

Histological and Biochemical Criteria of Pancreas in Diabetic Mice Due to Streptozotocin Exposure and the Potential Role of Saffron

Samir A. Nassar¹, Mahmoud R. Mahmoud², Hani M. M. Abd El-Salam³, Hagar A. Khalaf⁴

1-4: Zoology Department, Faculty of Science, Zagazig University, Zagazig, Egypt

Corresponding author: Samir A. Nassar

E-mail: sam59nas@gmail.com

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Abstract

Background: Diabetes mellitus (DM) is a chronic health problem, which has undesirable impacts on the quality of life in affected patients. The therapeutic potential of saffron have been documented over the last two decades. The concept of that saffron has a possible anti-diabetic activity is more closely-growing.

Objective: The aim of this study is to investigate the possible therapeutic potential of aqueous saffron extract (ASE) in diabetic mice via investigating some histological and immunohistochemical assessment for pancreas of different experimental groups.

Material and Methods: Animals were subjected to a single dose of STZ (65 mg/kg body weight). After 24 hours, animals with fasting blood glucose over than 300 mm/dl were considered diabetic. The experimental groups were categorized into; (G1) Normal control, (G2) Saffron, (G3) Diabetic and (G4) Diabetic + saffron. After the onset of diabetes, animals were treated with ASE (80 mg/kg body weight, orally) together with drinking water. After 45 days from the end of the experimental period, liver function tests, and histological studies were performed.

Results: The current results recorded a good phase of regeneration in both the exocrine and endocrine portions of pancreas as regards saffron supplementation after STZ- injection. Also, significant increase in AST, ALT and ALP together with a significant decrease in albumin, in the animals of diabetic group. However, ASE improved the biochemical pattern of all these parameters.

Conclusion: ASE administration at the level of experimental dose showed therapeutic potential on the biochemical and histological picture of treated diabetic mice.

Keywords: Pancreas, diabetes mellitus, physiology, histology, Saffron, mice

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Introduction

Diabetes mellitus is still one of the most important etiologies of death and disability in both developed and developing countries. According to the report by World Health Organization (WHO, 2015), a huge number of adults in the world suffer from diabetes and this disease will be the 7th leading cause of death in 2030. Diabetes is characterized by a reduction in the glucose uptake into muscle and adipose tissue, which leads to

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chronic extracellular hyperglycemia and several other complications. This life-threatening disease is mostly associated with high risk of atherosclerosis, hypertension, retinopathy, nephropathy, cataract formation, peripheral nerve damage, and liver apoptosis (Brownlee, 2001; Paneni *et al.*, 2013; Hayashi *et al.*, 2016; Karimi *et al.*, 2017). András *et al.* (2014) reported that DM results from loss of β -cells (T1DM) or resistance developed against insulin receptors (T2DM). Pathogenesis of diabetes is a complex interaction between various factors such as genetics, environment, metabolic disorders, and inflammatory mediators (Frances *et al.*, 2013). Recently, it is established that both types of diabetes mellitus, type I and II, have an impact on the number of β -cells and insulin secretion (Díaz-Villaseñor *et al.* 2006). This finding paid a great attention in targeting the pancreatic β -cell to give a good promise for increasing glycaemia management and slowing the development of diabetes complications (Garber, 2011). The histologic observations of islets from the pancreas of diabetic animals are characterized by degranulation of beta cells and inflammation, vacuolation together with a marked decrease in the number of islets (Adeyemi *et al.*, 2007; Aralelimath and Bhise, 2012).

Streptozotocin (STZ; N-nitro derivative of glucosamine) has been found to be a cytotoxic chemical to the pancreatic insulin-producing beta-cells causing their necrosis (Szkudelski, 2001; Takeshita *et al.*, 2006; Lenzen, 2008). Induction of experimental diabetes in rats using STZ has been found to be effective, convenient, and simple to use (Szkudelski, 2001). The histopathological evaluation showed that the average size of the pancreatic islets was significantly regressed in mice with T1DM compared to normal mice (Faridi *et al.*, 2019).

The ethanolic extract of saffron (*C. sativus L*) stigma recorded a hepato-protective effect in STZ-induced diabetic due to antioxidant effect of saffron (Rahbani *et al.*, 2012). According to Avicenna's book of the Canon of Medicine, there are various hepatoprotective herbs among the ancient medicinal plants, it seems that *C. sativus* has the high therapeutic potential on liver disorders (Shamsi *et al.*, 2014). A study by Mohajeri *et al.* (2008) determined that saffron ethanolic extract was able to reduce fasting blood glucose (FBG) and cause regenerative modification against damages in the endocrine cells of the pancreas in alloxan diabetic rats.

Aim: Therefore, it is an attempt to investigate a therapeutic role of saffron in hyperglycemia supporting the recent trend of alternative medicine. The onset which in turn may minimize the risk of diabetic complications that became direct cause for human death.

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 $25 \pm 2^\circ\text{C}$, under a 12-h light/12-h dark cycle and open access to food and water). 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. **Approval number:** ZU-IACUC/1/F445/2023

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)

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:

- **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).

- **Liver function tests:** Alkaline phosphate (ALP), alanine aminotransferase (ALT), aspartate aminotransferases (AST) and albumin.

For histology : 10% neutral formalin- fixed specimens of pancreas were used, dehydrated in ascending series of ethanol, cleared in xylene and embedded in paraffin wax. Section cutting was performed at 3-4 μ thickness, deparaffinized and processed for:

Hematoxylin and eosin (H & E) staining (Bancroft and Gamble, 2002).

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).

Results:

I- Histopathological results

The microscopic investigation of pancreas of normal control mice (unexposed) exhibited a normal histological architecture of pancreatic lobules separated by thin septa of connective tissue. The pancreatic lobules consist largely of the exocrine pancreatic acini. Pancreatic acini appeared with densely-stained pyramidal cells surrounding lightly-stained islets of Langerhans. The pyramidal cells with basal nuclei and apical acidophilic cytoplasm. The center of the islet comprises aggregates of small Beta-cells, while the periphery consist of large alpha-cells (Fig. 1,2). No histological changes were observed in pancreas of group 2 (saffron group) where similar obserations were obtained as compared to the control (Fig. 3).

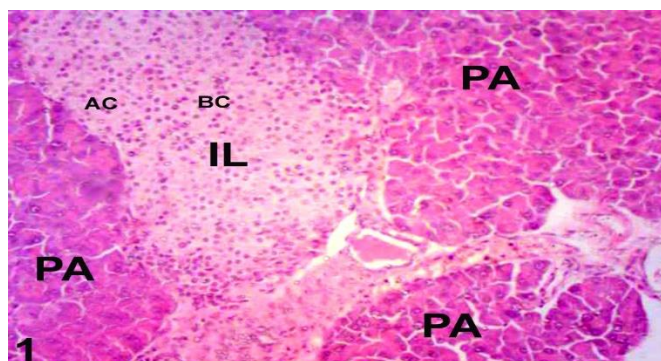


Fig.1: Section of pancreas from the control (un-exposed) group (H&E, X400) showing normal histological architecture of pancreatic acini (PA) grouped in lobules separated by interlobular septae and normal lightly-stained islet cells of Langerhans (IL) comprising alpha and beta cells (AC, BC).

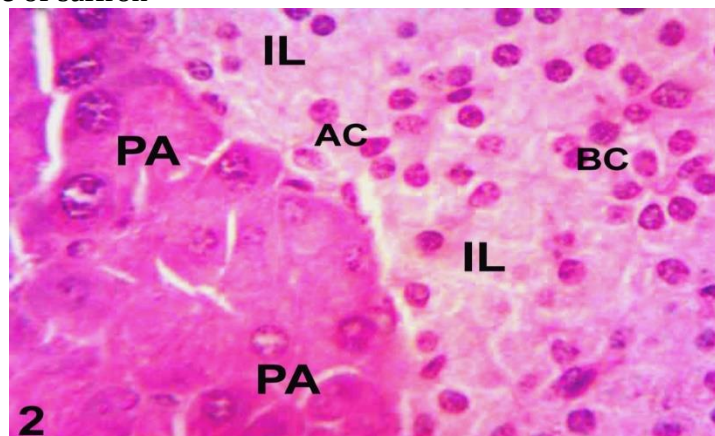


Fig.2: A magnified part of the last section (X1000) showing densely-stained PA with their pyramidal cells, basophilic nuclei and acidophilic cytoplasm and the component cells of the islet of Langerhans; alpha cells (AC) and beta cells (BC).

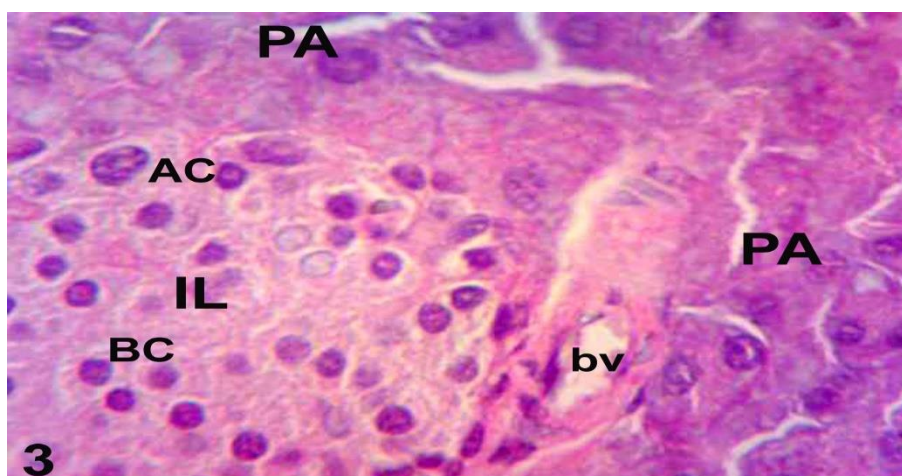


Fig. 3: Section of pancreas from G2 (saffron group) (H&E, X 1000) showing a normal histological pattern nearly similar to that of control.

However, pancreas of the diabetic mice revealed several histopathological changes in both of exocrine and endocrine cells. The exocrine acinar cells appeared necrotic, while islet cells appeared degenerated and shrunken with no clear demarcation between the exocrine and endocrine portions. Lymphocytic infiltration, congested blood vessel and vacuolization (Figs. 4,5). Investigating other section of pancreas from the diabetic group showed a atrophic changes in acinar cells which appeared severely-degenerated with concomitant hemorrhage. The islet of Langerhans appeared poorly-defined with highly -destroyed cells or almost entirely lost leaving large vacuoles and irregular boundary. (Fig. 6).

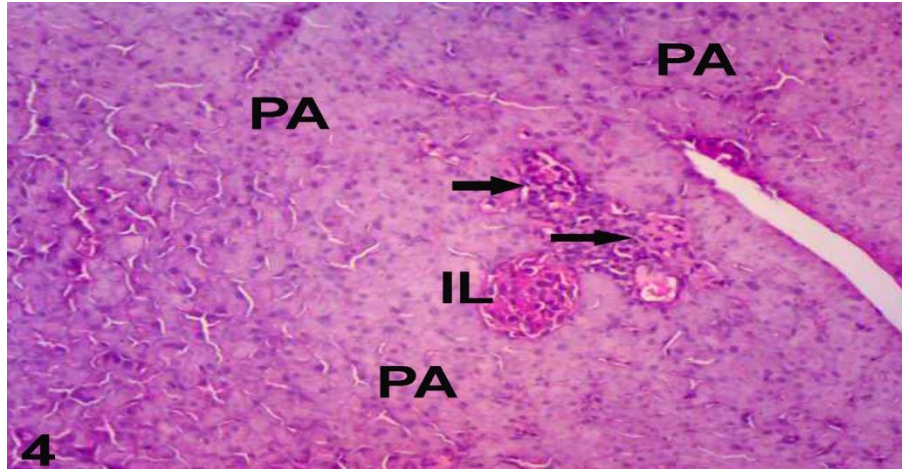


Fig. 4: Section of pancreas from G3, diabetic group, (H&E, X 400) showing necrotic pancreatic acini (PA), degenerated and shrunken islets of Langerhans (IL), some of them are highly-damaged (arrows).

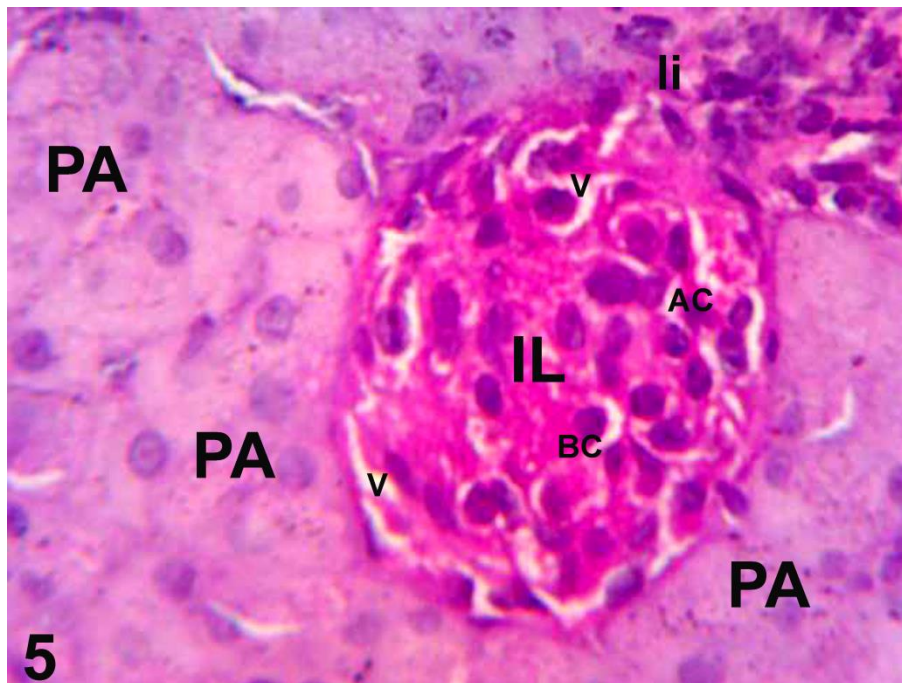


Fig. 5: Magnified part of the last section of pancreas from the diabetic group (X 1000) showing necrotic pancreatic acini (PA), shrunken islet of Langerhans (IL) with apoptotic alpha and beta cells (AC, BC) and invested by lymphocytic infiltration (li).

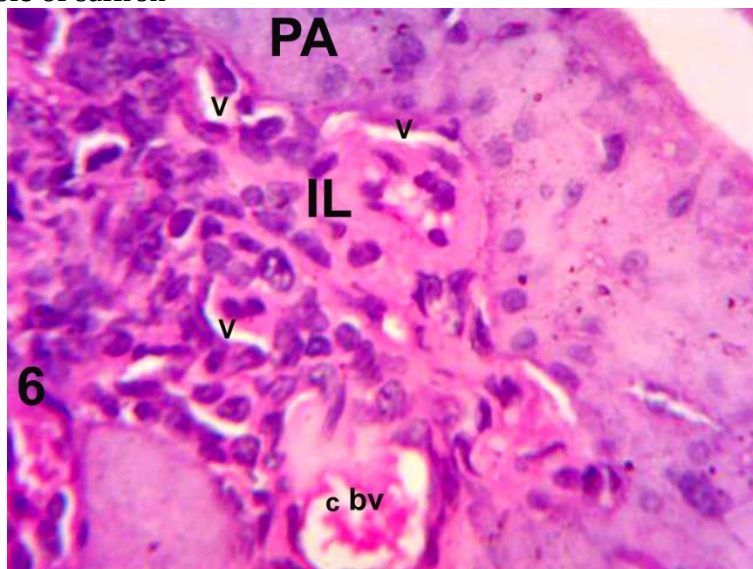


Fig. 6: Another magnified portion of the last photo, (H&E, X 1000) showing atrophic changes of the pancreatic acinar cells (PA) which appeared severely- necrotic The islet of Langerhans (IL) is poorly-defined with destructed cell, congested blood vessel (c bv). and irregular boundary.

On the other hand, animals of saffron+diabetic group exhibited marked evidence of partial cellular regeneration in the pancreatic parenchyma including PA and ilets of Langerhans reflecting some sort of improvement in the histological pattern due to saffron supplementation. Most of PA having intact basophilic nuclei and apical acidophilic cytoplasm except vacuolization and lymphocytic infiltration. Also, ilets of Langerhans showed partial regeneration to have some intact alpha cells and beta cells, while some necrotic areas are still present. The residual atrophic changes of acinar and ilelet cells appeared less severe. The border separating exocrine and endocrine portions became more distinct and regular in large part of its course.

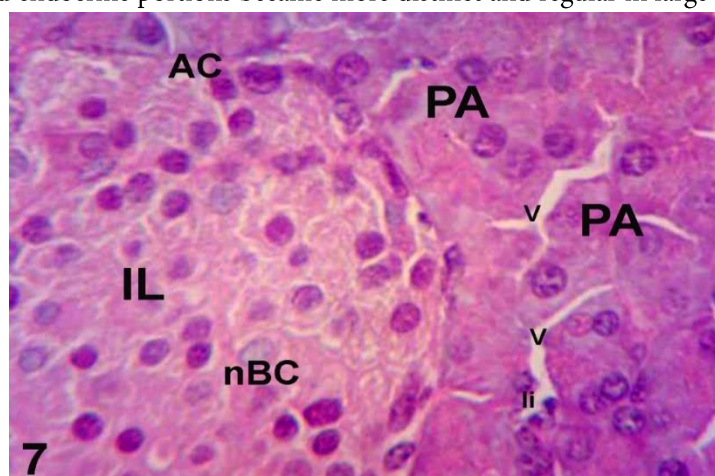


Fig. 7: Section of pancreas from G4, diabetic+ saffron group (H&E, X 1000) showing mild regeneration in both of the exocrine and endocrine portions except vacuolization (v). The border between exocrine and endocrine portions became more distinct and regular in part of its course. PA: pancreatic acini, IL: islets of Langerhans, Li: lymphocytic infiltration, v: vacuoles

II-Biochemical results:**Liver function:**

Transaminases (AST & ALT), ALP and albumin. exhibited a significant decrease ($p \leq 0.00025$, ≤ 0.00423 , ≤ 0.0075 & ≤ 0.00031 , respectively) in saffron group as compared to control. But this decreases still within normal range. Diabetic mice showed a significant increase in serum levels of AST, ALT & ALP ($p \leq 0.00001$, ≤ 0.00001 & ≤ 0.00001 , respectively) with a significant decrease ($p \leq 0.00001$) in albumin compared to the control group. G4 exhibited significant decreases in serum levels of AST, ALT & ALP ($p \leq 0.00001$, ≤ 0.00001 & ≤ 0.00001 , respectively) as compared to the diabetic group, while, serum levels of albumin showed a significant increase ($p \leq 0.02616$) as compared to the diabetic group (table 1).

Table1: Transaminases (AST & ALT) alkaline phosphatase (ALP) and albumin level in different experimental groups:

Groups Parameters		Normal control	Saffron	Diabetic	Saffron +Diabetic
AST	AM	125	112	208	158
	SD	1.82	3.97	7.73	5.18
	P-Value		0.00025	< 0.00001	< 0.00001
ALT	AM	82	74	179	128
	SD	2.91	3.46	6.10	4.64
	P-Value		0.00423	< 0.00001	< 0.00001
ALP	AM	110	100	173	129
	SD	5.76	3.11	4.04	3.21
	P-Value		0.0075	<0.00001	< 0.00001
Albumin	AM	3.66	3.12	2.46	2.8
	SD	0.15	0.13	0.17	0.22
	P-Value		0.00031	< 0.00001	0.02616

Discussion:

The present study was an attempt to evaluate the possible therapeutic potential of aqueous saffron extract (ASE) in streptozotocin (STZ)-induced diabetic albino mice by investigating the histological pattern and some biochemical parameters in pancreas of different experimental groups to elucidate the safety of saffron at certain dose for treating experimental 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). In the current experiment, pancreas of the diabetic mice revealed degenerated exocrine acinar cells which appeared

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necrotic, while islet cells appeared severely-degenerated and shrunken with no clear demarcation between the exocrine and endocrine portions. Lymphocytic infiltration, congested blood vessel and vacuolization could also demonstrated. However, saffron improved most of these changes. This is in agreement with other investigators. **Ali et al. (2016)** recorded that pancreas section of STZ-treated group showed degenerative changes in islets of langerhans, homogenization of the center and apparent relative reduction in the size and number of islets. Some of the islet cells showed vacuolated cytoplasm with nuclear changes as karyolysis, disappearing of nucleus as well as residue of destructed cells was observed. In other foci, irregular outline of the islet and an increased in reticent septa were noticed. Histopathology of the pancreas in diabetic rats showed a spectrum of changes ranging from moderate atrophy with reduced number of islet cells to severe atrophy with an occasional cell, mild to severe destruction of the islets of langerhans by lymphocytic infiltration. Islet cells showed vacuolation. Exocrine cells were similar to those of the vehicle-injected rat pancreas (**Mohajeri et al., 2009**). **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. 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. Destruction of pancreatic islets is the major determinant for the onset of hyperglycemia and development of complications in insulin-dependent diabetic patients (**Hosseini et al., 2015**). β -Cell dysfunction is a central feature of diabetes (**Fernández-Millán et al., 2015**). Located in the islets of the pancreas, β -Cell is unique as they are able to secrete insulin in response to the increases in plasma glucose. Many pathological mechanisms can impair the proper functioning of β -Cell, leading to an insufficient insulin secretory response, the underlying cause of DM (**Keane et al., 2015**).

The current results were in agreement with those of who reported that Although STZ's degenerative effects on the pancreatic islets were reversed by CUR and nCUR. In diabetic rats, they were able to lessen the islets of Langerhans morphological changes. Additionally, it has been suggested that CUR and nCUR can cure pancreatic beta cell damage from free radicals produced by oxidative stress and prevent membrane disruption (**Metaweia et al., 2023**).

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 Hajiaghaye, 2011; Elgazar et al., 2013**). The pre-administration of diabetic rats either with saffron extract showed significant improvement in albumin contents when compared with diabetic rats. These results may be due to their antidiabetic role which improved pancreatic β cells and increased serum insulin level (**Abou El-Soud et al., 2007; Arasteh et al., 2010 and Elgazar et al., 2013**). Saffron inhibitory effect on pancreatic lipase may be another mechanism which may act by reducing the absorption of fat and cholesterol (**Sheng et al., 2006**). Diabetic group had a constant high blood glucose level, which suggested that the beta cells of the pancreas were destroyed by STZ. However, a significant decrease in the level of blood glucose was seen in group treated with SAE (**Ashrafi et al., 2018**). **Ali et al. (2016)** recorded that Pancreatic tissue of the control group showed normal pancreatic structure. The pale stained islets of langerhans "the endocrine component" were scattered throughout the exocrine component with well define boundary. The islets are made up of irregular cords or masses of polygonal epithelial cells. Pancreatic sections of animals treated with either saffron showed normal pancreatic architecture with no observable pathological finding in the cells of islets of langerhans. However, histological examination of pancreatic tissue of saffron + STZ induced diabetic rat showed improvement in the islet

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structure and restoration of normal pancreatic architecture. The STZ-treated rats showed a significantly increase of plasma glucose level and decrease of plasma insulin level compared to that of control animals. Recovery of pancreatic β -cells after treatment with *Crocus sativus* L. stigma in alloxanized mild diabetic rats was prominent as their pancreases were histologically near normal after 1 month of extract administration, many islet cells were absent showing atrophy of cells, in the pancreas of the extract treated animals vacuolation of the islets cells was also not prominent, partially reversion also occurred in the damaged pancreatic islets of severe diabetic rats after treatment with *Crocus sativus* L. stigma, importantly, regeneration of pancreatic islets with normal appearance was recorded in the diabetic-rats after 14 days of ethanolic extract administration (Mohajeri *et al.*, 2009). Daily treatment with the hydroalcoholic extract of saffron could markedly restore the average size of the pancreatic islets in diabetic rats (Faridi *et al.*, 2019), more-over treatment with the hydro alcoholic extract of saffron significantly reduced the incidence of hypoglycemia and restored insulin secretion and histopathological changes in pancreas sections, treatment with saffron reduced lymphocyte proliferation index in the cells isolated from the pancreas of diabetic mice. Also, the extract of saffron markedly decreased the production of pro-inflammatory interleukin-17 (IL-17) increased anti-inflammatory IL-10 and transforming growth factor- β in the pancreatic cell population. Moreover, the production of proinflammatory nitric oxide and reactive oxygen substances were down-regulated by the saffron extract. Treated rats with 200 mg/kg b.wt of saffron extract had vacuulations 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 extract prevented deterioration in β -cell function and induced islet regeneration, probably via its antioxidant effect, in alloxan induced diabetic animals (Elgazar *et al.*, 2013). Another study showed that saffron extract in rat protects pancreas tissues and reduces the incidence of diabetes in this model (Assimopoulou *et al.*, 2005). The findings of one study indicated that saffron and its main ingredients (crocin, crocetin and safranal) may have anti-hyperglycemic and blood insulin level elevating effects without hepatic and renal toxicities in the alloxan-diabetic rats (kianbakht and Hajiaghace, 2011). Based on these results, the protective effect of saffron extract on pancreas of diabetic rats might be attributed directly to scavenging activity due to its major constituents including crocin, crocetin and safranal, these compounds as natural antioxidant prevent diabetes complications (Farkhondeh and Samarghandian, 2014). Extant evidence 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 to mitigate insulin resistance in prediabetic individuals (Yaribeygi *et al.*, 2019). Recently, many studies have shown that saffron and its main ingredients ameliorated the diabetogenicity of STZ and alloxan in animals by reacting with free radicals formed from STZ and alloxan during its interaction with the β -cell, or prevent radical formation (Taherei *et al.*, 2014). Saffron extract had anti-hyperglycemic and hypoglycemic effects in alloxan-diabetic and non-diabetic rats respectively.

Further, saffron extract increased serum insulin levels in alloxan-diabetic and non-diabetic rats and caused regeneration of β -cells in a

alloxan diabetic rats (Mohajeri *et al.*, 2009 and Arasteh *et al.*, 2010). Saffron extract increased serum insulin levels and caused regeneration of β -cells in diabetic rats (Evans, 2007). There are indications that crocin is effective in blood glucose lowering. The involvement of several mechanisms such as the stimulation of Langerhans islets or the insulin-sensitizing effects on peripheral muscles has been proposed. Most importantly,

it was suggested that the strong antioxidant properties of crocin by scavenging the ROS (Kianbakht and Hajiaghace, 2011).

Saffron petal extract improved the histological damages against STZ-induced nephropathy according to the study of Zarez *et al.* (2017). Saffron bioactive compounds also inhibit the production and activity of caspases family of proteins involved in various forms of cellular death, apoptosis, necrosis, pyroptosis and, finally, β -cell destruction and islet failure (Rojas *et al.*, 2018). Saffron extract may help β -pancreatic cells to increase insulin secretion and reduce elevated blood glucose levels (Assimopoulou *et al.*, 2005; Shirali *et al.*, 2013). Another study also demonstrated that saffron extract has hypoglycemic effects on healthy male rats its extract could increase their insulin secretion from pancreatic β -cells (Arasteh *et al.*, 2010).

Concerning the liver function parameters, Diabetic mice showed a significant increase in serum levels of AST, ALT & ALP with a significant decrease in albumin compared to the control group. G4 (saffron-treated group) exhibited significant decreases in serum levels of AST, ALT & ALP as compared to the diabetic group, while, serum levels of albumin showed a significant increase as compared to the diabetic group. This is in line with other investigators in previous studies. Altinoz *et al.* (2014) used AST and ALT as an indicator of diabetes induced liver injury. The results of Altinoz study demonstrated that AST and ALT levels increased significantly in DM group when compared to control group. These results are similar to the study of Arkkila *et al.* (2001) indicated that serum AST and ALT levels which are common indicators of liver diseases were increased in diabetic people when compared with the general population. Similarly, Ana Angelica *et al.* (2009) demonstrated that AST and ALT activities increased in the serum of diabetic animals. Liver functions tests in Elgazar *et al.* (2013) study included that activities of AST and ALT are cytosolic marker enzymes reflecting hepatocellular necrosis as they are released into the blood after cell membrane damage. Therefore, Elgazar used the activities of AST, ALT and ALP in the circulation as indicators of hepatic damage. his 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. Ana Angelica *et al.* (2009) Indicated that activities of AST, ALT were increased in the serum of diabetic animals. Hamden *et al.* (2008) demonstrated that increased generations of free radicals due to oxidative stress develop several adverse effects in diabetes mellitus such as hepatology 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 (Medina and Moreno-Otero, 2005). Ali *et al.* (2016) showed that the aminotransferases (AST and ALT) levels were significantly increased in the liver of STZ-exposed animals. Biochemical and histochemical results in Ali study of STZ induced diabetic rats showed significant decrease in the total protein and albumin content. Modaresi *et al.* (2010) stated that the consumption of saffron has an impact on the synthesis of serum proteins like albumin via changing the status of liver cell function. Arasteh *et al.* (2010) found that saffron extract, significantly decrease blood glucose level, cholesterol. Aqueous and ethanolic extracts of saffron stigmas and petals significantly decreased the levels of AST and ALT in plasma and reduced the incidence of liver lesions induced by CCl₄ (Iranshahi *et al.*, 2011). After crocin treatment, serum ALT levels decreased significantly in DM + crocin group compared with DM group. In addition, serum AST levels decreased, but it was not significant (Altinoz *et al.*, 2014). The results of animal study by Elgazar *et al.* (2013) indicated that saffron can reduce the ALT and AST levels in diabetic rats, ALP levels were decreased significantly by saffron consumption. These results may be due to their antidiabetic role which improved pancreatic β cells and increased serum insulin level which has anabolic effect in protein (Abou El-Soud *et al.*,

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2007; Arasteh *et al.*, 2010; Elgazar *et al.*, 2013). Also, the current results are consistent with those of Amatto *et al.* (2023) who reported that the oral use of *C. sativus* (saffron), depending on the pharmaceutical formulation, acts positively on the parameters of fasting blood glucose, glycated hemoglobin, systolic blood pressure, total cholesterol, aspartate aminotransferase and creatinine. Therefore, it is a promising therapeutic treatment for type-2 diabetes mellitus and its main metabolic and cardiovascular complications.

Conclusion:

The oral use of *C. sativus* (saffron), depending on the pharmaceutical properties, acts positively on the aspartate aminotransferase and alkaline phosphatase levels together with the histopathological pattern of pancreas. Therefore, it is a promising therapeutic treatment for type-1 diabetes mellitus and its main metabolic and endocrine complications.

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