our data is also in accordance with the data reported by Amin et al. (2010) who observed that when rats treated with high or low dose of tartrazine (500 mg/kg and 15 mg/kg respectively) a rising level of creatinine is observed.

The level of plasma glucose and creatinine on rats which consumed tartrazine significantly decreased compared to rats treated with saffron and followed by tartrazine. This result showed the protective effect of saffron against elevation of plasma creatinine and glucose. Jorns et al. (1999) indicated that during interaction with β -cell many substances act with free radicals formed from alloxan and can prevent radical formation or improve diabetogenic effect of alloxan in animals. Assimoupoulo et al. (2005) reported that saffron and its active constituents has shown significant radical scavenging activity and good antioxidant activity against free radicals and our finding confirms that consumption of aqueous extract of saffron had a major role including protective effect of vital tissues (liver, pancreas, kidney) which is similar to previous studies (Jorns et al., 1999; Assimopoulou et al., 2005; Liu et al., 2013). This effect could be attributing firstly to scavenging activity

of crocin and safranal and to regenerative properties of the extract. Crocin could selectively prevent the absorption of fats by inhibiting the action of pancreatic lipases (Hemmati et al., 2015). Further, saffron extract increased serum insulin levels and caused regeneration of β -cells in diabetic rats (Evans, 2007). The hypoglycemic effect of saffron extract seems to exert by mechanisms such as insulin resistance reducing, stimulating of glucose uptake by peripheral tissues and inhibition of intestinal glucose absorption (Kianbakht and Hajiaghaee, 2011; Elgazar et al., 2013).

One hundred μ l of extracts samples were injected into a 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 (Figure 5). The HPLC chromatogram of the saffron extract indicated crocin and its isomers as the major compound present in the extract with a percentage of safranal.

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 this analysis, different form of crocins were detected in our saffron samples. The HPLC analysis shows that the trans-crocin is the most abundant carotenoid compound in the extract. The finding agrees with previous study reporting the same carotenoids profile in saffron sample (Caballero-Ortega et al., 2007). This result led as to suggest that crocin might be the principal compound responsible of the antidiabetic, diabetogenic effect and antioxidant activities demonstrated previously.

Conclusion

From this study, we can conclude that oral administration of crude extract of stigmas from *Crocus sativus* Linn. has a significant beneficial effect. In fact, the consumption of this extract reduces blood glucose level and creatinine. These results showed the curative (antidiabetic) and protective (antidiabetogenic) effect of saffron against diabetes induced by tartrazine, therefore it has the potential to give therapeutic effect in diabetes diseases. Further studies are necessary to elucidate in detail the mechanism of action of this medicinal plant at the

cellular and molecular level. Therefore, saffron may be regarded as a useful therapy for diabetes mellitus.

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Conflict of interest

The authors declare no conflict of interest.

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