**Dunaliella salina** Suppress Oxidative Stress, Alterations in the Expression of Pro-Apoptosis and Inflammation Related Genes Induced by STZ in Diabetic Rats

Farouk K. El-Baz1, Wagdy K. B. Khali2, Hoda F. Booles3, Hanan F. Aly4, Gamila H. Ali4

1Plant Biochemistry Department, National Research Centre (NRC), 33 EL Bohouthist. (former EL Tahrir st.), Dokki, Giza, Egypt.
2Cell Biology Department, National Research Centre (NRC), 33 EL Bohouthist. (former EL Tahrir st.), Dokki, Giza, Egypt.
3Therapeutic Chemistry Department, National Research Centre (NRC), 33 El Bohouthist. (former EL Tahrir st.), Dokki, Giza, Egypt.
4Water pollution Research Department, National Research Centre (NRC), 33 EL Bohouthist. (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 present investigation is to study the protective effect of the *Dunaliella salina* extract against streptozotocin (STZ)-induced type 2 diabetes mellitus (T2DM) in male rats. Paraoxonase-1 (PON1), transforming growth factor-β (TGF-β), apoptosis assay as well as expression of the apoptosis and inflammatory related genes were assessed. The results revealed that the antioxidant biomarker PON1 decreased in diabetic rats, however, TGF-β level was elevated in diabetic rats. Whereas, the treatment of rats with STZ increased the apoptosis rates and the alterations of the pro-apoptosis (Eotaxin, Caspase-1 and Caspase-2) as well as inflammation (NF-κ B1 and AIF-1) related genes. Aside from, treatment of diabetic rats with *D. salina* ethanolic extract, they demonstrated an increase in PON1 level, while reduction in the elevated TGF-β level was monitored. However, treatment of STZ-injected rats with *D. salina* extract exhibited low apoptosis rates and decreased the alteration of the pro-apoptosis and inflammatory related genes induced by STZ in rats. The biological effect of *D. salina* could be attributed to its high level of 9-cis β-carotene which protect cells from the oxidative stress, hence, treatment of diabetic rats with *D. salina* extract declared therapeutic potential in hyperglycemia dominance which need further clinical trial for usage as nutraceutical product, in diabetic regimen.

**Keywords:** Dunaliella salina, type 2 diabetes mellitus, Paraoxonase-1, transforming growth factor-β, apoptosis.

**INTRODUCTION**

Diabetes mellitus (DM) is a global health problem that will rise in the following twenty years, with special hazards due to the increase number of diabetic patients in children and adolescents categories. Paraoxonase (PON), an enzyme linked to high-density lipoprotein (HDL), intones both roles of antioxidant and anti-inflammatory of HDL beside it may have a protective role in the prevention of these complications. Low activity of PON1 has been detected in diabetic status associated with high tendency for cardiovascular attack, as PON1 activity is dependent contributing risk factor for coronary artery disease. Chronic and low-grade inflammations are related to type 2 diabetes where, pro-inflammatory and anti-inflammatory cytokines are released mainly by the adipose tissue. Among these cytokines, transforming growth factor-β (TGF-β) which is played the central role in inflammatory process in T2DM. TGF-β1, acts as an anti-inflammatory immunemediator, inhibits or reverses the macrophages activation by interfering with signaling throughout toll-like receptor-dependent pathways. High levels of TGF-β1 and interleukin-1 receptor antagonist (IL-1Ra) were accompanied with T2DM, leading to diabetic complication hence, TGF-β1 may act in both sides.

Reactive oxygen species (ROS), acts as a reactive intermediate initiating liver apoptosis. The authors declared that, OH radicals increased in the diabetic rats liver play a serious role in the elevation of hepatic lipid peroxidation (LPO), leading to increment in pro-apoptotic events. Apoptosis, or programmed cell death, is a physiological process, genetically controlled and it essential for normal tissue development and homeostasis.

Eotaxin gene is one of the pro-apoptotic genes and it is encoding protein belong to cytokines. A recent study reported that eotaxin is a ligand for C-C chemokine receptor type 5 (CCR5) may explain the macrophage accumulation that occurred at 24 h. However, infiltrating leukocytes may often be sources of pro-inflammatory chemokines, and it is more likely that the delayed recruitment of macrophages into the tissue reflects secondary processes dependent upon the induction of other chemokines rather than being dependent directly upon the action of eotaxin or eotaxin-2.

One of the best-known the mediators of apoptosis are caspases, which are cysteine-dependent, aspartate-specific proteases involved in pro-inflammatory cytokine activation and are the effectors of apoptotic pathways. Sequential activation of caspases plays a central role in the execution-phase of cell apoptosis. Grant and Dixt reported that, caspase-1 controls the secretion of bioactive IL-1β throughout some, a multiprotein that is composed of NLRP3 (nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing-3) ASC...
(apoptosis associated speck-like protein containing a CARD) and procaspase-1. The authors added that, NLRP3 inflammasome is an important scout of metabolic dysregulation besides, it controls obesity-associated insulin resistance and pancreatic β-cell dysfunction.

Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) is a protein complex that controls transcription of DNA, cytokine production and cell survival. Moreover, it plays a vital role in DM development via its activation by pro-inflammatory cytokines, to arrange survival and death of β-cells. The authors added that, NF-κB activation induces both apoptosis and insulin resistance in T2DM. Also, NF-κB is involved in various major diabetic complications such as diabetic cardiomyopathy, retinopathy, nephropathy and neuropathy.

A very few research work has been carried out on *D. salina* in management of diabetes. The authors declared that, *D. salina* is a source of natural substances such as carotenoids with antioxidant therapy that lead to improve diabetic associated oxidative stress. However, this is the first study investigating the role of *D. salina* to inhibit pro-apoptotic expression and inflammation in type 2 diabetes. *D. salina* algal extract has been not investigated yet. Therefore, the present study aimed to evaluate the ameliorative efficacy of *D. salina* versus oxidative stress, alteration in the expression of apoptosis and inflammation related genes in STZ-induced diabetic rats.

**MATERIALS AND METHODS**

**Chemicals and reagents**

TRIzol reagent was bought from Invitrogen (Germany). The reverse transcription and PCR kits were obtained from Fermentas (USA). SYBR Green Mix was purchased from Stratagene (USA). All other chemicals used in this study were purchased from Merck, Sigma and Fluka brand chemicals.

**Cultivation of *D. salina***

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

**Ethanolic extract of *D. salina***

*D. salina* dried powder (100 g) was soaked in ethanol (80%) and shaken on an orbital shaker (Heidolph UNIMAX 2010) for 48 hrs at 150 rpm. Then, the obtained extract was filtered using a Buchner funnel and filter paper (Whatman No. 4). The algal residue was extracted three times with the same fresh solvent and extracts were combined. Combined extracts were concentrated and freed of solvent under reduced pressure, using a Rotary evaporator (Heidolph-Germany) at 40°C. The dried crude concentrated extracts were stored in a freeze (-20°C), until used for analyses.

**Biological experiment**

**Animals and experimental design**

Forty male albino rats weighted (150±20 g), were divided into four groups of 10 animals each, were used to investigate the antidiabetic effects of *D. salina* ethanolic extract and provided by the Animal House of the National Research Centre (NRC). **Group I**: normal control, **Group II**: diabetic control 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. After injection, animals had free access to food, water and were given 5% glucose solution to drink overnight to encounter hypoglycaemic shock. Animals were checked daily for the presence of glycosuria. Animals were considered to be diabetic if glycosuria was present for 3 consecutive days. After 3 days of STZ injection fasting blood samples were obtained and blood sugar was determined (≥300 mg/dl). **Group III**: diabetic + *D. salina* ethanolic extract orally administered 150 mg/kg body weight *D. salina* ethanolic extract for 15 days respectively, **Group IV**: diabetic + glibenclamide (daonil) orally administered antidiabetic glibenclamide 10 mg/kg body weight daily for 30 days. 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.

**Collection of blood serum samples**

At the end of the experiment, 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. The serum was obtained by centrifuging the blood samples at 3000 rpm for 10 min, and then the serum was used for the estimating of PON1 and TGF-β method.

**Biochemical estimations**

**PON1 and TGF-β assay**

Estimation of serum PON1 activity and TGF-β level was performed by ELISA; a sandwich enzyme immunoassay.

**Calculation**

\[
\text{% Change} = \frac{\text{Mean of Control} - \text{Mean of treated}}{\text{Mean of control}} \times 100
\]

\[
\text{% of Improvement} = \frac{\text{Mean of Treated} - \text{Mean of disease}}{\text{Mean of control}} \times 100
\]

**Apoptosis assay**

The tissue of liver (50 mg per sample) was made into single-cell suspensions according to method of Villalba. Cells apoptosis was determined by flow cytometry (FCM) assay using Annexin V/PI apoptosis detection kit. The single-cell suspension (1x10⁶ cells/mL) was suspended in 200 μL ice-cold binding buffer and then 10 μL horseradish peroxidase FITC labeled Annexin V and 5 μL propidium iodide (PI) were added. The cell suspension was incubated in darkness at room temperature for 15 min.
Apoptosis rate was determined by flow cytometer. In this study, both FITC and PI negative cells were considered as normal cells. FITC-single positive and PI negative cells were defined as early apoptotic cells, while both FITC and PI positive cells were considered as late apoptotic or necrosis cells.

**Gene expression analysis**

**Extraction of total RNA and cDNA synthesis**

Liver tissues of male rats were used to extract the total RNA using TRIzol® Reagent (Invitrogen, Germany) kit. The isolation method was carried out according to the manufacturer’s instructions of the above kit. Approximately 50 mg of the liver tissues were mixed with some drops of liquid nitrogen and homogenized in 1 ml of TRIzol® Reagent in autoclaved mortar. Afterwards, total RNA was dissolved and preserved in diethylpyrocarbonate (DEPC)-treated water up to use.

To assess the RNA yield and purity of the total RNA, RNAse-free DNase I (Invitrogen, Germany) was used to digest DNA contamination. A small drop of isolated RNA was examined photospectrometrically at 260 nm. The purity of total RNA was determined between 1.8 and 2.1 to be good purified when it examined by photospectrometer at the 260/280 nm ratio. To avoid RNA damaging, aliquots of RNA were prepared after isolation for either reverse transcription reaction or otherwise for storing at -80°C up to use.

To synthesize the complementary DNA (cDNA) isolated RNA from liver tissues was reverse transcribed into cDNA. The reaction volume was carried out in 20 µl. The reaction volume was prepared according to the instructions of the RevertAid™ First Strand cDNA Synthesis kit (MBI Fermentas, Germany). The reverse transcription (RT) reaction was performed for 10 min at 25°C. Afterwards, the tubes of the reaction were put in thermo-cycler machine for 60 min at 42°C, and then the reaction was terminated for 5 min at 99°C. The PCR products containing the cDNA were kept at – 20°C up to use for DNA amplification.

**Quantitative Real Time-PCR (qRT-PCR)**

A Step One Real-Time PCR System (Applied Biosystem, USA) was used to assess the copy of the cDNA of male rats to detect the expression values of the tested genes. To perform the PCR reaction, a volume of 25 µL of reaction mixtures was prepared containing 12.5 µl of SYBR® green (TaKaRa, Biotech. Co. Ltd.), 0.5 µl of 0.2 µM forward and reverse primers, 6.5 µL DNA-RNA free water, and 2.5 µl of the synthesized cDNA. The cDNA was propagated using reaction program consisted of 3 steps. In the first step the PCR tubes was incubated at 95°C for 3 min. In the second step the reaction program consisted of 50 cycles. Each cycle of them consisted of 3 sub-steps: (a) 15 sec at 95°C; (b) 30 sec at 60°C; and (c) 30 sec at 72°C in the third step the reaction program consisted of 71 cycles. The first cycle of them started at 60°C for 10 sec and then the followed cycles increased about 0.5°C every 10 sec up to 95.0°C. A melting curve of the reaction was performed for each qRT-PCR termination at 95.0°C to assess the quality of the primers.

To verify that the reaction of the qRT-PCR does not have any contamination PCR tubes containing non template control were used. The sequences of specific primer of the genes used are listed in Table (1).

The relative quantification of the target genes to the reference (β-Actin) was determined by using the 2^ΔΔCT method.

**Statistical analysis**

Data were analyzed by comparing values for different treatment groups with the values for individual control. All data were expressed as mean ± S.D. of rats in each group.

Significant differences between the groups were statically analyzed using SPSS computer program; one-way analysis of variance (ANOVA) combined with co-state computer program, where P<0.05 test was applied for the comparison among means according to the method of Steel and Torrie.20

All results were expressed as mean ± S.D. of the mean. Data were analyzed by one way analysis of variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS) program, version 11 followed by least significant difference (LSD) to compare significance between groups. Difference was considered significant when P <0.05.

**RESULTS**

**Effect of D. salina extract on PON1 activity**

In the present study, the PON1 activity of the different groups is presented in Table 2 which showed that, the mean of PON1 activity in normal control rats was determined within the normal range (10.12±g/ml). On contrast, diabetic rats showed significant decrease in the PON1 activity with percentage 40.21% as compared to normal control rats. However, the remediation of diabetic rats with D. salina extract and glibenclamide declared an increase in the PON1 activity with improvement percentages 11.56 and 15.90%, respectively.

The enhancement in PON1 activity illustrated that, treatment of diabetic rats with D. salina extract as well as glibenclamide is able to ameliorate PON1 activity comparing to normal rats.

**Effect of D. salina extract on TGF-β level**

Table (3) declared the effect of D. salina extract on TGF-β level in diabetic and diabetic-treated rats versus control one. It is noticeable that, marked increase in TGF-β level in diabetic rats with percentage 89.59%. D. salina extract and glibenclamide improved TGF-β level in diabetic-treated groups with percentages 37.83 and 47.20 %, respectively.
Table 1: Primer sequences used for qPCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer sequence (5′–3′)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eotaxin</td>
<td>F: CACCATGCAGCTCTCCACAG</td>
<td>Y08358</td>
</tr>
<tr>
<td></td>
<td>R: CAGTAGTGTGGGGATCTTCTT</td>
<td></td>
</tr>
<tr>
<td>Caspase-1</td>
<td>F: TCTAAGGGAGGACATCTTTTTC</td>
<td>U14647</td>
</tr>
<tr>
<td></td>
<td>R: TGGGCTATTCTAAGGGGAAAAC</td>
<td></td>
</tr>
<tr>
<td>Caspase-2</td>
<td>F: CCCCCTCTCGGTGTGTGAGTC</td>
<td>U77933</td>
</tr>
<tr>
<td></td>
<td>R: GCTGTAGTGTGGCTGTAATA</td>
<td></td>
</tr>
<tr>
<td>NF-κB1</td>
<td>F: CAAAGCAGAGATGTTGAGA</td>
<td>L26267</td>
</tr>
<tr>
<td></td>
<td>R: ATCATGCTCTCTTTGCGACAT</td>
<td></td>
</tr>
<tr>
<td>AIF-1</td>
<td>F: GCAGATTACGCCCCGCTGCTA</td>
<td>U17919</td>
</tr>
<tr>
<td></td>
<td>R: TGAGAAAGTCAGAATGAAA</td>
<td></td>
</tr>
<tr>
<td>β-actin</td>
<td>F: GGAGATTACTGCCCTGCTGCTG</td>
<td>Deng</td>
</tr>
<tr>
<td></td>
<td>R: GACTCATGTAACCTTGCTG</td>
<td></td>
</tr>
</tbody>
</table>

F: forward primer; R: reverse primer; (NF-κB1): Nuclear factor kappa beta p105; AIF-1: Allograft inflammatory factor-1

Table 2: Effect of *D. salina* extract on PON1 antioxidant biomarker in different groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>PON1 (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>Mean±S.D.</td>
<td>10.12±0.52</td>
</tr>
<tr>
<td>Diabetic rats</td>
<td>Mean±S.D.</td>
<td>6.05±0.07</td>
</tr>
<tr>
<td></td>
<td>% Change to control</td>
<td>40.21</td>
</tr>
<tr>
<td>Diabetic + <em>D. salina</em> extract</td>
<td>Mean±S.D.</td>
<td>7.22±0.10</td>
</tr>
<tr>
<td></td>
<td>% Change to control</td>
<td>28.65</td>
</tr>
<tr>
<td></td>
<td>% of improvement</td>
<td>11.56</td>
</tr>
<tr>
<td>Diabetic + glibenclamide</td>
<td>Mean±S.D.</td>
<td>7.66±0.19</td>
</tr>
<tr>
<td></td>
<td>% Change to control</td>
<td>24.30</td>
</tr>
<tr>
<td></td>
<td>% of improvement</td>
<td>15.90</td>
</tr>
</tbody>
</table>

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* extract on TGF-β anti-inflammatory biomarker in different groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>TGF-β (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>Mean±S.D.</td>
<td>250.23±27.74</td>
</tr>
<tr>
<td>Diabetic rats</td>
<td>Mean±S.D.</td>
<td>474.42±18.87</td>
</tr>
<tr>
<td></td>
<td>% Change to control</td>
<td>89.59</td>
</tr>
<tr>
<td>Diabetic + <em>D. salina</em> extract</td>
<td>Mean±S.D.</td>
<td>379.74±9.14</td>
</tr>
<tr>
<td></td>
<td>% Change to control</td>
<td>51.75</td>
</tr>
<tr>
<td></td>
<td>% of improvement</td>
<td>37.83</td>
</tr>
<tr>
<td>Diabetic + glibenclamide</td>
<td>Mean±S.D.</td>
<td>356.30±5.78</td>
</tr>
<tr>
<td></td>
<td>% Change to control</td>
<td>42.38</td>
</tr>
<tr>
<td></td>
<td>% of improvement</td>
<td>47.20</td>
</tr>
</tbody>
</table>

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.
Effect of *D. salina* on the apoptotic markers in STZ-injected rats

The effect of *D. salina* extract on the inhibition of apoptotic markers in STZ-induced diabetic rats is summarized in Figure (1). The results revealed that STZ-induced T2DM in male rats increased apoptosis rates to 522 % as compared to the normal untreated rats. In contrast, the results showed that *D. salina* supplementation reduced the apoptosis rates induced by the STZ, where the apoptosis rates declined to 312.2% in rats treated with *D. salina* extract compared with those in STZ-injected rats (DM) as shown in Figure (1). In addition, the effect of the antidiabetic drug on the apoptosis rates was decreased significantly, where the apoptosis rate was 273.2 compared with that in STZ-injected rats.

![Figure 1: Levels of apoptosis in liver tissues of different groups. Data are presented as mean ± S.D. followed by different superscripts are significantly different (P<0.05).](image1)

![Figure 2: Expression levels of Eotaxin mRNA in liver tissues of different groups. Data are presented as mean ± S.D. followed by different superscripts are significantly different (P<0.05).](image2)

![Figure 3: Expression levels of Caspase-1mRNA in liver tissues of different groups. Data are presented as mean ± S.D. followed by different superscripts are significantly different (P<0.05).](image3)

![Figure 4: Expression levels of Caspase-2 mRNA in liver tissues of different groups. Data are presented as mean ± S.D. followed by different superscripts are significantly different (P<0.05).](image4)

**Effect of *D. salina* extract on the alteration of gene expression in STZ-treated rats**

The expression values of the pro-apoptosis (Eotaxin, Caspase-1 and Caspase-2) and inflammation (NF-κB1 and AIF-1) related genes in liver tissues of male rats were quantified by real-time RT-PCR (Figures 2-6).

The results revealed that exposure of male rats with STZ increased the mRNA expression values of Eotaxin, Caspase-1, Caspase-2, NF-κB1 and AIF-1 genes to 254.6, 241.5, 268.9, 518.6, and 563.2, respectively compared with control rats (Figures 2-6).

On the other hand, expression values of Eotaxin, Caspase-1, Caspase-2, NF-κB1 and AIF-1 genes decreased to 254.7, 170.1, 189.7, 339.5 and 378.9, respectively, in STZ treated *D. salina* extract compared with STZ-injected rats (DM) (Figures 2-6). In addition, the effect of the glibenclamide drug on the gene expression alteration was decreased significantly, where the expression values of GLT-1 Eotaxin, Caspase-1, Caspase-2, NF-κB1 and AIF-1 genes decreased to 206.3, 164.9, 164.4, 306.9 and 339.5, respectively, in STZ -treated glibenclamide compared with STZ-injected.
erythrocyte declared activity decrease comparing significant predictor of T2DM protein and/or complex DISCUSSION

Figure 5: Expression levels of NF-κB1 mRNA in liver tissues of different groups. Data are presented as mean ± S.D. a,b,c followed by different superscripts are significantly different (P≤0.05).

Figure 6: Expression levels of AIF-1 mRNA in liver tissues of different groups. Data are presented as mean ± S.D. a,b,c followed by different superscripts are significantly different (P≤0.05).

DISCUSSION

Diabetes mellitus is regarding as a progressive and complex metabolic disorder described with inveterate hyperglycaemia resulting from impaired insulin secretion and/or insulin action that leads to carbohydrate, lipid and protein metabolism disorders.21 PON1 enzyme is an important protein which attracts a considerable interest because it is has the most of antioxidant properties.22 T2DM is a leading cause of insulin deficiency and a state of oxidative stress which consumed antioxidant resources of the body. PON1 is an antioxidant enzyme related to High Density Lipoprotein (HDL), which was found to decrease in T2DM. Hence, PON1 activity may be a predictor of cardiovascular disease (CVD) in T2DM.23,24

According to the recent diabetic results, PON1 revealed a significant reduction with percentage amounted 40.21% comparing with the normal group. These results run in parallel with those Ferretti.25 who found significant decrease in PON1 activity in diabetic state. This low activity may be rely on the hypothesis of Karabina26, who declared that, the decreased ability of HDL to protect erythrocyte membranes could be related to lipid composition of HDL and to this low PON1 enzyme activity. In diabetes condition, PON1 is dysfunctional because of glycation, reducing its ability to retard LDL and cell membrane oxidation contributing to the inflammation model of diabetes, resulting in the excess atherosclerosis common in this disease.27

Touching to diabetic rats treated with D. salina, the PON1 activity increased with improvement percentage reached 11.56% as compared to normal group. β-carotene in algae has the ability to inhibit LDL-oxidation in diabetes and protect against oxidative stress.28 Moreover, it restores the hepatic enzymes activity including, catalase, Glutathione peroxidase and superoxide dismutase, which in turn protects vital organs from xenobiotic and other damages.29 Considering to the current results, the improving effect of D. salina on PON1 activity may be attributed to the occurrence of β-carotene in D. salina alga that has the ability to reduce the elevated levels of lipid.

Concerning to TGF-β level, Table (3) indicated significant increase in TGF-β level of diabetic rats with percentage 89.59%. In the line with the current results, Yener30 found that TGF-β1 level in diabetic patients was higher than controls (29.84ng/ml vs 11.37ng/ml). The authors backed these findings to, hyperglycaemia causes an inflammatory reaction and the increase in TGF-β1 levels may mirror the compensative increases in subclinical inflammation, as the pathways of TGF-β have immunosuppressive properties. In addition, the hyperglycaemia is related to up-regulation of glucose transport-1 that leads to TGF-β over expression by mesangial tubular cells or infiltrating renal cells.31 Administration of diabetic rats with D. salina extract leading to reduction in TGF-β level with improvement percentage reached to 37.83%.

The present study revealed that STZ-induced diabetic rats exhibited high apoptosis rate in pancreatic tissue and changes in the apoptosis and inflammatory related genes in liver tissues. In agreement with these findings Saini32 reported that, the cytotoxic effect of STZ on beta cells in murine pancreatic beta cell line involves the activation of the apoptotic pathway. In addition, STZ has been shown to induce apoptosis in β-cells and modifies mouse islet loss after STZ injection, but the mechanisms involved of STZ are not well understood.33,34 On the other hand, Poh and Muniandy35 and Ferretti36 reported that, the apoptosis in human cells increased with STZ injection is due to the alteration in expression of caspase genes, whereas, high expression levels of caspase genes increase the apoptosis rates in human cells. Furthermore, several studies with STZ-induced diabetic rats reported that STZ injection reduced glutathione concentration.35 The glutathione antioxidant system has been proposed to be the most important intracellular defense of an organism against free radicals, and is also known to be affected by the generalized increase in oxidative stress associated with diabetes.36

The current study revealed that D. salina supplementation reduced the apoptosis rates and the alterations in the apoptosis and inflammatory related
genes in STZ-induced rats. This microalgae was selected due to its high carotenoid content and was submitted to an environmentally friendly extraction process with pressurized liquids.\textsuperscript{37,38} Its extract showed highest antioxidant activity \textit{in vitro} and \textit{in vivo}.\textsuperscript{39} It has been found that the protective effect of Dunaliella alga is attributed to the 9-cis \(\beta\)-carotene content.\textsuperscript{40} It has been found that 9-cis \(\beta\)-carotene inhibited the micronucleus formation in human lymphocytes \textit{in vitro}.\textsuperscript{40} Additionally, several authors considered that the 9-cis \(\beta\)-carotene protection is due to its antioxidant properties and inhibition of the metabolic activation of promutagens. The 9-cis \(\beta\)-carotene anti-mutagenic and promotional effects might result from its pro-oxidant activity under conditions of high oxidative stress and high concentration.\textsuperscript{41,42} Therefore, we can suggest that \textit{D. salina} is able to reduce apoptosis rates and alterations in the apoptosis and inflammatory related genes due to its high level of 9-cis \(\beta\)-carotene which protect the cells from the oxidative stress induced by STZ.

**CONCLUSION**

From the obvious results it can be concluded that, \textit{D. salina} extract is able to improve the activity of PON1 and reduce the elevated level of TGF-1\(\beta\) as well as it can prevent the apoptosis and the alterations in the apoptosis and inflammatory related genes induced by STZ.

The biological effect of \textit{D. salina} could be attributed to its high level of 9-cis \(\beta\)-carotene which protect the cells from the oxidative stress.

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