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Physiological and biochemical changes in serum of diabetic mice due to streptozotocin exposure and therapeutic role of saffron

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Abstract:Background: Diabetes mellitus is still one of the most important causes of death and disability worldwide. The therapeutic potential of saffron have been documented over the last two decades. The concept of that saffron has a possible anti-diabetic property is Fully-growing.

Aim of the work: the aim of this study is to assess the possible therapeutic potential of aqueous saffron extract (ASE) in diabetic mice by investigating some physiological and biochemical parameters of different experimental groups.

Material and Methods: A single dose of STZ (65 mg/kg body weight) were intraperitoneally injected to mice. After 24 hours, animals with fasting blood glucose over than 300 mg/dl were considered diabetic. The experimental groups were equally divided into; (1) Normal control, (2) Saffron, (3) Diabetic and (4) Diabetic + saffron. After the onset of diabetes, animals were treated with oral gavage of ASE (80 mg/kg body weight) along with drinking water. After 45 days from the end of the experimental period, measurement of blood glucose, body weight and lipid profile parameters were performed.

Results: The current results recorded an obvious increase in blood glucose levels and parameters of lipid profile, together with a concomitant decrease in body weight in the diabetic group. However, treatment with ASE improved the profile of all the tested parameters towards the normal status as compared to untreated diabetic ones.

Conclusion: The study suggests a therapeutic potential for ASE administration on the biochemical parameters in treated diabetic mice

Keywords:*Saffron, STZ, diabetes meletus, physiology, blood parameters, mice*

Introduction

Diabetes mellitus (DM) and its complications are a major health problem. It is estimated that the incidence of diabetes will reach to 300 million by 2025 **(Yazdanpanah *et al*., 2016; Zare Javid *et al*., 2017).**

Diabetes mellitus is a disorder of the metabolism of carbohydrates, proteins, and lipids, and its principal characteristic is hyperglycemia due to lower secretion, lack of insulin or resistance of insulin. Prolonged uncontrolled DM leads to certain microvascular complications such as nephropathy, neuropathy, retinopathy, and macrovascular complications, such as cardiovascular disease and stroke. Poor glycemic control is the main cause worldwide of end-stage chronic kidney disease, amputations, and blindness **(Didangelos**and**Karamitsos, 2009)**. Due to DM's increasing prevalence globally, it has already become a major cause of cardiovascular morbidity and mortality. Moreover, costs for DM treatment and its complications are substantial economic burden for many countries. Furthermore, patients with DM complications have a decreased quality of life and life expectancy **(Calcutt *et al*., 2009;Trisha, 2009)**. However, optimal control of plasma glucose and lipid concentrations can reduce the incidence of DM-related complications **(Brownlee, 2003)**

Type1diabetes (T1D) is the most common autoimmune disorder in childhood, but it can be detected at any age, even in the elderly **(Atalay *et al*., 2014)**. The prevalence of T1D has increased worldwide over the past two decades. Recent statistical studies showed that more than 231 million people worldwide suffer from this disease and are expected to reach 371 million in 2025 **(Rostambeigy*et al*., 2014)**.

Streptozocin (STZ) is a strong alkalizing agent which is capable of destroying beta cells of the pancreatic islets **(Faridi *et al*., 2019).**

Thus, there is an urgent need for alternative anti-diabetic remedies with better risk-benefit ratios and greater patient acceptability **(Shane-McWhorter, 2005; Zareba *et al*., 2005; Gilbert** and **Pratley, 2009).**

Saffron extracts have potent hypoglycemic effects, making them a primary nutraceutical for the treatment of both type 1 (insulin dependent) and type 2 (non-insulin dependent) diabetes **(Shirali *et al*., 2013; Milajerdi*et al*., 2018).**

Saffron active ingredients are able to exert antioxidant **(Nikbakht-Jam *et al*., 2015; Rahiman*et al*., 2018; Yaribeygi*et al*., 2018b)**, anti-inflammatory **(Yaribeygi*et al*., 2018b)**, memory enhancer **(Abe** and **Saito, 2000; Ghadrdoost*et al*., 2011)**, antitumor **(Hoshyar** and **Mollaei, 2017; Moradzadeh *et al*., 2018),** antidepressant (**Lopresti** and **Drummond, 2014; Jam *et al*., 2017; Shafiee *et al*., 2017)**, antiasthma **(Javadi *et al*., 2017)**, cough suppressant **(El-Alfy, 2017)**, cardiovascular protection **(Hatziagapiou**and **Lambrou, 2018)**, neuroprotection **(Wang *et al*., 2015)**, visual function improvement **(Riazi *et al*., 2017;Liou *et al*., 2018)** and sexual behavior potentiation effects **(Malviya *et al*., 2016; Sadoughi, 2017).**

Therefore, the **aim** of the present study was to investigate the possible therapeutic potential of saffron supplementation on biochemical and hematological parameter, due to streptozotocin-induced DM.

MATERIAL AND METHODS

- **Animals**

Twenty adult male Albino mice weighing 30-35 g, 12 weeks old were used in this study. Animals were housed under standard laboratory conditions (five rats per cage, an ambient temperature of 25 ± 2°C, under a 12-h light/12-h dark cycle and open access to food and water).

- **Ethical approval:**

This study was conducted in accordance with ethical procedures and policies approved by: Animal Care and Use Committee of Faculty of science, Zagazig University IACUC.

- **Animal grouping:**

After acclimatization period, animals were equally divided to 4 groups as follows: (G1) normal control, (G2) saffron, (G3) diabetic and (G4) diabetic + saffron. **Streptozotocin injection:** Streptozotocin (STZ) (Sigma-Aldrich, USA) was dissolved in distilled water for fast and functional induction of diabetes after 24 hours and

injected as a single dose intraperitoneally (65mg/kg BW) to G3 and G4 **(Al-Hariri *et al*., 2011; Nassar** and **Hashem, 2017)**

- **Animal weights:**

The tested animal weights were recorded to nearest (g) before treatment and through the experiment.

- **Testing of blood glucose:**

Blood samples were taken from the tail vein directly to the glucose strips for all studied groups. Glucose was determined by using a glucometer (Accu-Check, Roche, Germany); the upper limit of this assay is 33.3 mmol/L (600 mg/dL).

- **Blood sampling:**

In plain tubes the samples of freshly collected blood were allowed to clot and the serum was separated after centrifugation and used for the determination of the following tests:

1-Lipids profile (Triglyceride, cholesterol and total lipids).

2- Glucose level.

Preparation of aqueous saffron extract (ASE): The dried stigmas of *Crocus sativus* flower were obtained from Al-alawy Market, Jeddah, Saudi Arabia. Soak one gram of saffron in 100 ml distilled water. Homogenize in the same distilled water. After 2 hours, stir for 1 hour and filter. This aqueous extract was lyophilized and stored at 4°C until further use **(Premkumar *et al*., 2003).**

Ethics approval:

State authorities approved the experiment and followed Egyptian animal-protection rules of IACUC at Zagazig University with approval number: **ZU-IACUC/1/F/445/2023**

- **Statistical analysis**

Statistical analysis was done using analysis of variance (ANOVA), followed by t-test. Results were expressed as means ± standard deviation (SD). The value of $p \leq 0.05$ was used to indicate statistical significance. Analysis was done using "Statistical Package for Social Sciences" (SPSS) version: 25.0

RESULTS

1- Biochemical results:

a) The glucose onset

In the control group (G1), the blood glucose level recorded a normal range 131 mg/dl during the period of study. While, blood glucose level of saffron group exhibited non-significant decreases (122 mg/dl with $p \leq 0.07686$) but within normal range. However, glucose level showed a significant increase ($p \leq 0.00001$) in the diabetic group compared to normal one. The blood glucose level of G4 was significantly-reduced ($p \leq 0.00001$) by saffron administration as compared to the diabetic group (table 1).

Table 1: Glucose measurements for different experimental groups:

Groups / **Parameters**	**Normal Control (NC)**	**Saffron(S)**	**Diabetic(D)**	**Diabetic+Saffron(D+S)**
Average mean (AM)	131	122	403	267
Standard deviation (SD)	7.12	7.21	6.43	7.45
P Value		0.07686	< 0.00001	< 0.00001

b) The Body weights:

Body weight of the saffron group exhibited a non-significant ($p \leq 0.18691$) difference as compared to normal control group after 45 days of administration. However, the body weight was decreased significantly ($p \leq$

0.00535) in the diabetic group in comparison with that of the normal control group. Also, saffron extract was non-significantly (p ≤ 0.12832) increasing the body weight of animals of G4 as compared to the diabetic group (table2).

Table 2: Body weight measurements for different experimental groups:

Groups / Parameters	Normal Control (NC)	Saffron(S)	Diabetic(D)	Diabetic+Saffron(D+S)
Average mean (AM)	34	32	27	30
Standard deviation (SD)	1.95	2.41	3.65	3.43
P Value		0.18691	0.00535	0.12832

c) Lipid profile (Triglycerides, Cholesterol and Total lipid):

The three tested parameters recorded significant decrease (p ≤ 0.047, ≤ 0.00035 & ≤ 0,01727, respectively) in animals of saffron group as compared to control. But, the recorded decrease still within normal range. However, mice of diabetic group showed significant increase for these parameters (p ≤ 0.00001, ≤ 0.00001 & ≤ 0.00001, respectively) as compared to control group. Diabetes + saffron group exhibited significant decrease (p ≤ 0.00001, ≤ 0.00001 & ≤ 0.00001, respectively) as compared to the diabetic group approaching the normal status (table 3).

Table 3: Triglycerides, Cholesterol and Total lipid levels in different experimental groups:

Groups / Parameters		Normal control	Saffron	Diabetic	Saffron +Diabetic
Triglyceride	**AM**	73	69	144	95
	SD	3.04	2.3	3.39	5.12
	P-Value		0.047	< 0.00001	< 0.00001
Cholesterol	**AM**	119	110	163	135
	SD	2.39	2.30	4.16	4.04
	P-Value		0.00035	< 0.00001	<0.00001
Total lipid	**AM**	348	342	494	411
	SD	4.16	1.67	4.44	7.76
	P-Value		0.01727	<0.00001	< 0.00001

DISCUSSION:

The present study was an attempt to assess the possible therapeutic potential of aqueous saffron extract (ASE) in STZ-induced diabetic albino mice by investigating some biochemical and physiological parameters in sera of different experimental groups to test the safety of saffron at a certain dose for treating diabetic animals. The reason for the high prevalence of morbidity and mortality seen in diabetic patients is

the complications associated with the disease evident by the derangements in the biochemical, histopathological and hematological parameters as diabetes progresses **(Rashid** *et al***., 2019).Glucose onset**
Measurement of glucose status in this experiment revealed a significant increase in glucose onset in diabetic animals which returned toward the normal status by treatment with ASE recording a hypoglycemic effect for saffron.Where, consumption of ASE for 45 days, in the current study, showed a significant decrease in glucose status. These results are in agreement with those of other investigators who observed that blood glucose concentrations increased significantly in diabetic group compared to control group during the experimental period **(Altinoz***et al***., 2014).** Also**,Elgazar***et al***. (2013)** revealed that during experimental period, blood glucose level in untreated diabetic rats was significantly higher compared to the normal control rats. The diabetic control group had a constant high blood glucose level, which suggested that the beta cells of pancreas were destroyed by STZ **(Ashrafi** *et al***., 2018).** The results of **Altinoz***et al***. (2014)** study showed that oral administration of crocin eliminated hepatic injury by scavenging ROS in STZ -induced diabetic rats and decreased the blood glucose levels significantly by hypoglycemicactivity. STZ-treated rats showed a significantly increase of plasma glucose level and decrease of plasma insulin level compared to that of control animals. **(Ali** *et al***., 2016).** These results are in agreement with those of other investigators who reported that 100 mg of *crocus sativus* supplementation for 12 weeks significantly decreased fasting blood glucose (FBG) in subjects with metabolic syndrome **(Kermani** *et al***., 2017).** It is proved that treatment with the hydroalcoholic extract of saffron leads to several advantages in reducing diabetes so that these mice show a lower level of blood glucose than untreated diabetic ones and at the same time the consequences of diabetes, such as hypertriglyceridemia and hypercholesterolemia **(Faridi** *et al***., 2019). Karimi-Nazari** *et al***. (2019)** suggested that the principal mechanisms involved in the antidiabetic effect of saffron supplementation are its strong antioxidant and anti-inflammatory properties. Improvement of the glycemic profile by saffron components can prevent diabetic complications by inhibition of hyperglycemia induced pathophysiologic molecular pathways. Saffron contains more than 150 chemical compounds including vitamins (such as thiamine and riboflavin), amino acids (alanine, proline, and aspartic acid), polysaccharides, flavonoids, and carotenoids (including crocin and crocetin), which are known as key regulators of the antioxidant and inflammatory responses **(Setayesh** *et al***., 2021). Mohajeri** *et al***. (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 saffron extract and its active constituent significantly decreased serum glucose. The active constituent of *Crocus sativus L.* has antioxidant properties which may be very helpful to reduce defects in insulin secretion hence it prevents diabetes complications **(Evans, 2007)**. Additionally, **Mohajeri** *et al***. (2009)** study showed that saffron extract augmented insulin secretion in diabetic rats. These data were in accordance with **Hemmati** *et al***. (2015)** who demonstrated that hydroalcoholic extracts of saffron increased adiponectin levels, therefore, decreased diabetes by the carotenoid crocin (the active ingredient of saffron). Streptozotocin causes a constant high blood glucose which proves that beta cells of pancreas are destroyed. However, diabetic rats treated with SAE showed a significant reduction in the level of blood glucose. Previous studies have also reported the hypoglycemic effects of saffron along with reversing weight loss. In other words; Saffron ethanolic and aqueous extract have the potential to regulate of insulin, improve blood glucose and weight loss in diabetic rats **(Mohajeri** *et al***., 2009; Arasteh** *et al***., 2010; Kianbakht**and**Hajiaghaee, 2011; Elgazar***et al***., 2013)**. A study by **Mohajeri** *et al***. (2008)** determined that the saffron ethanolic extract was able to reduce FBG and cause regenerative modification against damages in the endocrine cells of the pancreas in alloxan diabetic rats. In this context, the suggested hypoglycemic mechanism of saffron involves reduction of insulin resistance and prevention of intestinal glucose absorption **(Xi** *et al***., 2007).** Some reports have also shown that saffron can stimulate glucose uptake in skeletal muscles and adipose tissues **(Yang** *et al***., 2003; Youn** *et al***., 2004).** Moreover, studies indicate the potent hypoglycemic effects of saffron and its bioactive ingredients/β carotenes **(Shirali** *et al***., 2013).** Improvement of the glycemic profile by saffron components can prevent diabetic complications by inhibition of hyperglycemia-induced pathophysiologic molecular pathways **(Yaribeygi***et al***., 2018b; Yaribeygi***et al***.,**

2018a). Saffron significantly lowers plasma glucose and insulin levels and effected improvement in the serum glycemic profile **(Arasteh** *et al***., 2010; Shirali** *et al***., 2013).**

Body weight:

In the current investigation, administration of animals with STZ dissolved in distilled water resulted, effectively, in the induction of diabetes within 24 hours with a significant decrease in body weight of experimental animals which increased and returned toward the normal state by treatment with ASE. These results are in agreement with those of other investigators who recorded that after STZ-induction, body weight of diabetic rats begins to decrease **(Jones** *et al***., 2000; Pushparaj** *et al***., 2000; Muruganandan***et al***., 2005).** During diabetes, due to defect in insulin secretion or function, the excessive glucose production during gluconeogenesis is not useful for the body; therefore, diabetic individuals will lose weight and muscle wasting occurs **(Pushparaj** *et al***., 2000).** During the sub-chronic assessment, mice body weights were observed, where the elevation of diabetic mice body-weights are one of the indicators of alleviation of diabetes symptoms **(Raafat** and **El-Lakany, 2018).** Moreover, **Talebanzadeh***et al***. (2018)** reported that the hypoglycemic effects of saffron which reverses gluconeogenesis is another underlying mechanism involved in the prevention of weight loss in diabetes **(Mohajeri** *et al***., 2008).** Also**, Samarghandian***et al***. (2016a)** reported that saffron extract treatment did not significantly influence body weight within groups of non-diabetic animals. But, saffron extract significantly enhanced body weight in diabetic rats at the end of experimental period. **Altinoz***et al***. (2014)** found that crocin prevent body weight loss in streptozotocin-induced diabetic rats. **Guneli***et al***. (2008)** showed that natural and chemical agents which eliminate free radicals and prevent this body weight loss of rats. In diabetes, both muscle atrophy and loss of weight are the consequence of the body impairment to use the excessive glucose produced during gluconeogenesis **(Shirwaikar***et al***., 2004)**. Body weight loss is mainly observed during short- and long-term experimental diabetes studies **(Coldiron** *et al***., 2002; Obrosova***et al***., 2003). Altinoz***et al***. (2014)** detected a significant weight loss when compared DM group with control group.

Lipid profile:

In the present study, STZ-exposed mice exhibited abnormal lipid profile characterized by elevation of total lipid (TL), triglyceride (TG) and cholesterol (Cho) which may be attributed to disturbed metabolism, and these results are in agreement with those of other investigators **(Elgazar***et al***., 2013)** who observed thatalloxan-induced diabetic rats had significantly higher serum levels of TL, TG and Cho as compared to control group. Increased plasma total lipid, Cho and TG levels in diabetes, may be related to the changes in lipid metabolism and structure. Recently, **Farombi**and **Ige (2007)** demonstrated that plasma cholesterol and TG levels were increased significantly in diabetic rats induced by alloxan. **Jarald** *et al*. (**2008)** 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**(Granner, 1996)** and increased lipid peroxidation which is associated with hyperlipidaemia (**Morel** and **Chisolm, 1998)**. 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 lipase, which hydrolysis triglycerides. However, in diabetic state lipoprotein lipase is not activated due to insulin deficiency resulting in hypertriglyceridemia and hypercholestermia**(Sharma***et al*., **2003)**. Furthermore, **Ali** *et al***. (2016)** reported that STZ-exposed rats exhibited abnormal metabolic pattern, characterized by increased total lipid, total cholesterol, triglycerides in serum and liver tissue, increased serum LDL-c, v LDL-c and reduced serum HDL-c. The results of the present study, indicated that saffron is effective to prevent hyperlipidemia due to diabetes, where ASE consumption for 45 day of experiment was able to decrease TG, TL and Cho level in a normal range. Saffron inhibits elevation of the serum lipid profile keeping total lipid, triglyceride and cholesterol at healthy levels. The

results of**Samarghandian***et al***. (2013)** studyindicate that safranal is also effective to prevent hyperlipidemia due to diabetes. Safranal inhibits elevation of serum lipid profile by controlling oxidative and nitrosative systems. Saffron has been reported to help lower cholesterol and keepcholesterol at healthy levels **(Arasteh** *et al*., **2010).** On the other hand, **Azimi** *et al***. (2014)** showed that consumption of saffron tea for 8 weeks decreased serum cholesterol levels significantly. In animal studies, on diabetic rats, saffron was significantly effective to lower serum cholesterol levels **(Arasteh** *et al***., 2010; Elgazar***et al***., 2013; Samarghandian***et al***., 2013; Shirali** *et al***., 2013; Hemmati** *et al***., 2015).** Saffron consumption in diabetic rats cause significant decreases in TG level **(Samarghandian***et al***., 2013; Shirali** *et al***., 2013).** Regarding the hypolipidemic effects of saffron, **Sheng** *et al.***, (2006)** indicated that crocin has lipid lowering properties and selectively inhibits the activity of pancreatic lipase as a competitive inhibitor. Moreover, **He** *et al***., (2005)** found that crocin has a potent hypotriglyceridemic and hypocholesterolemic activity in atherosclerotic quails. Therefore, saffron is beneficial for curing of cardiovascular disorders (**Mohajeri** *et al***., 2008)** 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 ca2+ concentration in endothelial cell and activating superoxide dismutase **(Xiang** *et al***., 2006)** (2) Inhibitory effect on pancreatic lipase. It may act by reducing the absorption of fat and cholesterol through inhibiting pancreatic lipase activity **(Sheng** *et al***., 2006).** The active components of saffron can alter molecular mechanisms by affecting transcription factors, growth factors and diverse intracellular signaling pathways **(Samarghandian***et al***., 2016b; Yang** *et al***., 2017; Yaribeygi***et al***., 2018b).** C. sativus (saffron) powdered plant, extract, and crocin have potential as an adjunct treatment for T2DM, improving control of metabolic and clinical parameters. However, C. sativus extract seems to be superior because it was effective in more parameters and did not induce adverse effects **(Amatto** *et al***., 2023).** The incidence of DM is sharply increasing worldwide which represents a critical burden or patients and for the society as well due to micro- and macro-vascular complications. The stress factors for the development of vascular disease in subjects with DM are obesity, the presence of poor glycemic control, dyslipidemia, imbalance of oxidant/antioxidant, and inflammation. **(Samarghandian***etal***., 2016a)** indicate that intraperitoneal injection of saffron extract significantly ameliorated the adverse metabolic effects in rats treated with STZ. **Assimoupoulo***et al***. (2005)** reported that saffron and its active constituents exhibited scavenging and good antioxidant activity against free radicals. **Lahmass***et al***. (2017)** results confirmed a major protective effect for vital organs (liver, pancreas, kidney) which is in agreement with previous studies **(Jorns** *et al***., 1999; Assimopoulou***et al***., 2005; Liu** *et al***., 2013)**. This effect could be attributed firstly to scavenging activity of crocin and safranal and to the regenerative properties of the extract. Crocin could selectively prevent the absorption of fats by inhibiting the action of pancreatic lipases **(Hemmati** *et al***., 2015)**. The hypoglycemic effect of saffron extract seems to exerted 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)**. Finally, treatment of diabetes and its complications mainly depend on chemical or biochemical agents; however, a case of total recovery from diabetes has never been reported **(Ivorra** *et al***., 1989; Li** *et al***., 2004).**

CONCLUSION

Administration of ASE showed significant therapeutic potential on the biochemical parameters of diabetic mice which may suggest a functional role in diabetic medication. The current study suggests that saffron is a safe and efficacious natural product that might serve as an adjunct to routine antidiabetic medications, and also as a dietary supplement.

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