European Journal of Biological Sciences 5 (1): 14-22, 2013
ISSN 2079-2085

DOI: 10.5829/idosi.ejbs.2013.5.1.7224

Anti-Hyperglycemic Effect of Saffron Extract in Alloxan-Induced Diabetic Rats

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Abstract: Saffron (*Crocus sativus*) from iridaceous genus is a stable grass and is considered as an important herb in medical, cosmetics and hygienic industries. The aim of present study was to investigate the effect of given oral administration of saffron water extract with different dosages on alloxan-induced diabetic rats. Thirty five male albino rats of Sprague-Dawley strain weighing 200 ± 5 g were divided into five groups of equal number and weight. Group I, normal control rats; group II, diabetic control rats; and groups III, IV and V, diabetic rats, given orally saffron extract by tube feeding at levels of 200, 400 and 600 mg/kg of body weight, respectively. Oral administration of saffron extract at the three different doses caused significant increase in body weight and serum insulin level in all treated diabetic groups, while significantly reduced blood glucose levels as well as the improvement in lipid profile and liver and kidney functions compared to the positive control group. Histological study showed that pancreas sections of rats from positive control group had hypertrophy and hyperplasia of β-cells of islets of langerhans associated with pyknosis of their nuclei. However, treated rats with 200 mg/kg b.wt of saffron extract had vaculations of acinar epithelial lining in pancreas. Slight hypertrophy of islets of langerhans was demonstrated in pancreas of treated rats with 400 mg/kg b.wt. Apparently normal histological structure of pancreas was found in treated group with 600 mg/kg b.wt. In conclusion administration of saffron extract reduced blood glucose level and the incidence of different complications as results of hyperglycemia. Saffron have an advantage due to the presence of associated bioactive compounds with antioxidant properties which may exert further health promoting effects.

Key words: Saffron · Diabetes Millets · Kidney Functions · Liver Functions · Lipid Profile

INTRODUCTION

Saffron (*Crocus sativus*) from iridaceous genus is a stable grass and its flowers are purple. Saffron is considered as an important herb in medical, cosmetics and hygienic industries [1].It has a sweet smell and a biting taste which has been in use from long times before as a seasoning and food color [2]. Considering contradictory reports about toxicity of saffron, there has been confusion on applying it as an herbal medicine. Some reports suggest that saffron is fully nontoxic herb [3].

Regarding therapeutic characteristics, saffron is beneficial for curing nervous pains, asthma, rheumatism, cough, gastric disorders, sleeplessness, uterus chronic hemorrhage, feminine disorders, fever, influenza and cardiovascular disorders and brain damages [4]. In traditional medicine, saffron has been utilized with various applications such as sexual potential stimulant [5], antidepression [6], sedative, anti inflammation, regulating menstruation and increasing factor of body transpiration [7]. It has also been cleared that saffron improves the memory functions and has anti tumorous properties and some removal effects on free radicals [8]. Researchers have demonstrated that saffron's extract reduces ischemia in kidney and skeletal muscles [9].

Diabetes mellitus is the most prevalent metabolic disorder. If it is not duly treated, it will lead to serious complications such as atherosclerosis, retinopathy, nephropathy, neuropathy and etc., which are the main causes of morbidities and mortalities [10]. Insulin and oral anti-hyperglycemic drugs is the cornerstone of the diabetes treatment, although they have important adverse effects and cannot always maintain glycemia and prevent diabetes complications significantly [11]. Applying medicament herbs has been popular from ancient times among people and in recent years a multilateral approach

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has emerged on using medicines with natural and especially herbal origin [12].Thus there is a continuing need for alternative anti-diabetic remedies with better risk- benefit ratios and greater acceptability. Plants have always been sources of drugs and many of the existing drugs have originated from plants directly or indirectly. Therefore, the aim of present study was to determine the effect of given orally administration of saffron water extract with different dosages on alloxan-induced diabetic rats.

MATERIALS AND METHODS

Material

Saffron: Saffron (*Crocus sativus*) was obtained from local herbs and medicinal plants market, Makka, KSA.

Rats and Diet: Thirty five male albino rats of Sprague-Dawley strain weighing 200 ± 5 g were obtained from the Laboratory Animal Colony, Medicine College, Umm Al Qura University, KSA. Basal diet constituents were purchased from Baghafar Company for Pharmaceutical and Chemical, Jada, KSA.

Chemicals: Alloxan, kits for serum biochemical analysis of insulin, total lipids (TL), triglycerides (TG), total cholesterol (TC), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP),urea nitrogen, uric acid and creatinin were purchased from Baghafar Company for Pharmaceuticals and Chemicals, Jada, KSA.

Methods

Preparation of Saffron Extract: The powdered saffron were extracted with 90% ethyl alcohol and concentrated at low temperature (50°C) using a Rotary evaporator apparatus (manufactured in Basil, Switzerland). Dried ethanol extract was dissolved in a mixture of carboxy methylcellulose and few drops of Tween 80 as a suspending agent to obtain 10% concentration liquid extract.

Preparation of Basal Diets: The basal diet (AIN-93M) was prepared according to Reeves *et al.* [13]. Diet was formulated to meet recommended nutrients levels for rats. Diabetes was induced by a single subcutaneous injection of alloxan dissolved in sterile normal saline at a dose of 150 mg/kg of body weight according to the method described by Buko*et al.* [14]. Non-diabetic control rats were injected with an equivalent amount of slain solution. Diabetic rats were kept for the next 24 hours on 10% glucose solution to prevent hypoglycemia. Seventy-two hours after injection with alloxan, the diabetic rats were confirmed by measuring the 4-h fasting blood glucose level from the tail vein. Animals with a blood glucose level from 300 mg/dl were considered diabetic and included in the experiment.

Experimental Design: All animals were housed in plastic cages at 25 ± 5°C, with a relative humidity of 55 ± 5%, an alternating 12-hour light-dark cycle and were given free access to basal diets and water *ad libitum*. The animals were allowed to acclimatize to the laboratory environment for 7 days and then were randomly assigned to five groups of equal number and weight (seven animals each) as follows: Group I, normal control rats (negative control group) fed on the basal diet only; Group II, diabetic control rats (positive control group) fed on the basal diet only; Groups III, IV and V, diabetic rats, given orally saffron extracts by tube feeding at levels of 200, 400 and 600 mg/kg of body weight, respectively. Body weight was evaluated during experimental period.

Determination of blood glucose levels during experimental period (4 weeks) was done in blood samples collected from tail veins of the rats after the animals had been fasted for 12 hr and determination of the blood glucose levels were carried out at intervals of zero, first, second, third and fourth week by using a single touch Glucometer (Ascensia ENTRUST, Bayer) based on glucose oxidase.

Biochemical Analysis

Serum Insulin Assay: The serum insulin level was measured by a sensitive rat insulin radioimmunoassay kit (Diamond Co, Hannover, Germany).

Lipid Profile Assay: Serum concentration of TL was determined calorimetrically using spectrophotometer apparatus adjusted at 520nm as described by kit instructions (Randox Co., Ireland). Serum concentrations of TG and TC were determined using enzymatic methods as described in the instructions provided with the kits (Analyticon® Biotechnologies AG, Germany). The absorbance of the tested samples was read using spectrophotometer adjusted at 546nm.

Determination of Liver Functions: Serum AST, ALT and ALP activities were determined using colorimetric methods as described in the kits instruction (Diamond Co, Hannover, Germany). The absorption of the test samples were read at 505nm for AST and ALT and at 510nm for ALP.

Kidney Function Assay: Serum urea nitrogen and uric acid concentrations were determined by enzymatic colorimetric method and creatinin was determined using colorimetric kinetic as described in the kits instruction (Diamond Co, Hannover, Germany).

Histopathological Examination: Pancreas and kidney of the scarified rats were taken and immersed in 10% formalin solution. The fixed specimens were then trimmed, washed and dehydrated in ascending grades of alcohol. Specimens were then cleared in xylol, embedded in paraffin, sectioned at 4-6 microns thickness and stained with Haemtoxylin and Eosin stain for histopathological examination as described by Carleton, [15].

Statistical Analysis: All data were expressed as means ± SD. Significant differences among the experimental groups were determined by one-way analysis of variance using the SPSS statistical analysis program. Statistical significance was considered at $p<0.05$.

RESULTS

Body Weight: Effect of oral administration of saffron extract at doses of 200, 400,600 mg/kg b.wt on body weight in diabetic rats is recorded in Table 1. Results indicated that at the initial of experiment, there was no significant difference in body weight (g) among all groups ($p<0.05$). During the experimental period at the end of first, second, three and fourth week, mean ± SD values of body weight (g) of positive rats were significantly ($p<0.05$) reduced compared to the negative control group. Oral administration of saffron extract at the three different doses caused significant increased in body weight (g) in all treated diabetic groups compared to the positive group. The increase in body weight in treated diabetic rats was more detectable with increasing different doses of saffron extract.

Blood Glucose Level: Presented results in Table 2 revealed that initial blood glucose levels after induction with alloxan were significant ($p<0.05$) increased in all diabetic rats as compared to the normal control rats. During the experimental period, higher significant difference in blood glucose level was found in the positive group compared to the normal group ($p< 0.05$). Oral administration of saffron at the three different doses (200, 400 and 600 mg/kg of b.wt) caused significant ($p<0.05$) decrease in blood glucose levels at the end of the first, second, third and fourth week, compared to the positive control group. The decrease in blood glucose level during treatment was more detectable with increasing doses of saffron extract.

Serum Concentration of Insulin: Effect of oral administration of saffron extract at the three different doses on serum insulin in diabetic rats is showed in Figure (1). Results indicated that serum insulin level of the diabetic positive control group significantly decreased after 4weeks of alloxan administration compared with the normal control group ($p < 0.05$). The oral administration of saffron extract increased the levels of blood insulin in treated diabetic rats significantly ($p<0.05$) compared with the untreated control group.

Serum Concentrations of Total Lipids, Triglycerides and Total Cholesterol: Serum concentrations of TL, TG and TC in diabetic rats are presented in Table 3. Tabulated results revealed that positive control group had significant ($p<0.05$) increased in serum levels of TL, TG and TC compared to the negative control group. According to the results, treated diabetic rats with different doses of saffron extract had significant ($p<0.05$) decreased in serum levels of TL, TG and TC, compared to the positive control rats. Treated group with 600mg/kg b. wt had the lowest levels of serum TL, TG and TC, which was significant ($p<0.05$) decreased compared to the other treated groups.

Liver Functions Assay: Serum concentrations of AST, ALT and ALP as indicator of liver functions are recorded in Table 4. Data revealed that positive control group had significant increased ($p<0.05$) in serum concentrations of AST, ALT and ALP compared to the negative control group. Oral administration of saffron extract caused significant increased ($p<0.05$) in serum concentrations of AST, ALT and ALP, compared to the positive diabetic group. The decreased in serum concentrations of AST, ALT and ALP were more detectable with increasing the dose of saffron extract.

Kidney Functions Assay: Data in Table 5 illustrated that the effect of oral administration of saffron extract on kidney functions as indicated by serum concentrations of BUN, UA and Cr. Results revealed that positive control group had significant increased in serum levels of BUN, UA and Cr ($p<0.05$) compared to the normal control group. However, oral administration of saffron extract at the three different doses caused significant decreased in serum concentrations of BUN, UA and Cr compare to the positive control group.

Table 1: Body weight of diabetic rats administered different levels of saffron

Groups		Body weight (g) during experimental period as Mean ± SD				
		Initial	First week	Second week	Third week	Fourth week
Normal group (-ve)		204.07±0.61	210.86±0.90b	215.79±0.70b	221.57±0.53b	226.00±0.82ab
Diabetic group (+ve)		204.00±0.50	199.57±0.98d	198.00±0.58e	196.00±0.82e	193.43±0.53d
Diabetic groups treated with saffron at levels of:	200mg/kg	204.29±0.57	209.26±0.78c	212.43±0.45c	217.36±0.56d	219.50±0.82c
	400mg/kg	203.86±0.69	210.00±0.56bc	216.43±0.98b	220.14±0.96c	225.57±0.79b
	600mg/kg	203.86±0.69	212.14±0.90a	219.00±0.82a	223.86±0.96a	226.64±0.38a

Means with different superscripts letters are significant at p<0.05.
A uses harmonic mean sample size = 7 rats.

Table 2: Glucose concentrations in serum of diabetic rats administered different levels of saffron

Groups		Blood glucose level (mg/dl) during experimental period as Mean ± SD				
		Initial	First week	Second week	Third week	Fourth week
Normal group (-ve)		95.39±0.43b	94.56±0.80e	94.56±0.89e	94.05±0.67e	95.16±0.43e
Diabetic group (+ve)		457.57±0.98a	552.00±0.82a	527.43±0.79a	503.29±0.49a	499.75±0.98a
Diabetic groups treated with saffron at levels of:	200mg/kg	457.43±0.98a	398.57±0.79b	299.43±0.79b	242.00±0.41b	161.00±0.82b
	400mg/kg	457.21±0.57a	386.86±0.50c	286.57±0.53c	219.86±0.69c	133.14±0.69c
	600mg/kg	457.14±0.90a	373.29±0.49d	262.57±053d	194.86±0.48d	99.71±0.76d

Means with different superscripts letters are significant at p<0.05.
A uses harmonic mean sample size = 7 rats.

Table 3: Lipid profile of diabetic rats administered different levels of saffron

Animal groups		Serum levels (Mean ± SD)		
		TL (mg/dl)	TG (mg/dl)	TC (mg/dl)
Normal group (-ve)		321.42±0.65e	79.98±0.45e	69.15±0.75e
Diabetic group (+ve)		386.44±0.89a	121.38±0.82a	94.17±0.78a
Diabetic groups treated with saffron at levels of:	200mg/kg	351.99±0.80	104.34±0.49	89.64±0.61
	400mg/kg	343.91±0.70	100.61±0.39	84.44±0.45
	600mg/kg	326.68±0.69	93.31±0.47	79.21±0.37

Means with different superscripts letters are significant at p<0.05.
A uses harmonic mean sample size = 7 rats.

Table 4: Liver functions of diabetic rats administered different levels of saffron

Animal groups		Serum levels as Mean ± SD		
		AST (U/L)	ALT (U/L)	ALP (U/L)
Normal group (-ve)		17.14±0.12e	14.26±0.14e	38.10±0.17e
Diabetic group (+ve)		27.42±0.16a	22.86±0.39a	52.01±0.76a
Diabetic groups treated with saffron at levels of:	200mg/kg	23.09±0.30b	19.28±0.28b	43.98±0.75b
	400mg/kg	21.69±0.39c	18.05±0.49c	39.54±0.51c
	600mg/kg	18.61±0.36d	15.90±0.50d	37.89±0.28d

Means with different superscripts letters are significant at p<0.05.
A uses harmonic mean sample size = 7 rats.

Table 5: Kidney functions of diabetic rats administered different levels of saffron

Animal groups		Serum levels as Mean ± SD		
		BUN (mg/dl)	UA (mg/dl)	Cr (mg/dl)
Normal group (-ve)		3.40±0.08	31.00±0.34	0.88±0.01
Diabetic group (+ve)		6.54±0.13	60.64±0.55	2.31±0.09
Diabetic groups treated with saffron at levels of:	200mg/kg	5.41±0.08	51.81±0.33	1.88±0.01
	400mg/kg	4.93±0.20	45.61±0.51	1.31±0.08
	600mg/kg	3.61±0.13	35.30±0.98	1.16±0.05

Means with different superscripts letters are significant at p<0.05.
A uses harmonic mean sample size = 7 rats.

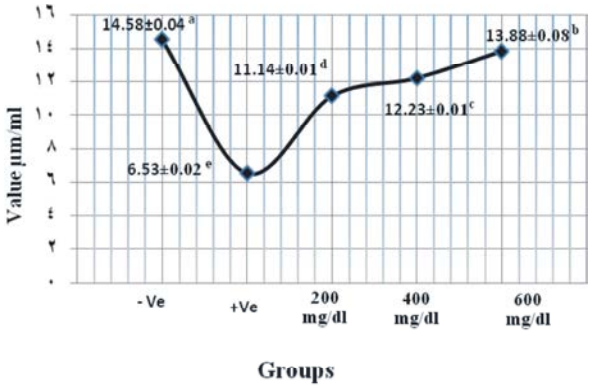

Fig. 1: The effects of saffron extract treatment on insulin levels in test groups of rats. Results denote mean ± SD (n = 7). The different letters in this figure represent the significant differences groups ($p < 0.05$)

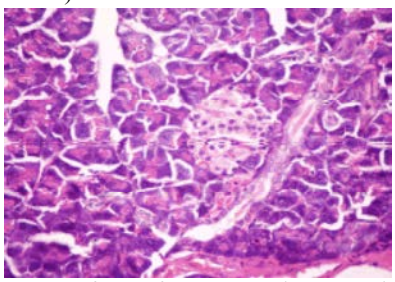

Fig. 2: Pancreas of rats from normal group showing no histological changes (H and E X400)

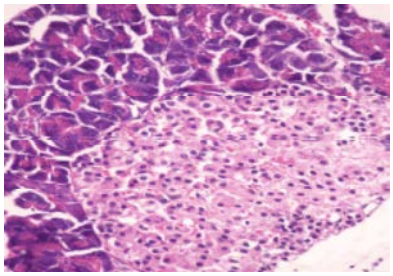

Fig. 3: Pancreas of rats from positive control group showing hypertrophy and hyperplasia of β-cells of islets of langerhans associated with pyknosis of their nuclei (H and E X400)

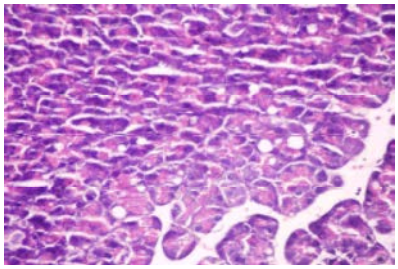

Fig. 4: Pancreas of rats from treated group with 200mg/kg b.wt of saffron extract showing vaculations of acinar epithelial lining (H and E X 400)

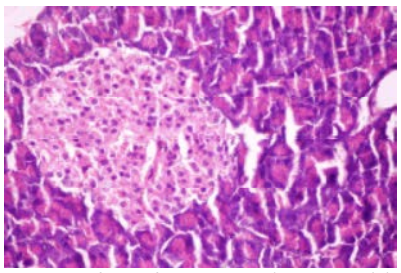

Fig. 5: Pancreas of rats from treated group with 400mg/kg b.wt of saffron extract showing slight hypertrophy of islets of langerhans (H and E x400)

Histopathological Study: Pancreas of rats from normal group showed normal histological structure Figure 2. However, pancreas sections of rats from positive control group showed hypertrophy and hyperplasia of β-cells of islets of langerhans associated with pyknosis of their nuclei as shown in Figure 3. Treated rats with 200 mg/kg b.wt of saffron extract had vaculations of acinar epithelial lining in pancreas (Figure, 4). Slight hypertrophy of islets of langerhans was showed in pancreas of treated rats with 400mg/kg b.wt as shown in Figure 5. Apparent normal histological structure of pancreas was showed in treated group with 600 mg/kg b. wt.

DISCUSSION

The present study aimed to investigate the hypoglycemic effect of saffron extract in diabetic male rats.

Results revealed that during experimental period, blood glucose level in untreated diabetic rats was significantly higher compared to the normal control rats. Histopathological examination of pancreas sections from positive control rats was agreed with Laxmi *et al.* [16] who showed that there was extensive damage of the langerhans in alloxan induces diabetic rats. High blood glucose level cause deterioration of pancreatic β cells due to oxidative stress. Alloxan induced diabetic rats had significantly higher serum levels of TL, TG and TC as compared to the normal control group. Increased plasma total lipid, TC and TG levels in diabetes, may be related to the changes in lipid metabolism and structure. Recently, Farombi and Ige, [17] demonstrated that plasma cholesterol and TG levels were increased significantly in diabetic rats induced by alloxan. Jarald *et al*. [18] mentioned that alloxan induced diabetic rats showed significant hyperlipidaemia and hypercholesterolemia as compared to the control. Previous study demonstrated that in diabetic rats, the utilization of impaired

carbohydrate leads to accelerate lipolysis, resulted in hyperlipidaemia [19] and increased lipid peroxidation which is associated with hyperlipidaemia [20]. The elevation in serum level of total lipid is usually elevated in diabetes millets, such an elevation represents as risk factor for coronary heart disease. This abnormal high level of serum lipid is mainly due to the decrease in the action of lipolytic hormones in the fat depots due to the action of insulin. Under normal circumstances, insulin activates the enzyme lipoprotein lipase, which hydrolysis triglycerides. However, in diabetic state lipoprotein lipase is not activated due to insulin deficiency resulting in hypertriglyceridemia and hypercholestermia [21].

Liver functions tests in the present study included serum AST, ALT and ALP. The activities of AST and ALT are cytosolic marker enzymes reflecting hepatocellular necrosis as they are released into the blood after cell membrane damage. Therefore, we used the activities of AST, ALT and ALP in the circulation as indicators of hepatic damage. In the current study data revealed that positive control group have significantly higher level of serum AST, ALT and ALP compared to that of the negative control group. Liver sections of positive control rats showed congestion of hepatic sinusoid, vacuolization of hepatocytes and necrosis of sporadic hepatocytes. These results was agreed with Arkkila *et al.* [22] who reported that elevated activities of serum AST and ALT is a common sign of liver diseases and observed frequently among people with diabetes than in the general population. Ana Angelica *et al.* [23] indicated that activities of AST, ALT were increased in the serum of diabetic animals. Hamden *et al*. [24] demonstrated that increased generations of free radicals due to oxidative stress develop several adverse effects in diabetes mellitus such as hepathology and nephropathy disorders. Therefore, the oxidative stress is a common pathogenetic mechanism contributing to initiation and progression of hepatic damage in a variety of liver disorders [25]. Hyperglycemia increases the generation of free radicals by glucose auto-oxidation and the increment of free radicals may lead to liver cell damage. The increase in oxygen free radicals in diabetes could be primarily due to the increase in blood glucose levels and secondarily due to the effects of the diabetogenic agent alloxan [26].

The present study indicated that untreated diabetic rats had significant increase in serum levels of BUN, UA and Cr as compared to that of the normal control rats. These results agreed with Verma and Bordia [27] who indicated that increased kidney functions are signs of kidney dysfunctions in the diabetic disease compared to control. These results confirmed by Uladimir [28] who revealed that hyperglycemia are associated with long-term damage, dysfunction and failure of various organs, especially kidneys. Recently, Jarald *et al.* [18] showed that diabetic rats had a significant increase in creatinin and urea levels as compared to the normal animals. Kidney dysfunctions in the diabetic rats may be related to the generation of reactive oxygen species and lipid peroxidation which are associated with tissue injury following ischemic insult. In addition, Shah *et al.* [29] reported that increased oxidative stress and reduce antioxidative ability in diabetes results in renal tubular injury, proteinuria and leads to gradual loss of renal function. The diabetic rats had higher values of plasma BUN than control rats [30].

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. The present results agreed with Mohajeri *et al.* [12] who reveled that saffron extract had anti-diabetic effects and its efficiency in the reduction of blood sugar and fat amount is also proven. Recently, Arasteh *et al.* [31] reported that saffron extract had anti-hyperglycemic and hypoglycemic effects in alloxan-diabetic and non-diabetic rats, respectively. Further, saffron extract increased serum insulin levels and caused regeneration of β-cells in alloxan diabetic rats. Lozano *et al*. [32] reported that saffron extract has various compounds like α-Krustyn, crocins including the crocin and tricrocin, pykrvkrvsyn and safranal. These active constituent have antioxidants properties which may be very important in mitigating impaired insulin secretion and action in insulin resistance and prevent diabetes complications [33]. In a recent study, Mohajeri *et al.* [34] showed that saffron extract induced significantly lower in blood glucose and increased serum insulin in diabetic rats. The hypoglycemic effect of saffron extract seems to exert by mechanisms such as insulin resistance reducing [35], stimulating of glucose uptake by peripheral tissues [36] and inhibition of intestinal glucose absorption [37].

Regarding the hypolipidemic effects of saffron, Sheng *et al.* [38] indicated that crocin has lipid lowering properties and selectively inhibits the activity of pancreatic lipase as a competitive inhibitor. Moreover, He *et al.* [39] founded that crocin has a potent hypotriglyceridemic and hypocholesterolemic activity in atherosclerotic quails. Therefore, saffron is beneficial for curing of cardiovascular disorders [12]. Several mechanisms for the hypolipidemic effects of saffron extract and its constituents have been proposed: (1)

Inhibitory effects on the levels of malondialdehyde, oxygen free radical and intracellular ca^{2+} concentration in endothelial cell and activating superoxide dismutase [40]. (2) Inhibitory effect on pancreatic lipase. It may act by reducing the absorption of fat and cholesterol through inhibiting pancreatic lipase activity [38].

With regard to the improvement of liver and kidney functions effect of saffron extract may be related to the antioxidant properties and its effect in reducing blood glucose level and radical scavenging effect. These results was agreed with Cam *et al.* [41] who reported that the hypoglycemic and antioxidant of saffron prevent oxidative stress and preserve liver function as was observed by low rates in AST, ALT, ALP, total and direct bilirubin.

CONCLUSIONS

In conclusion, all the above complications in diabetic rats may be related to the oxidative stress results from increased blood glucose level. Oxidative stress may cause oxidative damage of cellular membranes and changes in the structural and functional integrity of sub-cellular organelles and may produce effects that result in various complications in diabetic disease. Chronic elevation of blood glucose will eventually lead to tissue damage, with consequent often serious disease, while evidence of tissue damage can be found in many organ and systems. However, oral administrations of saffron extract reduce blood glucose level and the incidence of different complications as results of hyperglycemic. Saffron have an advantage due to the presence of associated bioactive compounds with antioxidant properties which may exert further health promoting effects.

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