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ANTIDIABETIC EFFICACY OF *DUNALIELLA SALINA* EXTRACT IN STZ-INDUCED DIABETIC RATS

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ABSTRACT

The hypoglycemic role of *Dunaliella salina* in comparison with the reference antidiabetic drug glibenclamide was studied. Rats were intraperitoneally injected with a single dose of STZ (45 mg/kg body weight). *D. salina* ethanolic extract was administrated to rats at a dose 150 mg/kg body weight. Specific biochemical parameters were studied including blood glucose level, pancreatic function; α-amylase, liver function enzymes; alanine and aspartate aminotransferases (ALT, AST), alkaline phosphatase (ALP). Beside, oxidative stress biomarkers including nitrite level (NO), lipid peroxidation (MDA), glutathione levels (GSH) and superoxide dismutase (SOD), were determined. In addition, total protein content and albumin level were evaluated. The results clearly indicated significant increment in blood glucose level, AST, ALT and ALP enzyme activities, NO and MDA levels in diabetic rats. While, α-amylase activity and GSH level were decreased in hyperglycemic rats. However, albumin level, total protein content and SOD activity showed no significant difference in diabetic rats. Liver and pancreas of diabetic rats at a cellular level declared degenerative changes, massive area of leucocytic cells infiltrations and congested central vein. Treatment of diabetic rats with ethanolic extract of *D. salina* significantly decreased blood glucose level , AST, ALT, ALP enzyme activities as well as NO and MDA levels. Also, enhancement in α-amylase and GSH levels was noticed as a result of *D. salina* administration to diabetic rats. Moreover, noticeable improvement in liver and pancreas architectures post treatment of diabetic rats with *D. salina* ethanolic extract. In conclusion, the oral administration of *D. salina* extract in STZ-induced diabetic rats could have promising ameliorating effects in controlling hyperglycemia, counteract disease and delaying its complication.

KEYWORDS:*Dunaliella salina*, **Antidiabetic, STZ, Liver function, Oxidative stress, Liver and pancreas histology**

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INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic diseases in which a person has elevated levels of blood sugar, either due to the body does not produce enough amounts of insulin or because the receptors cells are not sensitive to the produced insulin.[1] It is accompanied with disturbances in the metabolism of carbohydrate, fat and protein resulting from defects in insulin secretion, insulin action or both. [2] DM has many symptoms such as thirst, polyuria, blurring of vision and polyphagia in addition it has severe complexities represented in keto-acidosis or non-ketotic hyperosmolarity. [2] According to the 2009 report of the World Health Organization (WHO), high blood plasma ranked first in the list of leading universal hazards for death and accounted for 7.5 million pearson in the world in 2004. [3] In the last century, leading science evolved cure for different illnesses but this lead drove to the flourishing of some common morbid and mortal diseases. [2] Streptozotocin (STZ), a naturally occurring nitrosourea, has a wide use to induce insulin dependent diabetes mellitus in experimental animals due to its toxic irreversible destruction effects on islet β-cells. [4] The STZ selectivity towards pancreatic β-cell toxicity and diabetic condition is related to the similarity between STZ and glucose moiety in chemical structure which enables STZ to penetrate the plasma membrane and enter the β-cell. [5] Microalgae are considered as a unicellular microorganism which can grow in fresh or salt water and have varied shapes with a diameter or length of 3-10 μm. [6] Microalgae have bioactive compounds that can be sourced directly from primary metabolism (proteins, fatty acids, vitamins, and pigments) or can be synthesized from secondary metabolism and can present antifungal, antiviral, antialgal, antienzymatic, or antibiotic activities [7].*Dunaliella salina*, a unicellular halophilic green microalga, knowing as a source of β-carotene having various applications in the health and nutritional products. [8] *D. salina*, containing cis and trans isomeric forms of β-carotene, act as good antihepatotoxic agent when compared to synthetic all trans β-carotene. The hepatoprotective effect of *D. salina* may be due to the presence of these isomeric forms of carotene and other oxygenated carotenoids (xanthophylls). [8] β-carotene rich algae has a protective role in the reduction of oxidative stress besides, it can restore the activity of hepatic enzymes such as, catalase, peroxidase and superoxide dismutase, resulting in the protecting of vital organs against xenobiotic and other damages. [9] Moreover, natural antioxidant products such as *D. salina* microalga with high content in carotenoids have a possible rule in improving oxidative stress associated with diabetes. [10] Hence, the current research was designed to evaluate the possible ameliorating effects of *D. salina* for lowering blood glucose level, improving pancreatic and liver architectures as well as functions, oxidant-antioxidant imbalance associated with diabetes in STZ induced-diabetic rats.

MATERIAL AND METHODS

(i) Material

Reagents and standards:STZ was purchased from Sigma-Aldrich, India. All chemicals in the present study are of analytical grade, products of Sigma, Merck and Aldrich. All kits were the products of Biosystems (Alcobendas, Madrid, Spain), Sigma Chemical Company (St. Louis, MO, USA), Biodiagnostic Company (Cairo, Egypt).

(ii) Methods

Cultivation of *D. salina*:The organism was grown in conical flask 5 litres containing BG11 nutrient media according to Stanier et al. [11] (Table 1). The culture was harvested by centrifugation, dried at 40^{o}C and then grounded into homogeneous fine powder.

Ethanolic extract preparation of *D. salina*: For the preparation of the ethanolic extract, 100 g of *D. salina* powder was soaked in ethanol (80%) and shacked on shaker (Heidolph UNIMAX 2010) for 48 hrs at 150 rpm. The extract was filtered using a Buchner funnel and Whatman No. 4 filter paper and the algal residue was re-extracted with the addition of fresh ethanol for another two times. Combined filtrates were concentrated using Rotary evaporator (Heidolph-Germany) at 40°C under vacuum. The resulting dry extract was evaporated on a rotary vacuum evaporator to dryness. The dry extract was stored at -20˚C in a freeze and kept for further analysis. [12]

1. Biological experiment

a. Experimental animals:Male albino rats (n=50) weighted (150±20 g), were obtained from the Animal House of the National Research Centre (NRC). Animals were quarantined and allowed to acclimate for one week before beginning experimentation. They were housed 10 per cage under temperature controlled environment (26-29°C) with a fixed light/dark cycle with free access to water and food. All procedures of the present study were performed according to the Ethical Committee of the NRC, Egypt, provided that the animals will not suffer at any stage of the experiment.
b. Induction of diabetes model:For the evaluation of STZ diabetic effect, Type 2 diabetes was induced by intraperitoneally injection of a single dose of STZ (45 mg/kg body weight) dissolved in 0.01M citrate buffer immediately before use.[13] After STZ injection, rats had free access to food, water and were given 5% glucose solution to drink overnight to encounter hypoglycaemic shock. [14] Rats were checked daily for the presence of glycosuria. Rats were considered to be diabetic if glycosuria was present for 3 consecutive days. [15] After 3 days of STZ injection fasting blood samples were obtained and blood sugar was determined (≥300 mg/dl).
c. Experiment:Animals were divided into 5 groups (10 rats each), as followed;

Group 1: Considered as normal, healthy control rats. Group 2: Considered as normal rats treated with *D. salina* ethanolic extract, Group 3: Considered as diabetic group; hyperglycemic rats were used for the experiment and classified as follows: Groups 4:

Considered as diabetic rats orally administered 150 mg/kg body weight *D. salina* ethanolic extract for 15 days [10] respectively, Groups 5: Considered as diabetic rats orally administered antidiabetic glibenclamide reference drug 10 mg/kg body weight daily for 30 days. [16] Collection of blood, organs and tissue samples: Rats were fasted overnight (12-14 hours), anesthetized by diethyl ether and blood collected by puncture of the sublingual vein in clean and dry test tube, left 10 minutes to clot and centrifuged at 3000 rpm for serum separation. The separated serum was used for biochemical analysis of glucose, α-amylase, liver function; aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP), albumin level and total protein content.After blood collection, rats of each group were sacrificed, liver and pancreas were removed immediately (a part was fixed in 10% formalin for histopathological examination). The liver tissue was homogenized in 5-10 volumes of appropriate medium using electrical homogenizer, centrifuged at 3000 rpm for 15 min, the supernatants (10%) were collected and placed in Eppendorff tubes then stored at -80°C and quantified for the determination of oxidative stress markers; nitric oxide (NO) and malondialdehyde (MDA) as well as non-enzymatic antioxidant glutathione reduced (GSH), antioxidant enzyme superoxide dismutase (SOD) spectrophotometrically according to the commercial instructions of the kits.

d. Biochemical determinations:Glucose was determined in blood serum using colorimetric kits. [17] Inhibitory activity of α-amylase was estimated. [18] AST and ALT enzyme activities were assayed. [19] ALP enzyme activity was determined. [20] Total protein content and albumin were determined according the method of Bradford [21] and by diagnostic kits respectively. Oxidative stress and antioxidants biomarkers (NO, MDA, GSH and SOD) were determined in liver tissue homogenate. NO was determined according to the method described by Moshage et al. [22] Moreover, liver MDA level was estimated according to the method of Satoh [23], GSH was determined according to the method of Beutler et al. [24] While, SOD enzyme activity was determined according to the method of Mohanty et al. [25].
e. Histopathological examination: Collected tissues were fixed in 10% buffered formalin for 24 hrs for fixation. Then processed in automatic processors, embedded in paraffin wax (melting point 55-60 °C) and paraffin blocks were obtained. After embedding in wax, tissues were cut into serial sections at 6 µm thicknesses were prepared and the sections were analyzed *via* haematoxylin and eosin staining (H&E staining) [26]. The cytoplasm stained shades of pink and red and the nuclei gave blue colour. The slides were examined and photographed under a light microscope (x400 magnification).
f. Statistical analysis:Statistical analysis is carried out using SPSS computer program (version 8) combined with co- state computer program, where unshared letters are significant at P ≤0.05.

RESULTS AND DISCUSSION

1. *Effect of D. salina extract on blood glucose level and α-amylase activity*

Table (2) demonstrated the blood glucose level and α-amylase activity in serum of normal and different therapeutic groups. It is obvious that, there is no significant difference in blood glucose levels and α-amylase activity between normal control and normal rats treated with *D. salina* ethanolic extract. However, diabetic rats exhibited significant increase in blood glucose level with percentage change 170.82%. However, significant inhibition in α-amylase enzyme activity with percentage change 45.94% was detected in diabetic rats. On the other hand, diabetic-treated rats with *D. Salina* extract and glibenclamide standard drug declared reduction in blood glucose level with improvement percentages 166.36 and 167.92%, respectively comparing to normal control rats. Significant increment in α-amylase enzyme activity was observed post *D. salina* ethanolic extract as well as glibenclamide antidiabetic drug treatments with amelioration percentages 49.87and 50.19%, respectively. Diabetes mellitus is such a disease that characterized by hyperglycemia, hyperglycemia or high blood sugar is a condition in which the amount of blood circulates glucose raises. [2] DM is not only remarked with abnormal blood glucose level, but it also progressed to affect other complications such as, the macrovascular (coronary artery disease and cerebrovascular disease) besides, the microvascular levels (renal failure, blindness, limb amputation, neurological complications and pre-mature death). [27] In the present study, elevated level of glucose in diabetic rats was detected with percentage 170.82% comparing to the normal rats.These results are parallel to those reported by Garabadu and Krishnamurthy [28], who stated that a single injection of STZ (45 mg/kg) caused an increase in blood glucose level of diabetic rats. This is may be attributed to, STZ induction causes death of pancreatic β-cell throughout the alkylation of DNA leading to reduced synthesis and release of insulin. [29]Treating of diabetic rats with *D. salina* extract as well as glibenclamide improved the level of glucose with percentages reached to 166.36 and167.92%, respectively.However, inhibition α-amylase enzyme activity was observed in the diabetic rats comparing to normal control one. These results are in accordance with those reported by Aughsteen et al. [30] as they declared low serum amylase activity associated with insulin deficiency in type 2 diabetic patients. In concomitant with Nakajima et al. [31] declared that, the low serum amylase level may reverberate the abnormalities in both metabolism and glucose level which are associated with impaired insulin action resulting from insulin resistance and/or unsuitable insulin secretion. Administration of *D. salina* extract led to increase in the activity of α-amylase with improvement percentage49.87%. Moreover, glibenclamide drug also showed enhancement in α-amylase activity with improvement percentage 50.19%.It was found that; *D. salina* green alga contains a mass of carotenoids that have varied applications in health and nutrition. [8] Carotenoids cover a class of natural pigments which have been epidemiologically

related to a lower hazard for various diseases. [32] Fucoxanthin a marine carotenoid present in brown marine seaweeds, diatoms, macro and microalgae revealed anti-diabetic activity *via* uncoupling protein 1 expression in white adipose tissue (WAT) mitochondria leading to oxidation of fatty acids and heat production in WAT. [33] Moreover, fucoxanthin improved the insulin resistance and decreased blood glucose level by up-regulation of glucose transporter 4 (GLUT4) in skeletal muscle [33]. Hence, the hypoglycemic effect of *D. salina* may be explained on the basis of the occurrence of carotenoids and enhancement of glucose metabolism.

2. ***Liver function enzyme activities***

Table (3) showed liver function enzyme activities; AST, ALT and ALP in serum of control and therapeutic groups. No change was recorded in serum AST activity of normal rats treated with *D. salina* extract. With respect to diabetic rats, marked increase in all enzyme activities; AST, ALT and ALP were detected with percentages 52.45, 32.23and 107.17%, respectively. Treated-diabetic rats with *D. salina* extract demonstrated inhibition in AST, ALT and ALP enzyme activities comparing to control rats with improvement percentages 53.41, 23.13and 100.65%, respectively. While, glibenclamide treated-diabetic rats recorded amelioration percentages 50.67, 11.69 and 89.07% respectively, comparing to normal control rats. The liver is involved in type 2 diabetes (T2DM) pathogenesis as it presents a vital role in the glucose conservation in normal levels so, the hepatic dysfunction coming from insulin resistance is proposed as driving to T2DM. [34] The authors added that, liver enzymes including; AST, ALT are indicators on liver function. Regarding to the current results, diabetic rats declared significant increase in the liver enzyme activities; AST, ALT and ALP with percentages 52.45, 32.23 and 107.17%, respectively. These increments in liver enzymes are considered as an indicator for the hepatic damage. However, no change was observed in albumin level and total protein content in diabetic rats. In a good agreement with the current results, Ademiluyi and Oboh [35] revealed significant elevation in AST, ALT and ALP levels in diabetic rats comparing to normal control rats. The elevated activities of AST, ALT and ALP liver enzymes in the circulation is related to the infiltration of these enzymes from liver cytosol into the blood stream. [36] Administration of *D. salina* extract to diabetic rats resulted in noticeable reduction in these enzymes activity with improvement percentages 53.41, 23.13 and 100.65% for AST, ALT and ALP, respectively. Furthermore, glibenclamide drug improved the elevated levels of AST, ALT and ALP with percentages; 50.67, 11.69 and 89.07%, respectively. The ameliorative effect of *D. salina* extract in AST, ALT and ALP activities of diabetic rats may be associated with its highly content of carotenoids resulting in prohibiting the hepatic injury [37].

3. ***Albumin level and total protein content***

No significant difference was observed in albumin level and total protein content in normal control rats treated with *D. salina* extract. Also, no detectable changes in albumin and total protein content either in diabetic or diabetic-treated rats with *D. salina* (Table 4).

4. ***Oxidative stress markers and non-enzymatic antioxidant***

It can be easily noticed that, NO, MDA, GSH and SOD levels revealed no significant difference in normal rats post supplemented with *D. salina* extract comparing to normal untreated one (Table 5).In response to diabetic state, NO and MDA levels showed elevated values with percentages187.90 and 57.75%, respectively comparing to normal control rats. While, GSH level recorded significant reduction with percentage 36.70%, comparing to normal control rats. However, SOD enzyme activity declared insignificant change comparing to control rats. Treatment of diabetic rats with *D. salina* ethanolic extract improved the levels of NO, MDA as well as GSH with percentages 384.48, 42.12 and 36.74%, respectively. For glibenclamide, the percentages of amelioration recorded 166.21, 30.45 and 39.59%, respectively for NO, MDA and GSH.Oxidative stress is a status implicated in diabetes complications where, continued reactive oxygen species (ROS) generate that lead to the induction of changes in antioxidant enzymes activities in variable tissues and highly production of free radicals [29]. According to diabetic condition, NO and MDA levels were significantly elevated with increase percentages187.90 and 57.75%, respectively comparing to normal control rats. On contrast, GSH level reduced in diabetic rats with percentage 36.70%. These results run with the results indicated by Moraes et al. [38] and El-Baz et al. [39] who observed that, MDA level increased in diabetic group while, GSH level decreased. In diabetes, oxidative stress may rises from the autoxidation of glucose, the peroxidation of lipids, the glycation of proteins besides, the reduction in antioxidant enzymes activities. [40] In addition to, Coskun et al. [41] related the decreased level of GSH in diabetic to its exploitation in relieving the oxidative stress. *D. salina* extract improved NO, MDA and GSH levels in diabetic rats with percentages 384.48, 42.12 and 36.74%, respectively. This enhancement effect of *D. salina* extract may rely on the base of Murthy et al. [8] who explained that, *D. salina* carotenoids has the ability to enhance or maintain hepatic enzymes activity, that are implied in the combating of ROS. Moreover, carotenoids can produce antioxidant, anti-inflammatory, anti-cancer effects towards metabolic disorders including; diabetes and coronary disease. [42]

5. ***Histopathological investigations of pancreas***

Normal rats showed healthy acini (Figure 1a). Diabetic rats revealed focal massive area of leucocytic cells infiltrations (Figure 1b). Treatments of diabetic rats with *D. salina* extract (Figure 1c) showed congested blood vessels. While, treated-diabetic rats with drug showed dilated blood vessel (Figure 1d).

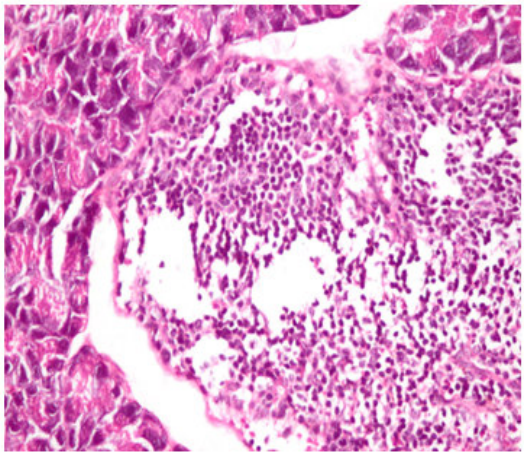

Figure 1a
Pancreas of control rats showing apparently healthy acini (H&E X 400).

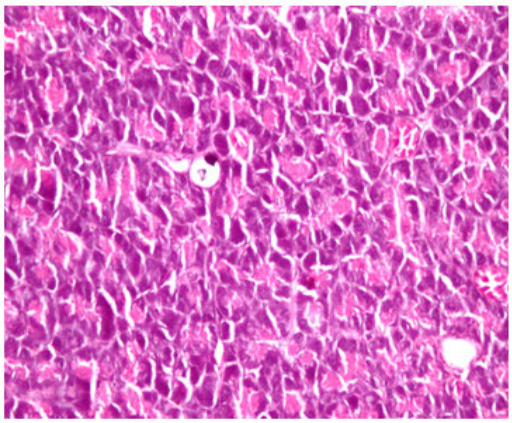

Figure 1b
Pancreas of diabetic rats showing focal massive area of leucocytic cells infiltrations (arrow head) (H&E X 400).

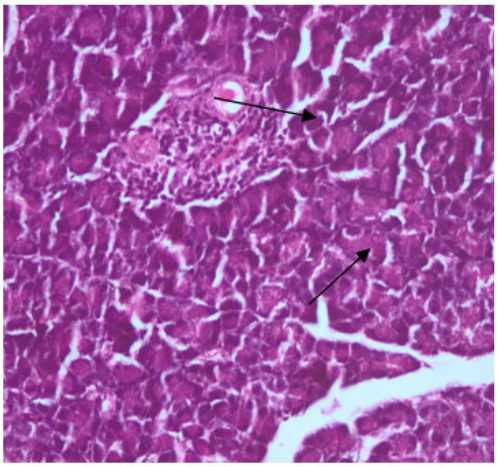

Figure 1c
Pancreas of diabetic rats treated with D. salina extract showing normal pancreatic acini, islets and ducts (H&E X 400).

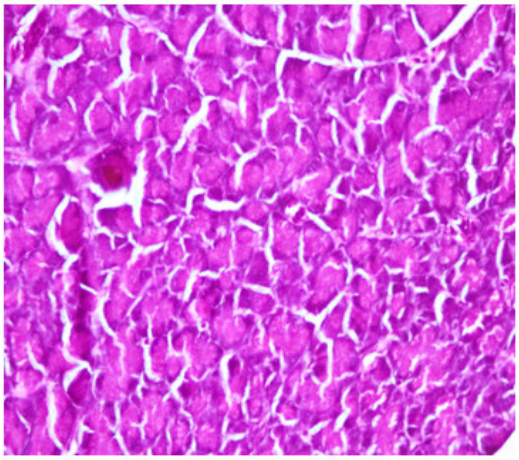

Figure 1d
Pancreas of diabetic rats treated with drug showing normal pancreatic acini, islets and ducts (H&E X 400).

6. ***Histopathological investigations of liver***

Normal rats revealed congested central vein (Figure 2a). In diabetic rats (Figure 2b), congested central vein and blood sinusoids were observed. Treatments of diabetic rats with *D. salina* extract (Figure 2c) showed congested central vein (arrow head) and blood sinusoids together with leucocytic cells permeation. While, treated-diabetic rats with drug (Figure 2d) showed congested central vein.

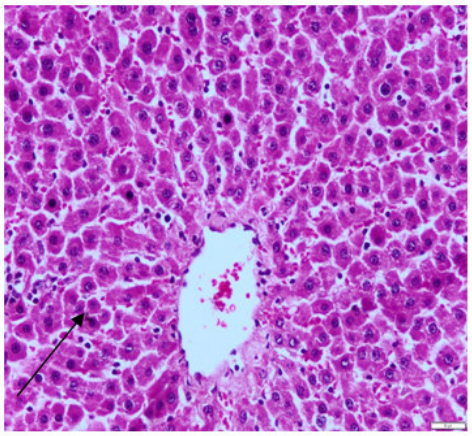

Figure 2a
Liver of control rats showing normal hepatic parenchyma; central veins, hepatocytes, blood sinusoides and portal areas (arrow) (H&E X 400).

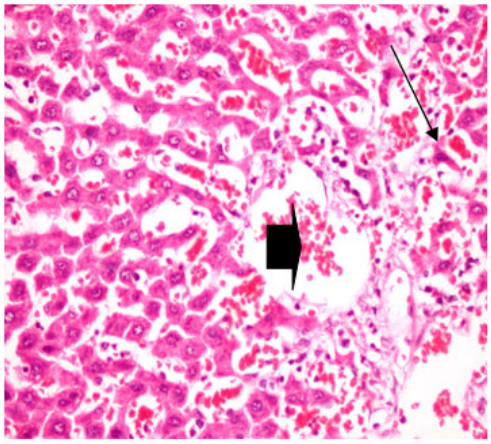

Figure 2b
Liver of diabetic rats showing congested central vein (arrow head) and blood sinusoids (arrow) (H&E X 400).

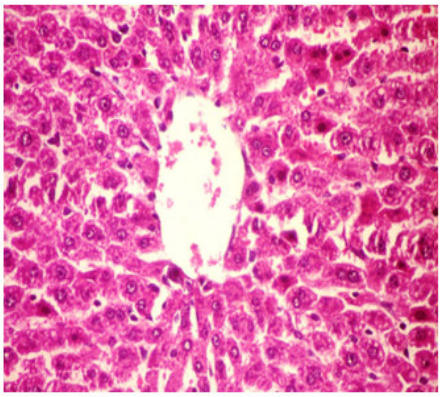

Figure 2c
Liver of diabetic rats treated with D. salina extract showing normal hepatic parenchyma; central veins, hepatocytes, blood sinusoides and portal areas (H&E X 400).

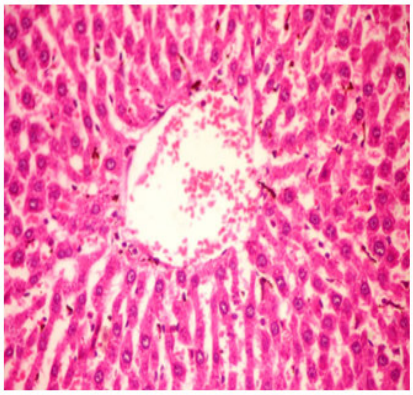

Figure 2d
Liver of diabetic rats treated with drug showing normal hepatic parenchyma; central veins, hepatocytes, blood sinusoides and portal areas (H&E X 400).

Considering, the histopathological investigations of pancreas, focal massive area of leucocytic cells infiltrations. The pancreatic observations of the present study are in concomitant with the results of El-Baz et al. [39] and Kakkar et al. [43], who observed inflammatory cells infiltration in STZ-induced diabetic rats. While, congested central vein and blood sinusoids were observed in the diabetic liver architecture. The presented hepatic examination is agreed with that reported by Sheweita et al. [44], who declared, STZ-diabetic rats revealed severe pathological changes including congestion and dilation of hepatic sinusoids.Treatments of diabetic rats with *D. salina* extract showed an enhancement in both pancreatic and hepatic architectures of diabetic rats. Regarding to the study of Kakkar et al. [43], who attributed the structural damage of pancreas and liver to the oxidative stress. Antioxidants, compounds that have the ability to delay, inhibit or prevent oxidation, help in diseases prevention by *in vivo* or *in vitro* reacting with free radicals leading to the decrease of oxidative stress. [45] β-carotene is considered as one of the strongest antioxidant that having a powerful antioxidant properties with wide applications such as medicine, pharmacy and cosmetics. [46] So, the repairing effect of *D. salina* extract on pancreatic and hepatic architectures may be due to the presence of antioxidant carotenoids.

Table 1
BG11 nutrient composition for growing D. Salina

Macronutrient	Mg/l
$NaNO_3$	1500.000
K_2HPO_4	40.000
$MgSO_4.7H_2O$	75.000
$CaCl_2.7H_2O$	36.000
Citric acid	6.000
$NaCO_3$	20.000
Na_2EDTA	1.000
Ferric ammonium citrate	6.000
Micronutrient	g/l
H_3BO_3	2.860
$MnCl_2.4H_2O$	1.810
$ZnSO_4.7H_2O$	0.222
$Na_2MoO_4.2H_2O$	0.390
$CuSO_4.5H_2O$	0.079
$Co(NO_3)_2.6H_2O$	0.0494

Add 1ml/L into the culture medium from the micronutrient.After autoclaving and cooling pH of medium is about 7.

Table 2

Effect of D. salina ethanolic extract on blood glucose level and α-amylase activity in normal, STZ-induced diabetic and diabetic-treated rats

Groups	Parameters	Glucose (mg/dl)	α-amylase (U/l)
Normal control	Mean±S.D.	112.25±2.21[b]	28.23± 1.53[a]
Normal + *D. salina* extract	Mean±S.D.	111.15±3.50[b]	27.93± 1.03[a]
	% Change to control	0.97	0.95
Diabetic rats	Mean±S.D.	304.00±34.01[a]	15.26±0.06[b]
	% Change to control	170.82	45.94
Diabetic + *D. salina* extract	Mean ±S.D.	117.25±8.34[b]	29.34±1.10[a]
	% Change to control	4.45	3.93
	% of improvement	166.36	49.87
Diabetic + glibenclamide drug	Mean ±S.D.	115.50±10.84[b]	29.43±0.60[a]
	% Change to control	2.89	4.25
	% of improvement	167.92	50.19

Data presented as mean ± SD, n=10. Statistical analysis is carried out using Co-state and SPSS computer programs (version 7), where unshared letter is significant at P ≤ 0.05.

Table 3

Effect of D. salina ethanolic extract on serum AST, ALT and ALP enzyme activities in normal, STZ-induced diabetic and diabetic-treated rats

Groups	Parameters	AST (U/ml)	ALT (U/ml)	ALP (U/l)
Normal control	Mean±S.D.	156.20±1.80[c]	72.07± 0.08[e]	52.10± 0.16[d]
Normal + *D. salina* extract	Mean±S.D.	157.00±1.00[c]	72.13± 0.05[e]	52.15± 0.11[d]
	% Change to control	1.27	0.08	0.09
Diabetic rats	Mean±S.D.	238.14±0.63[a]	95.30±0.20[a]	107.94± 3.81[a]
	% Change to control	52.45	32.23	107.17
Diabetic + *D. salina* extract	Mean ±S.D.	154.71±1.09[c]	78.63[d]±2.43	55.53±0.21[cd]
	% Change to control	0.95	9.10	6.58
	% of improvement	53.41	23.13	100.65
Diabetic + glibenclamide drug	Mean ±S.D.	158.99±1.84 [b]	86.87±0.43[b]	61.53±3.01[b]
	% Change to control	1.78	20.53	18.09
	% of improvement	50.67	11.69	89.07

Data presented as mean ± SD, n=10. Statistical analysis is carried out using Co-state and SPSS computer programs (version 7), where unshared letter is significant at P ≤ 0.05.

Table 4

Effect of D. salina ethanolic extract on serum albumin level and total protein content in normal, STZ-induced diabetic and diabetic -treated rats

Groups	Parameters	Albumin (g/dl)	Protein (g/dl)
Normal control	Mean±S.D.	4.21±0.08[a]	6.09±0.14[a]
Normal + *D. salina* extract	Mean±S.D.	4.47±0.11[a]	6.02±0.12 [a]
	% Change to control	0.06	1.14
Diabetic rats	Mean±S.D.	4.14±0.07[a]	7.15±0.50[a]
	% Change to control	1.66	17.40
Diabetic + *D. salina* extract	Mean±S.D.	4.09±0.05[a]	6.79±0.36 [a]
	% Change to control	2.80	11.49
	% of improvement	0.96	10.00
Diabetic + glibenclamide drug	Mean±S.D.	4.18±0.14[a]	6.89±0.15 [a]
	% Change to control	0.71	13.13
	% of improvement	0.96	4.20

Data presented as mean ± SD, n=10. Statistical analysis is carried out using Co-state and SPSS computer programs (version 7), where unshared letter is significant at P ≤ 0.05.

Table 5

Effect of D. salina ethanolic extract on oxidative stress and antioxidant biomarkers in normal, STZ-induced diabetic and diabetic- treated rats

Groups	Parameters	NO (µmol/l)	MDA (µmol/g.tissue)	GSH (mmol/g.tissue)	SOD (U/g.tissue)
Normal control	Mean±S.D.	34.48±1.02[d]	8.57±0.15[d]	8.79±0.02[b]	16.75±0.12[a]
Normal + *D. salina* extract	Mean±S.D.	34.22±1.00[d]	8.21±0.11[d]	8.70±0.04[b]	16.34±0.10 [a]
	% Change to control	0.75	4.20	1.02	2.44
Diabetic rats	Mean±S.D.	99.27±0.83[a]	13.52±0.35[a]	5.56±0.05[c]	16.97±0.14 [a]
	% Change to control	187.90	57.75	36.70	1.31
Diabetic + *D. salina* extract	Mean±S.D.	33.30±0.14[d]	9.91±0.60[c]	8.79 ±0.08[b]	16.90±0.06 [a]
	% Change to control	3.42	15.63	0.00	0.89
	% of improvement	384.48	42.12	36.74	0.41
Diabetic + glibenclamide drug	Mean±S.D.	41.96±1.23[c]	10.91±0.30[b]	9.04±0.09 [a]	16.92±0.05 [a]
	% Change to control	21.69	27.30	2.84	1.01
	% of improvement	166.21	30.45	39.59	0.29

Data presented as mean ± SD, n=10. Statistical analysis is carried out using Co-state and SPSS computer programs (version 7), where unshared letter is significant at P ≤ 0.05.

CONCLUSION

The present results confirmed that *D. salina* ethanolic extract is rich in carotenoids which exhibited antihyperglycemic property and may provide promising supplements and neutraceutical with a strong cure for diabetes.

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CONFLICT OF INTEREST

Conflict of Interest declared none.

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