Original Research Article **Biochemistry**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

THERAPEUTIC POTENTIAL OF DUNALIELLA SALINA EXTRACT ON LIPID ACCUMULATION AND ADHESION MOLECULES IN HYPERGLYCEMIC RATS

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ABSTRACT

The present study aims to investigate the antidiabetic effects of *Dunaliella salina* ethanolic extract. The antihyperlipidemic and anti-adhesion role of *D. salina* extract was evaluated comparing to glibenclamide antidiabetic drug. Lipid profile; total lipid (TL), total cholesterol (TC) and triglycerides (TG), adhesion molecules (ICAM-1 and VCAM-1) possessed a significant increase in STZ-induced diabetic rats model. *D.salina* ethanolic extract remediation of diabetic rats ameliorating TC, TG, ICAM-1 and VCAM-1 towered normal control levels. From histopathological point of view STZ-induced diabetic rats showed deteriorative modifications in the cardiac architecture. While, supplementation of diabetic rats with *D. salina* extract demonstrated an improvement in heart tissue. Hence, it could be deduced that, *D. salina* extract appeared therapeutic approaches in hyperglycemia dominance.

KEYWORDS: VCAM-1, ICAM-1, *Dunaliella Salina*, Diabetes, Lipid profile

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INTRODUCTION

Diabetes mellitus (DM) acted as the most common endocrine disorder that leads to various and dangerous complications including micro and macro-vascular diseases. [1] Type 2 diabetes mellitus (T2DM), an inveterate metabolic defect that is described by hyperglycemia, rises from the lack in sensitivity to the hormone insulin which mostly exist in adipose tissues, liver and skeletal muscles. [2] Jointly, diabetes and lipid profile are considered as the leading predictors for metabolic disturbances including dyslipidaemia (lipid abnormalities) and cardiovascular diseases. [3] There is a relation between lipid abnormalities and diabetes where, insulin resistance or deficiency can affect on the key enzymes and pathways of lipid metabolism. [4] Dyslipidemia; coming from hyperglycemia in diabetic state can lead to chronic risks such as, coronary artery disease, stroke and peripheral vascular disease. [5] Moreover, disturbance in the level of glucose in T2DM patients usually joined with low levels of high-density lipoprotein HDL, elevated levels of low-density lipoprotein (LDL) as well as hypercholesterolaemia and hypertriglyceridaemia. [6] T2DM is described by high happening of vascular complications, oxidative stress and dyslipiodemia. [7] In diabetes condition, the elevated levels of soluble adhesion molecules such as, ICAM-1 and VCAM-1 which considered as a marker of endothelial dysfunction related to insulin resistance.[8] Microalgae have different therapeutic bioactive compounds such as proteins, polysaccharides, lipids, vitamins, enzymes, sterols, which can be gained from the biomass and have pharmaceutical and nutritional importance. [9] Microalgal bioactive compounds are represented in two forms; primary bioactive compounds such as proteins, fatty acids, vitamins, and pigments, or secondary bioactive compounds such as cyanovirin, oleic acid, linolenic acid, palmitoleic acid, vitamin E, B12, β-carotene, phycocyanin, lutein, and zeaxanthin that have antimicrobial, antioxidant and anti-inflammatory activities against different diseases. [10,11] Microalga *D. salina* is considered as the best commercial source of β-carotene besides, it's different species accumulate variable and considerable amounts of worthy compounds including; vitamins, carotenoids, glycerol, minerals, lipids and proteins. [12] *D. salina* carotenoids have potential antioxidants that reduce the oxidative damage initiated by reactive oxygen species (ROS) which as a consequence leading to the protection against the risk of humans cancer and degenerative diseases. [10] Hence, the objective of this research is to investigate the potential hypolipidemic, and anti-adhesion effects possessed by *D. salina* microalgae for possible control in T2DM.

MATERIAL AND METHODS

(i) ***Chemicals and reagents***

In the present study, all chemicals 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) Preparation of D. salina ethanolic extract

For obtaining the ethanolic extract, 100 g of *D. salina* powder was soaked in ethanol (95%) 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. [13]

(iii) Biological experiment

1. Animals

Male albino rats (n=50) weighted (150±20 g), were used for the evaluation of anti-diabetic effects of *D. salina* ethanolic extract and provided by the Animal House of the National Research Centre (NRC) and housed in a temperature controlled environment (26-29°C) with a fixed light/dark cycle for one week as an adaptation period to acclimatize under normal combination with free access to water and food. The present study is approved by the Ethical Committee of the NRC, Egypt, provided that the animals will not suffer at any stage of the experiment.

2. Experimental design

Fifty rats were selected for this study and divided into five groups of ten rats each as follows:

Group 1: Normal, healthy control rats. Group 2: Normal rats treated with *D. salina* ethanolic extract, Group 3: Is considered as diabetic group; where T2DM 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. [14] After injection, animals had free access of food, water and were given 5% glucose solution to drink overnight to encounter hypoglycaemic shock. [15] Animals were checked daily for the presence of glycosuria. Animals were considered to be diabetic if glycosuria was present for 3 consecutive days. [16] After 3 days of STZ injection fasting blood samples were obtained and blood sugar was determined (≥300 mg/dl). Hyperglycaemic rats were used for the experiment and classified as follows: Groups 4: Diabetic rats oral administered 150 mg/kg body weight *D. salina* ethanolic extract [17] for 15 days respectively, Groups 5: Diabetic rats administered orally antidiabetic glibenclamide reference drug 10 mg/kg body weight daily for 30 days. [18]

3. Blood sample preparations

After treatments, 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 lipid profile; total lipids (TL), TC and triglycerides (TG), vascular markers including adhesion molecules; Intercellular adhesion molecule (ICAM-1) and vascular adhesion molecule (VCAM-1). After blood collection, rats of each group were sacrificed, heart was removed immediately

(a part was fixed in 10% formalin for histopathological examination).

4. Biochemical investigations

TL level was determined according to the method of Zollner and Kirsch. [19] TC level was estimated. [20] TG level was determined. [21] Estimation of serum adhesion molecules; ICAM-1 and VCAM-1 levels was performed by ELISA; a sandwich enzyme immunoassay.

5. Histopathological examination

The heart specimens obtained from the normal and treated groups of animals 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. Sections of 6 µm thicknesses were prepared and stained with Haematoxylin and Eosin (H&E) stain. [22] 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).

6. 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

1. Lipid profile

Lipid profile; TL, TC and TG in normal control and different treated groups were recorded in Table (1). It can be easily noticed that, there is no change in TL, TC and TG levels in normal rats treated with *D. salina* extract as compared to untreated normal control one. Diabetic group showed increase in TL, TC and TG levels with percentages change 37.00, 67.00 and 69.73%, respectively. Diabetic-treated rats with *D. salina* extract showed reduction in TL, TC and TG levels as compared to normal control rats with percentages of improvement 24.00, 67.00 and 68.00%, respectively. While, the percentages of improvement reached to 41.86, 67.00 and 68.00%, respectively for TL, TC and TG in glibenclamide-treated diabetic rats.

2. Adhesion molecules

Table (2) showed the effect of *D. salina* extract on adhesion molecules levels in normal, STZ-induced diabetic and diabetic-treated rats. The present results demonstrated an insignificant change in ICAM-1 and VCAM-1 levels post *D. salina* extract supplemented to normal rats comparing to untreated normal control one. Diabetic rats showed significant increase in both ICAM-1 and VCAM-1 levels with percentages 405.05 and 138.44%, respectively. ICAM-1 level was significantly improved in diabetic-treated rats with *D. salina* extract and glibenclamide with percentages 98.31 and 201.68%, respectively. Also, diabetic rats treated with *D. salina* extract and glibenclamide declared amelioration in VCAM-1 levels with percentages 137.56 and 107.33%, respectively comparing to normal control rats.

3. Histopathological investigations of heart

Heart of normal rats showed congested blood vessel (Figure 1a) while, heart of diabetic rats showed intermuscular leucocytic cells infiltration (Figure 1b). Diabetic-treated rats with *D. salina* extract showed congested blood vessel in cardiac tissue (Figures 1c). However, in cardiac tissue of treated-diabetic rats with glibenclamide revealed area of muscular hyalinosis and congested blood vessel (Figure 1d).

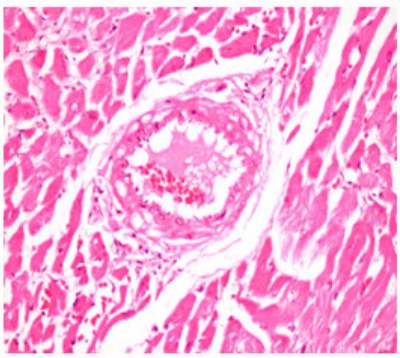

Figure 1a
Heart of control rats showing congested blood vessel (arrow), (H&E X 400).

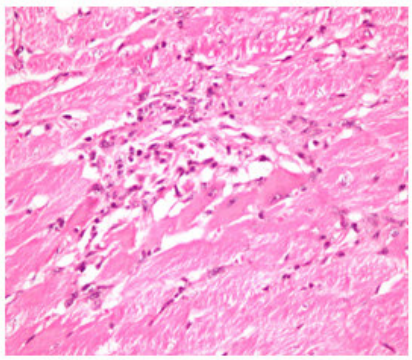

Figure 1b
Heart of diabetic rats showing intermuscular leucocytic cells infiltration (arrow) (H&E X 400).

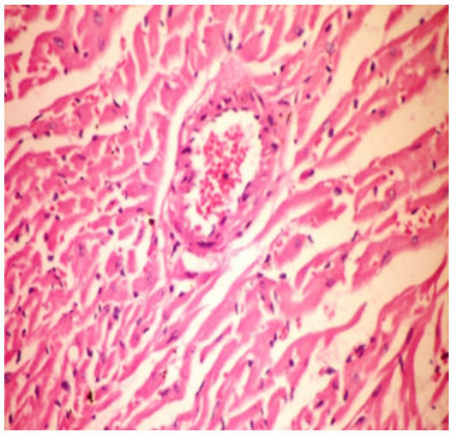

Figure 1c

Heart of diabetic rats treated with Dunaliella salina extract showing congested blood vessel (arrow), (H&E X 400)

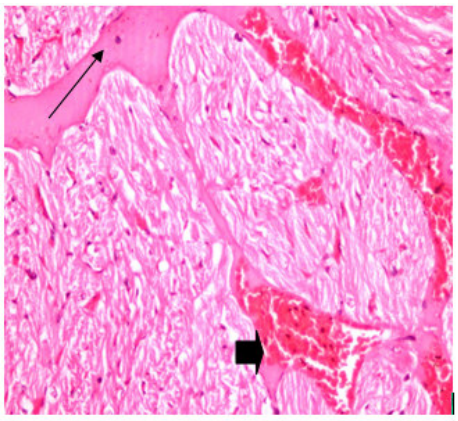

Figure 1d

Heart of diabetic rats treated with drug showing area of muscular hyalinosis (arrow) and congested blood vessel (arrow head) (H&E X 400).

Table 1

Effect of D. salina ethanolic extract on lipid profile level (TL, TC and TG) in normal, diabetic and therapeutic groups

Groups	Parameters	TL (g/dl)	TC (mg/dl)	TG (mg/dl)
Normal control	Mean±S.D.	17.77^{c}± 0.56	18.13^{c} ±0.07	13.05^{b}±0.04
Normal + *D. salina* extract	Mean±S.D.	17.90 ^{c}±0.42	18.21^{c}±0.03	13.0^{b}±0.11
	% Change to control	0	0	0
Diabetic rats	Mean±S.D.	24.44^{a}±0.33	30.40^{a} ±0.07	22.15^{a}±0.06669.73
	% Change to control	37	67	
Diabetic + *D. salina* extract	Mean±S.D.	20.00^{b}±0.05	18.23^{c} ±0.04	13.21^{b}±0.12
	% Change to control	12.00	0	1
	% of improvement	24	67	68
Diabetic + glibenclamide drug	Mean±S.D.	17.00^{c}±0.17	18.22^{c} ±0.05	13.22^{b}±0.13
	% Change to control	4.33	0	1.00
	% of improvement	41.86	67	68

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 2

Effect of D. salina ethanolic extract on adhesion molecules level (ICAM-1 and VCAM-1) in normal, diabetic and therapeutic groups

Groups	Parameters	ICAM-1(ηg/ml)	VCAM-1 (ηg/ml)
Normal control	Mean±S.D.	4.75±0.47^{e}	9.13±0.16^{d}
Normal + *D. salina* extract	Mean±S.D.	4.89±0.55^{e}	9.19±0.07^{d}
	% Change to control	2.94	0.65
Diabetic rats	Mean±S.D.	23.99±1.84^{a}	21.77±0.32^{a}
	% Change to control	405.05	138.44
Diabetic + *D. salina* extract	Mean±S.D.	19.32±1.50^{bc}	9.21±0.03^{d}
	% Change to control	306.73	0.87
	% of improvement	98.31	137.56
Diabetic+glibenclamide drug	Mean±S.D.	14.41±1.15^{d}	11.97±0.10^{b}
	% Change to control	203.36	31.10
	% of improvement	201.68	107.33

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.

DISCUSSION

In diabetes mellitus, dyslipidemia is characterized by the elevated levels of triglycerides, low-density lipoprotein cholesterol (LDL-C) and the decreased levels of high-density lipoprotein cholesterol (HDL-C). [23] The authors appended that, fifty percent of diabetic patients are highly exposed to elevated levels of lipids leading to complications such as, cardiovascular, renal disorders and coronary heart disease. Additionally, hyperglycemia in DM is accompanied with dyslipidemia presented in the elevated level of TC, LDL cholesterol and TG. [24] According to the present results, diabetic rats showed significant increase in TL, TC and TG levels with percentages 37.00, 67.00 and 69.73%, respectively comparing to normal control rats. These results are run in parallel with those reported by El-Baz et al. [25], who found TG, TC and TL levels were significantly raised in diabetic rats. In diabetic condition, the shortage in insulin secretion after injection with STZ impaired lipogenic activity where, insulin plays a serious role in lipogenesis stimulating high level of lipid in plasma. [26,27] Along with, the rise in TC levels may be

explained on the rule of Shankarprasad et al. [28], who hypothesized that, diabetes hyperphagia stimulates the increased activity of 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase in the intestine causing an increase in cholesterol synthesis and consequently, leading to raised levels of cholesterol in plasma. Moreover, the hypertriglyceridemia in diabetic condition may be attributed to highly rates production of triglyceride rich with very low-density lipoprotein (VLDL) by the liver and to the low rates of TG removal by peripheral tissues-primarily adipose tissue and muscle. [28] The remediation of diabetic rats with *D. salina* ethanolic extract caused reduction in the levels of TL, TC and TG. This lowering effect may be build to the fact that, β-carotene has the ability to regulate the expression of HMGCoA reductase in the liver of rats, suppress the cellular cholesterol synthesis and increased macrophage LDL receptor activity. [29] The authors appended that, this effect can lead to enhanced clearance of LDL from the plasma, and thus carotenoids may be recognized as hypocholesterolemic agents. On the other hand, β-carotene reduces the lipid content of mature adipocytes where, it is implied in the control of body fat reserves in mature adipocytes. [30] Also, β-carotene is metabolized to retinoic acid (RA), that decreases PPAR-alpha and CCAAT/enhancer-binding protein expression, which are considered as the key lipogenic transcription factors. [30] Different forms of adhesion molecules such as ICAM-1, VCAM-1 and selectins, demonstrated in diabetes status, are suggested to have a role in the endothelial activation. [31] Regarding to diabetic rats, significant increase in ICAM-1 and VCAM-1 levels was obtained (405.05 and 138.44%, respectively), comparing to control rats. These results are in agreement with Matsumoto et al. [32] and El-Baz et al. [33] who found that, the serum levels of VCAM-1 and ICAM-1 were increased in type 2 diabetes. This may rely on the fact that, the highly content of glucose, free fatty acids (FFAs) and insulin resistance lead to impair in NO bioavailability resulting in impaired endothelial function. [34] In addition, high glucose content induces a signalling pathway mediated by protein kinase C resulting in an increase of adhesion molecules. [35] Diabetic-treated rats with *D. salina* extract revealed improvement in ICAM-1 and VCAM-1 levels with percentages 98.31and 137.56%, respectively. Armoza et al. [36], declared, carotenoids significantly improved the endothelial function by decreasing the expression of ICAM-1 and VCAM-1. The amelioration in endothelial dysfunction may be attributed to; there is a relation between carotenoids level and the decreased risk of cardiovascular dysfunction (CVD) *via* the modulation of atherogenic processes related to the vascular endothelium. [37] In DM patients, oxidative stress that affects on the metabolism of carbohydrate, lipid and protein raises and is suggested to encourage the endothelial cell dysfunction and arouse atherosclerosis progression. [38, 39] Carotenoids are tiptop physically and chemically active quenchers against ROS-mediated disorders. [40] Hence, the cardio-protective effects of carotenoids may arise from the antioxidant activity that is performed in; reduction of lipid peroxidation, apoptotic cell death and expression of inflammatory mediators. [7] DM induces both cardiovascular diseases and heart failure besides; it may drive to diabetic cardiomyopathy (DCM). [41] Reducing the ability to retard LDL contributing to diabetes, result in the excess atherosclerosis common. [42] The mechanism is that, the small particles of LDL can rapidly enter the arterial wall and it may be toxic to endothelial cells. [43] Histopathological examination refers to the histological examination, the heart of diabetic rats' demonstrated intramuscular leukocyte cells infiltration. This may relies on the fact that, immune cell-mediated inflammation is considered as the central mechanism resulting in vascular disease in diabetes status. [44] The authors added that, endothelium chronic inflammation in diabetic condition raises a constant infiltration and gathering of leukocytes at the location of endothelial cell injury. Moreover, the hyperglycemia stimulates the cardiac dysfunction throughout the direct toxic effects on cardiomyocytes. [45] So, the structural change in cardiomyocytes could be due to the degeneration of the structural protein in mitochondria of the cytoplasm that occurred in protein degradation related to diabetes [45].Medicament of diabetic rats with *D. salina* ethanolic extract enhanced the architectures of cardiac tissue that appeared normal. According to the recent observations of TL, TC and TG levels, *D. salina* extract improved the raised levels of these biomarkers in diabetic status compared to normal rats. The improvement in cholesterol or blood lipids including, HDL, LDL and triglycerides may diminish the CVD complications associated with diabetes by 20- 50%. [46] B-carotene in algae has the ability to inhibit the LDL-oxidation in diabetes and protect against oxidative stress. [47] Moreover, it restores the hepatic enzyme activity including, catalase, peroxidase and superoxide dismutase, which in turn protects vital organs from xenobiotic and other damages. [48] Therefore, the improvement in the accumulated fat levels may be the factor that led to the progress in cardiomyocytes.

CONCLUSION

The results of the currently work declared that treatment with D. *Salina* extract showed reduction in lipid profile, adhesion molecules possessed a significant increase. While supplementation of diabetic rats with D. *Salina* extract demonstrated improvement in heart tissue. It could be deduced that D. *Salina* extract appeared a therapeutic approaches through hyperlipidmic and anti-adhesive effect.

ACKNOWLEDGMENT

This work was supported and funded by the project entitled "Biodiesel production from algae as a renewable energy source". Funding organization: Research Development and Innovation programme (RDI), Funding Program: EU-Egypt Innovation Fund, 2014-2016.

CONFLICT OF INTEREST

Conflict of Interest declared none.

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