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Original Article

Treatment With Saffron Extract of the Diabetogenic Rats Induced by the Food Colorant Tartrazine

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Abstract

This study evaluated the antidiabetic and antidiabetogenic effect of saffron against diabetes induced by artificial dye Tartrazine on normal male rats. Rats were divided into 5 groups consisted of six rats and treatment was performed daily and orally. 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 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 group2 and group3 compared to control group. Treatment with saffron decrease creatinine level. 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.

Introduction

Diabetes mellitus is a metabolic disorder characterized by a persistent hyperglycaemia and considered as a major health risk in the world. The estimation of number of diabetic adults in the world will increase to 300 million by the year 2025 (1, 2). 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 (3). As a public health problem, T2DM is an important endocrine and metabolic disorder, and the

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incidence is on growth all over the world, especially in China (4). 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 T2DM such as *Crocus sativus* (5, 6) *Alluim sativum* (7) and *Trigonella foenum-graecum* (8).

Tartrazine is an orangecoloured, 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 (9, 10). The results of some studies showed that Tartrazine had the carcinogenetic and mutagenetic effects (11-15). Tartrazine also increase blood glucose level and plasma creatinine, cholesterol, triglyceride (16).

Saffron is a spice derived from the flower of *Crocus sativus* Linn., it's a genus in the family Iridaceae. Saffron is one of the highest priced and the most used spices around the world for flavouring and colouring food (17). To treat diseases traditionally, a crude extract of pistils of *Crocus sativus* in water was administered orally alone or with other medicinal plants (18). 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 (19). The ancient Romans had hoped to benefit from its reputed ability to prevent hangovers by scraping stigma into their wine (20).

As a medicinal plant, saffron is used in traditional Persian medicine, for throat problems, depression, menstrual disorders and inflammation (21). In Ayurvedic medicine, saffron was used to treat asthma, arthritis, colds and as an aphrodisiac, adaptogenic, antispasmodic, carminative, expectorant and sedative (22). It has been used in the remedy against eye diseases, scarlet fever, asthma, smallpox, colds and heart disease (23, 24). Numerous studies have demonstrated that saffron have anti-oxidant (25, 26), and crocin have beneficial effects in the treatment of neurodegenerative disorders such as Alzheimer's disease (27), also saffron or its active constituents has demonstrated an antinociceptive effect, as well as acute and/or chronic anti-inflammatory activity (28). Aqueous extract of saffron and its constituent showed an aphrodisiac activity in normal male rats (29). Recently, it was found that saffron extract, exhibited significant decreased of blood glucose level, cholesterol (30), anti-hyperglycemic effects (6, 31).

Many people use traditionally the macerate of saffron in water (crude extract) to treat diseases, 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. Dried milled powder of stigmas of *Crocus sativus* L. was macerated for 12 hours in distilled water before usage and crude extract was used to treat male rats.

Chemicals

Tartrazine (CAS 1934210, Purity 86.7%), was purchased from Alfa Aesar (Germany), SigmaAldrich (Japon) 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 at 440 nm. A Waters Symetry® C18 (4,6 µm x 250 mm) 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 (32). 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. 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.

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 2 Glucometer 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 (33, 34). Plasma concentrations of ALT and AST were determined by the method of (35) and biochemistry determinations were performed by using ILab 300 (Instrumentation Laboratory Corporate Headquarters, Barcelona, Spain).

Statistical analysis

All data were 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

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 I, treatment with Tartrazine and saffron did not affect metabolic parameters like pH and urine volume and the difference was

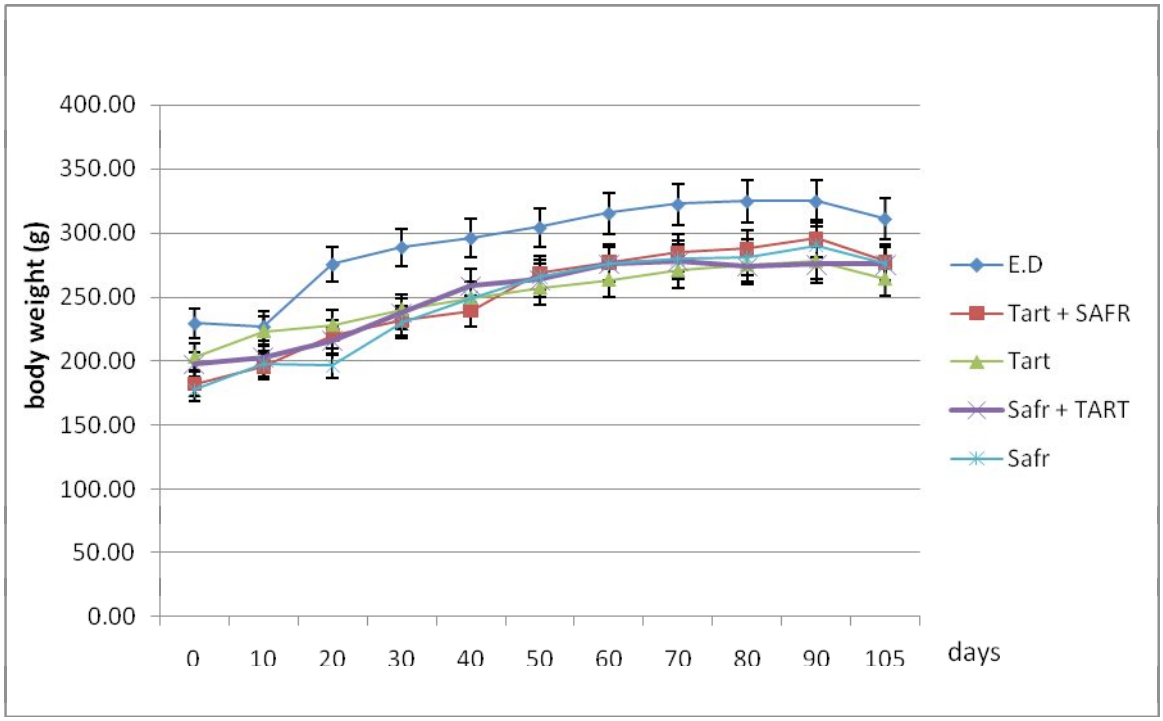

Fig. 1 : Body weights of Wistar rats for 105 days treated orally with Tartrazine and Saffron. ED: Rats treated with distilled water, Tart: Rats treated at the dose of 10 mg/kg Tartrazine, Tart + safr: Rats treated at the dose of 10 mg/kg Tartrazine + 120 mg/kg saffron, Safr + Tart: Rats treated at the dose of 120 mg/kg saffron + 10 mg/kg Tartrazine and Safr: Rats treated at the dose of 120 mg/kg saffron.

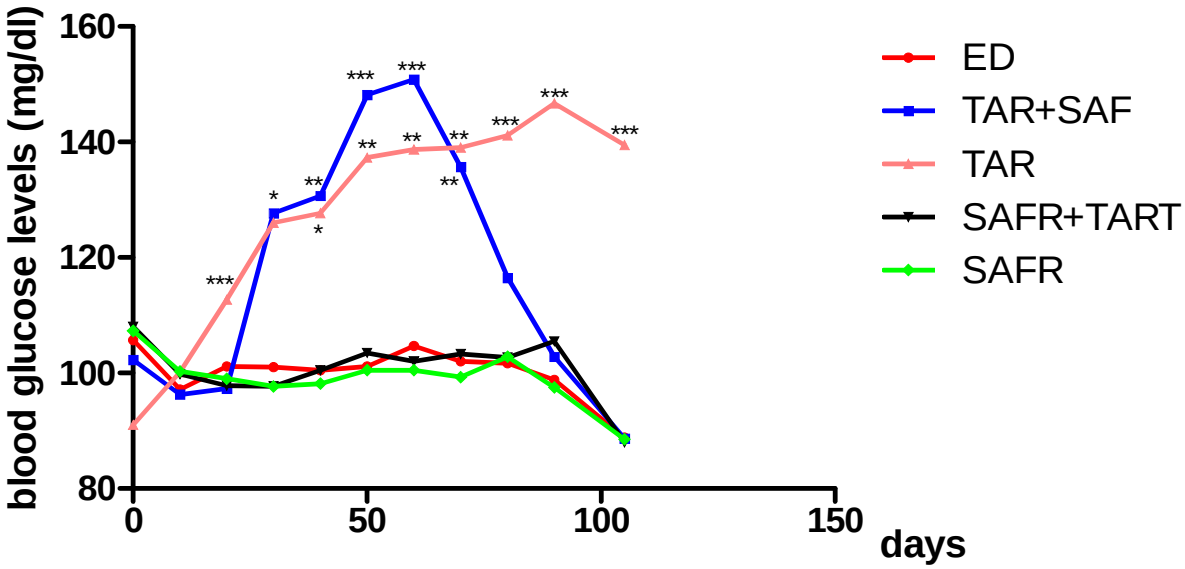

Fig. 2 : Variation of blood glucose levels during experimental period (105 days). ED: Rats treated with distilled water, Tart: Rats treated at the dose of 10 mg/kg Tartrazine, Tart + safr: Rats treated at the dose of 10 mg/kg Tartrazine + 120 mg/kg saffron, Safr + Tart: Rats treated at the dose of 120 mg/kg saffron + 10 mg/kg Tartrazine and Safr: 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 controls. **p<0.01 highly significantly different from controls. *p<0.05 significantly different to group control.

TABLE I: 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 (10 mg) + saffron (120 mg)*	*Saffron (120 mg) + Tartrazine (10 mg)*	*Saffron (120 mg/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.

significant on consumption of food and water; also, the difference between liver, right kidney and heart weight is not significant (Table II).

The levels of glucose and creatinine were significantly increased in all groups treated with 10 mg/kg b.w of Tartrazine compared to control group. The level of creatinine was 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 glucose and creatinine, 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 glucose, but it influnced on creatinine levels.

After 105 days of treatment with Tartrazine and saffron, significant difference was observed on 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 statistically not significant.

The results presented in Fig. 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

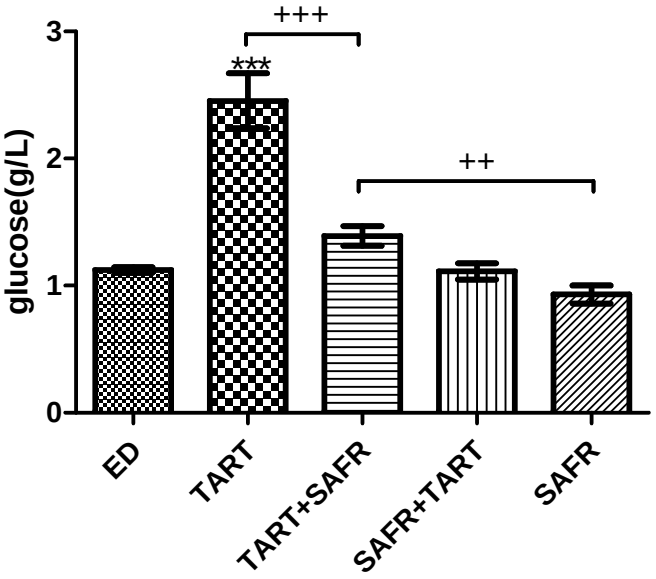

Fig. 3: Effects of Tartrazine and saffron on plasma glucose level. ED: Rats treated with distilled water, Tart: Rats treated at the dose of 10 mg/kg Tartrazine, Tart + safr: Rats treated at the dose of 10 mg/kg Tartrazine + 120 mg/kg saffron, Safr + Tart: Rats treated at the dose of 120 mg/kg saffron + 10 mg/kg Tartrazine and Safr: 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)

decreased compared to group 3 and there was significant difference observed between the group 2 and group 4.

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

TABLE II : Organ weight of Wistar rats sacrificed on day 105 of subchronic treatment and feeding with Tartrazine and saffron.

Metabolic parameters	*Control group*	*Tartrazine (10 mg/kg)*	*Tartrazine (10 mg) + saffron (120 mg)*	*Saffron (120 mg) + Tartrazine (10 mg)*	*Saffron (120 mg/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 III : Effects of Tartrazine and saffron on plasma AST and ALT. Control group treated with distilled water, Group 1 treated at the dose of 10 mg/kg Tartrazine, Group 2 treated at the dose of 10 mg/kg Tartrazine + 120 mg/kg saffron, Group 3 treated at the dose of 120 mg/kg saffron + 10 mg/kg Tartrazine and Group 4 treated at the dose of 120 mg/kg saffron.

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

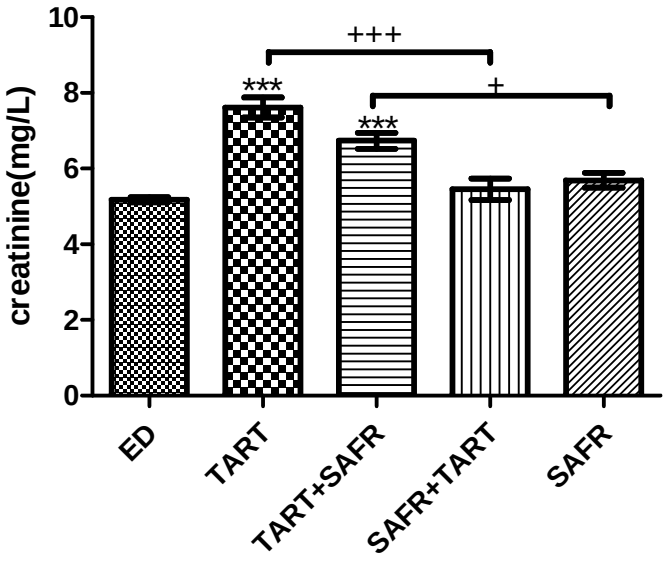

Fig. 4 : Effects of Tartrazine and saffron on plasma creatinine level. ED: Rats treated with distilled water, Tart: Rats treated at the dose of 10 mg/kg Tartrazine, Tart + safr: Rats treated at the dose of 10 mg/kg Tartrazine + 120 mg/kg saffron, Safr + Tart: Rats treated at the dose of 120 mg/kg saffron + 10 mg/kg Tartrazine and Safr: 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).

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 (32). The figure 5 depicts the HPLC chromatogram of the saffron extract at 440 nm. We identified four major peaks of carotenoids:

- Peak 1: crocin-1-*trans* with a retention time of 16,86 and 110,68±0.16 mg/g dry extract;
- Peak 2: crocin-3-*trans* with a retention time of 20,4 and 23.6±0.001 mg/g dry extract;
- Peak 3: crocin-4-*trans* with a retention time of 28,66 and 32,13±0.02 mg/g dry extract;
- Peak 4: crocin-3-*cis* with a retention time of 29,52 and 33,04±0.09 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

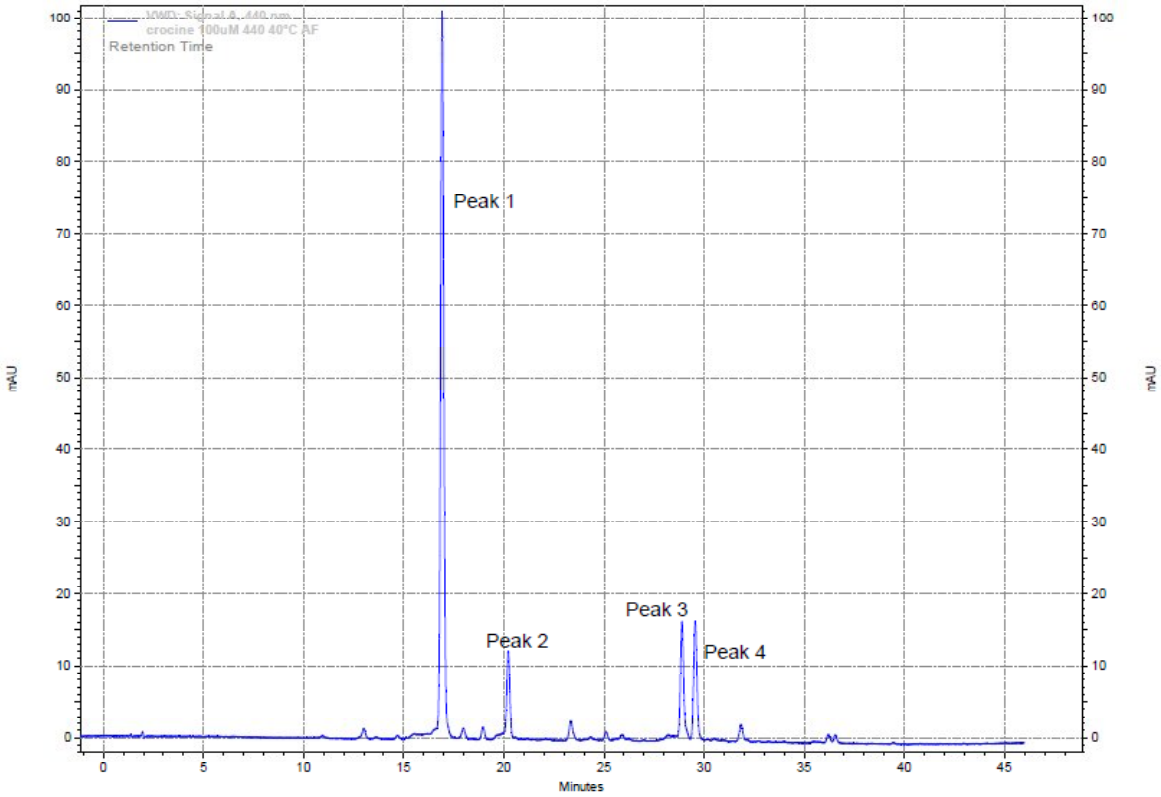

Fig. 5 : HPLC chromatogram of extract of saffron with different peaks of various components of the stigma at 440 nm. A Waters Symetry® C18 column, a linear gradient of methanol (10–100%) in water (15% of acetonitrile), and a flow rate of 1 ml/min were used for qualitative determinations.

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 (16) 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 (36) 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 b.w of Tartrazine and followed by 120 mg/kg b.w 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 who is responsible for these protective effects. Mohajeri (5) exhibited that the ethanolic extract of saffron has significantly decreased blood glucose levels and increased serum insulin in diabetic rats, also Arasteh (30) 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 (37). In a recent study, Mohajeri showed that saffron extract augmented insulin secretion in diabetic rats (36). This data was in accordance with Hemmati who

demonstrated that hydroalcoholic extracts of saffron increased adiponectin levels therefore the decrease of diabetes by carotenoids crocin, the active ingredients of saffron (38).

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's 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 (31).

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 (16, 39). Furthermore our data is also in accordance with the data reported by Amin who observed that when rats treated with high or low dose of Tartrazine (500 mg/kg b.w, 15 mg/kg b.w respectively) a rising level of creatinine (40).

The level of plasma creatinine on rats which consumed Tartrazine significantly decrease compared to rats treated with saffron and followed by Tartrazine. This result showed the protective effect of saffron against elevation of plasma creatinine. Jorns indicated that during interaction with â-cell many substances act with free radicals formed from alloxan and can prevent radical formation or improve diabetogenic effect of alloxan in animals and Assimoupoulo reported that saffron and its active constituents (41) 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) (2, 41, 42). This effect could be attributing firstly to scavenging activity of crocine and safranal and to regenerative properties of the extract.

One hundred µL of extracts 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. 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 crocin-4-trans and peak 4 was crocin-3-cis. 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 (32). This result led as to suggest that crocin might be the principal compound responsible of the antidiabetic, diabetogenic effect and antioxidant activities demonstrated previously.

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. While it has the potential to give therapeutic effect in diabetes. 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.

Conflicts of Interest

The authors declare no conflict of interest.

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