Research Article

Dunaliella salina Alleviates Renal Dysfunction and Suppresses Inflammatory Cytokines in STZ-induced Diabetic Rats

Farouk K. El-Baz*[1], Hanan F. Aly[2], Sayeda M. Abdo[3], Rehab Mahmoud[3], Safaa A. Saad[1]

[1]Plant Biochemistry Department, National Research Centre (NRC), 33 El Bohouth st., (former El Tahrir st.), Dokki, Giza, Egypt.
[2]Therapeutic Chemistry Department, National Research Centre (NRC), 33 El Bohouth st., (former El Tahrir st.), Dokki, Giza, Egypt.
[3]Water pollution Research Department, National Research Centre (NRC), 33 El Bohouth st. (former El Tahrir st.), Dokki, Giza, Egypt.
***Corresponding author's E-mail:** fa_elbaz@hotmail.com

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ABSTRACT

The aim of the current research is to study the effect of *Dunaliella salina* ethanolic extract on renal function and inflammatory cytokines in streptozotocin (STZ)-induced diabetic rats. Urea, creatinine, tumor nerosis factor (TNF)-α and interleukin (IL)-1β levels as well as histopathological investigations of renal tissue were estimated. The present results indicated, significant increase in urea, creatinine and inflammatory cytokines; TNF-α and IL-1β levels in diabetic rats. In addition, the histological examination of diabetic renal tissue declared degenerative changes in its architecture. Administration of *D. Salina* ethanolic extract to diabetic rats demonstrated significant decrease in blood urea, creatinine, TNF-α and IL-1β levels. Moreover, treatment of diabetic rats with *D. salina* extract preserved renal architectures that appeared normal. Hence, it could be concluded that *D. salina* extract may alleviate renal function, suppresses the inflammatory cytokines and recovers renal architectures in STZ-induced diabetes.

Keywords: *Dunaliella salina,* STZ, diabetes, renal function, inflammatory cytokines, TNF-α, IL-1β.

INTRODUCTION

The increased spread of diabetes led to a lot of macro and microvascular complications in heart, brain, vision and kidney such as coronary heart disease (CHD), stroke, visual impairment, diabetic kidney disease (DKD) and end stage renal disease (ESRD).[1] Diabetes is the most common reason in kidney failure, where it accounts nearly 44% of new cases and can lead to chronic kidney disease (CKD).[2] CKD represents a common complication in diabetes state that may result in mortality and cardiovascular disease.[3] As a result of diabetes advance, more albumin leaks into the urine leading macroalbuminuria or proteinuria stage.[4] When the albumin amount increases in urine, the kidneys' filtering function usually drop and the body retains various wastes leading to, the raise of blood pressure.[4]

Inflammation is associated with insulin resistance where, specific proinflammatory cytokines induce inflammatory response. These proinflammatory cytokines is the reason of β-cell death throughout the induction of mitochondrial stress and other responses.[5] Type 2 diabetes mellitus (T2DM) is associated with metabolic dysregulation and chronic inflammation whereas, inflammatory cytokines including IL-1, IL-6, IL-18 and TNF-α, are involved in the progression of diabetes.[6] For example, proinflammatory cytokine IL-1β partially inhibits the function of β-cell and enhances Fas-triggered apoptosis through activating the transcription factor nuclear factor (NF)-κB.[7]

Inflammatory cytokines pathways have central mechanisms in DKD where, cytokines and inflammatory mediators such as TNF-α, TGF-β and ILs affect on sodium excretion, renal blood flow and glomerular filtration rate (GFR).[8] Proinflammatory cytokines lead to kidney damage *via* their disorders in transporters and potassium (K+) ion channels from the nephron that changes the trans epithelial transport of solutes and water in kidney.[9] Also, TNF-α completely minimizes renal blood flow and GFR besides, it induces natriuresis in mice.[10]

Moreover, TNF-α develops the renal cells damage in diabetes by different biological ways including the activation of second messenger systems, transcription factors, cytokines synthesis, growth factors, receptors, cell adhesion molecules, enzymes involved in the synthesis of other inflammatory mediators, acute phase proteins and major histocompatibility complex (MHC) proteins.[11]

Microalgae are a promising feedstock of secondary metabolites for successful commercial applications including carotenoids (astaxanthin, lutein and β-carotene) and polyunsaturated fatty acids such as eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids.[12,13]

D. salina is a microalga with high content of carotenoids. Different carotenoids synthesized within marine organisms, for example, β-carotene, astaxanthin and fucoxanthin have altitude antioxidant activity.[14] The same authors added that, antioxidants possess protective roles versus excess reactive oxygen species (ROS), oxidative rancidity and peroxidation products (superoxide anions, hydroxyl radicals and hydrogen peroxide).

Few studies have examined the effect of *D. salina* in diabetes so; the present study is undertaken to appraise the improving effect of *D. salina* extract supplementation on renal function, inflammatory cytokines as well as renal architectures in diabetic rats induced by STZ.

MATERIALS AND METHODS

Chemicals and reagents

Chemicals used 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).

Cultivation of *D. salina*

The organism was grown in conical flask 5 litres containing BG11 nutrient media according to Stanier.[15] The culture was harvested by centrifugation, dried at 40°C and then grounded into homogeneous fine powder.

Preparation of *D. salina* ethanolic extraction

About 100 g of *D. salina* powder were continuously extracted by soaking in ethanol (80%) and shacked on shaker (Heidolph UNIMAX 2010) up to 48 hours at 150 rpm. The extract was filtered by using Whatman No. 4 filter paper and the residue was re-extracted with the addition of fresh ethanol (80%) for another two times. Combined filtrates were concentrated in Rotary evaporator (Heidolph-Germany) at 40°C under reduced pressure until the solvent has been removed. The dried extracts were stored at -20°C in a freeze and kept for further analysis.[16]

Biological experiment

Animals and rout of administration

The male albino rats (150±20 g) were provided by the Animal House of the National Research Centre (NRC) and housed in group of 10 rats per cage and maintained in controlled environment condition at 26-29°C and were provided 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 study was approved by by the Ethical Committee of the NRC, Egypt, provided that the animals will not suffer at any stage of the experiment.

Experimental design and diabetes induction

The rats were divided into four groups of 10 rats each.

Group I - Normal control rats

Group II - STZ injected rats (45 mg/kg b.wt) where type2 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.[17] After injection, animals had free access to food, water and were given 5% glucose solution to drink overnight to encounter hypoglycaemic shock.[18] Animals were checked daily for the presence of glycosuria. Animals were considered to be diabetic if glycosuria was present for 3 consecutive days.[19] After 3 days of STZ injection fasting blood samples were obtained and blood sugar was determined (≥300 mg/dl).

Group III - STZ + ethanolic extract of *D. salina* (150 mg/kg b.wt) for 15 days.[20]

Group IV - STZ + standard drug glibenclamide (10 mg/kg b.wt) daily for 30 days.

Sample preparations

Overnight fasted animals were sacrificed under slight diethyl ether anesthesia and blood collected by puncture of the sublingual vein in clean and dry test tube. Blood was left 10 minutes to clot and centrifuged at 3000 rpm to obtain serum. The separated serum was used for biochemical analysis of renal function (urea and creatinine), inflammatory cytokines; TNF-α and IL-1β.

Biochemical estimations

Determination of urea[21], creatinine[22] were assayed in serum by diagnostic kit method. Estimation of serum inflammatory cytokines; TNF-α and IL-1β were performed by ELISA; a sandwich enzyme immunoassay.

Histopathological examination

After blood collection, rats of each group were sacrificed. Kidney were removed immediately (a part was fixed in 10% formalin for histopathological examination). The kidney specimens obtained from different animals groups 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.[23] 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).

Statistical analyses

Values are expressed as Mean ± S.D. The data were statistically analyzed using analysis of variance (ANOVA) and Co-state computer program.

Data from the test groups were compared with their respective controls and differences at $p<0.05$ were considered to be significant.

RESULTS AND DISCUSSION

Effect of *D. salina* ethanolic extract on renal function

Administration of STZ resulted in a significant increase in urea and creatinine levels with percentage increase amounted to 34.49 and 84.37%, respectively as compared to normal control group.

However, after the treatment of diabetic rats with 150 mg/kg b.wt of *D. salina*, urea and creatinine levels significantly decreased with percentages of improvement reached to 31.88 and 79.68%, respectively compared with normal control group as shown in Table (1).

Regarding to the results of glibenclamide drug, urea and creatinine levels also decreased with percentages of improvement amounted 12.14 and 63.28%, respectively.

Effect of *D. salina* ethanolic extract on inflammatory cytokines

Table (2) showed the effect of *D. salina* extract on control, diabetic and diabetic-treated groups. Marked increase in TNF-α and IL-1β levels were noticed in diabetic rats with percentages 248.93 and 46.19%, respectively. *D. salina* extract and glibenclamide improved the level of TNF-α level with percentages reached to 27.76 and 87.69%, respectively. In addition, *D. salina* extract and glibenclamide reduced the elevated level of IL1-B with improvement percentages 46.70 and 32.48%, respectively.

Table 1: Effect of *D. salina* ethanolic extract on urea and creatinine levels in different groups

Groups	Parameters	Urea (mg/dl)	Creatinine (mg/dl)
Normal control	Mean±S.D.	30.64^{e}±0.08	1.28^{c}±0.05
Diabetic rats	Mean±S.D. % Change to control	41.21^{a}±1.28 34.49	2.36^{a}±0.03 84.37
Diabetic + *D. Salina* extract	Mean±S.D. % Change to control % of improvement	31.44^{d}±0.15 2.61 31.88	1.34^{c}±0.08 4.68 79.68
Diabetic + glibenclamide drug	Mean±S.D. % Change to control % of improvement	37.49^{b}±0.15 22.35 12.14	1.55^{b}±0.04 21.09 63.28

Values are expressed as mean ± SD for 10 rats. 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 inflammatory cytokines in normal and STZ-induced diabetic rats

Groups	Parameters	TNF-α (ρg/ml)	IL-1β (ηg/ml)
Normal control	Mean±S.D.	109.40^{e}±7.00	1.97^{d}± 0.050
Diabetic rats	Mean±S.D. % Change to control	381.73^{a}±7.25 248.93	2.88^{a}±0.08 46.19
Diabetic + *D. salina* extract	Mean±S.D. % Change to control % of improvement	351.36^{c}±1.01 221.17 27.76	1.96^{d}±0.04 0.00 46.70
Diabetic + glibenclamide drug	Mean±S.D. % Change to control % of improvement	285.79^{d}±4.89 161.23 87.69	2.24^{b}±0.06 13.70 32.48

Values are expressed as mean ± SD for 10 rats. Statistical analysis is carried out using Co-state and SPSS computer programs (version 7), where unshared letter is significant at P ≤ 0.05.

Renal histopathological examination

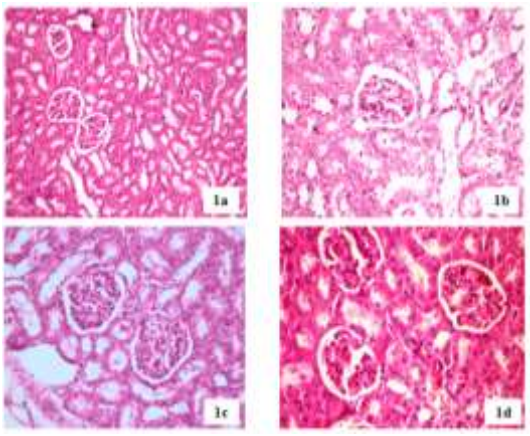

Figure 1: Histopathological changes in kidney Figure 1 (a, b, c and d) revealed the histopathological examination of renal tissues.

1a: Normal histological section of rat kidney showing normal renal tubules and renal glomeruli (H&E X 400).

1b: Histological section of diabetic rat kidney showing massively degenerated renal tubules (H&E X 400).

1c: Histopathological section of diabetic rat kidney treated with *D. salina* extract showing normal renal tubules and renal glomeruli (H&E X 400).

1d: Histopathological section of diabetic rat kidney treated with glibenclamide drug showing normal renal tubules and renal glomeruli (H&E X 400).

Kidney of normal rats showed normal interstitial blood vessel (Figure 1a) while, kidney of diabetic rats showed massively degenerated renal tubules (Figure 1b).

Diabetic-treated rats with *D. salina* extract showed congested blood vessel with normal renal tubules and

glomeruli (Figures 1c). However, diabetic-treated rats with glibenclamide revealed congested interstitial blood vessel with normal renal tubules and glomeruli (Figure 1d).

In diabetes, hyperglycemia is attributed to the long-term damage, dysfunction and failure of various organs including eyes, kidneys, nerves, heart and blood vessels[24]. DM is the most common endocrine disorder that leads to cute complications such as retinopathy, angiopathy, nephropathy and neuropathy, neurological disorders because of disturbance glucose exploitation[24]. Experimental and clinical observation declared that, lipids and particularly oxidized lipids are the main reason of glomerular kidney injury.[24]

Plasma urea and creatinine are markers of glomerular filtration rate (GFR) however; creatinine level is a more sensitive signal of kidney function as compared to urea level.[25] With respect to diabetic rats in the present results, there was significant increase in the levels of urea and creatinine with percentages 34.49 and 84.37%, respectively which considered markers of renal insufficiency. These increments may be illustrated on the findings of Ramya and Prasanna[25] who observed elevated levels of urea and creatinine in diabetes status. They added that, there is a strong link between blood sugar level and urea level where, hyperglycaemia is one of a major cause of renal damage development that leading to an increase in urea level. Remediation of diabetic rats with *D. salina* extract improved the levels of urea and creatinine with percentages 31.88 and 79.68%, respectively.[26] suggested that *D. salina* methanol extract induces the increase of antioxidant enzymes activity and inhibition of lipid peroxidation. So, this decreasing effect of *D. salina* extract in urea and creatinine levels may be due to the antioxidant properties since ROS may be involved in the impairment of GFR.[27,28]

In type 2 diabetes, chronic hyperglycemia encourages an inflammatory state, where cytokines increase may result in the destruction of the pancreatic β-cells and malfunction of the endocrine pancreas.[29] TNF-α is an inflammatory adipocytokine that plays a key role in the development of insulin resistance.[30] Also, IL-1β has an inverse relation with decreased insulin concentration.[31] Concerning to diabetic group, the inflammatory cytokines TNF-α and IL-1β possessed an elevated levels (248.93 and 46.19%, respectively) as compared to normal control rats. The present results are in concomitant with the results of **Al-Dahr and Jiffri**[32] who reported an increment in TNF- α level in all diabetic patients. In addition, **Mojtaba**[31] found elevation in IL-1β level in type II diabetic patients which was far higher than the non-diabetic counterparts. The authors backed this elevation in IL-1β to β-cell dysfunction.

Diabetic group treated with *D. salina* extract showed reduction in both levels of TNF-α and IL-1β with improvement percentages 27.76 and 46.70 %, respectively as compared to normal control group. This amelioration may be attributed to the fact that, antioxidants have the ability to suppress inflammatory markers like TNF- α and IL-1β.[33]

In addition, *D. salina* carotenoid extract possessed a significant antioxidant activity than all-trans forms of α-carotene, β-carotene, lutein and zeaxanthin in all antioxidant assays.[34] The authors linked between cis forms of carotenoids, especially 9- or 90-cis-b-carotene and crucial roles for the antioxidant capacities of the algal extract.

On the other hand, histopathological observations of diabetic kidney revealed massively degenerated renal tubules (Figure 1b). These results are in harmony with those previously reported by **Turoni**[35], who stated that diabetes is associated with degeneration in renal tubules. STZ affects on kidney tissues at varying degrees in different species *via* internalization through glucose transporter 2 (GLUT2) that is found on kidney; besides; it is destructed in the diabetic condition due to hyperglycemia grows both the expression and activity of GLUT2 receptors in the kidney proximal tubules.[36,37,38]

Whereby to diabetic rats treated with *D. Salina* extract, the histological investigations revealed normal renal tubules and renal glomeruli (Figure 1c). Severe oxidative stress is associated with inflammation in CKD where, chronic inflammation shares in diabetes nephropathy (DN) by the direct effect of proinflammatory mediators on cellular signaling and creating a state of oxidative stress.[39] The authors also explained that, the antioxidant and anti-inflammatory effects may protect kidney against the progression of diabetic nephropathy induced by inflammation. Abdel-Daim[40] suggested that, *D. salina* displayed a significant modulatory effect on acetic acid-induced colitis rats due to its high antioxidant enzymes activity, inhibition of lipid peroxidation and inflammation markers. Hence, the ameliorative effect of *D. Salina* extract on diabetic kidney may be due to the suppression of oxidants and inflammatory markers.

CONCLUSION

The data obtained from the present study clearly demonstrated that, the oral administration of *D. salina* ethanolic extract may be useful in the management of diabetes induced inflammation as well as nephropathy which need further extensive research to use as a supplements ameliorating diabetic complication.

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