Research Article

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

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ABSTRACT

The aim of the present investigation is to study the protective effect of the *Dunaliella slina* 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 β1 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 slina*, 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.[1] 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.[2] 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.[3] 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.[4] Among these cytokines, transforming growth factor-β (TGF-β) which is played the central role in inflammatory process in T2DM.[4] TGF-β1, acts as 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.[5]

Reactive oxygen species (ROS) induced by drugs such as anticancer drugs lead to considerable cellular damage and to a point of no return in apoptosis.[6] In the model of STZ-mediated hyperglycemia, the hydroxyl (OH), reactive oxygen species (ROS), acts as a reactive intermediate initiating liver apoptosis.[7] 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.[8] 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.[9] Sequential activation of caspases plays a central role in the execution-phase of cell apoptosis. Grant and Dixit[10] 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.[11] 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.[11]

A very few research work had been carried out on *D. salina* in management of diabetes.[12] 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.[13] The culture was harvested by centrifugation, dried at 40°C and then grounded into homogeneous fine powder.

Ethanolic extract of *D. salina*

D. salina dried powder (100 g) was soaked in ethanol (80%) and shacked 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.[14] After injection, animals had free access to 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). **Group III:** diabetic + *D. salina* ethanolic extract orally administered 150 mg/kg body weight *D. salina* ethanolic extract[12] for 15 days respectively, **Group IV:** diabetic + glibenclamide (daonil) orally administered antidiabetic glibenclamide 10 mg/kg body weight daily for 30 days.[17] 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

$$\%\ Change = \frac{Mean\ of\ Control - Mean\ of\ treated}{Mean\ of\ control} \times 100$$

$$\%\ of\ Improvement = \frac{Mean\ of\ Treated - Mean\ of\ disease}{Mean\ of\ control} \times 100$$

Apoptosis assay

The tissue of liver (50 mg per sample) was made into single-cell suspensions according to method of Villalba.[18] Cells apoptosis was determined by flow cytometry (FCM) assay using Annexin V/PI apoptosis detection kit. The single-cell suspension (1×10^6 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 $2^{-\Delta\Delta CT}$ 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 (10.12ηg/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

Gene	Primer sequence (5′–3′)	References
Eotaxin	F: CACCATGCAGCTCTCCACAG	Y08358
	R: CAGTAGTGTGTTGGGGATCTTCTT	
Caspase-1	F:TCTAAGGGAGGACATCCTTTCTC	U14647
	R: TGGGCTATTTCTAAAGGGCAAAAC	
Caspase-2	F:CCCTTCTCGGTGTGTGAGTC	U77933
	R: GCTGGTAGTGTGCCTGGTAAA	
NF-κB1	F:CAAGCAGGAAGATGTGGTGGA	L26267
	R: ATCATGTCCTTCTTTGGCAGCTA	
AIF-1	F:GCGAATGCTGGAGAAACTTGG	U17919
	R: TGAGAAAGTCAGAGTAACTGAACG	
***β*-actin**	F:GGAGATTACTGCCCTGGCTCCTA	Deng[19]
	R: GACTCATCGTACTCCTGCTGCTG	

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

Groups	Parameters	PON1 (ηg/ml)
Normal control	Mean±S.D.	10.12^{a}±0.52
Diabetic rats	Mean±S.D. % Change to control	6.05^{e}±0.07 40.21
Diabetic + *D. salina* extract	Mean±S.D. % Change to control % of improvement	7.22^{c}±0.10 28.65 11.56
Diabetic + glibenclamide	Mean±S.D. % Change to control % of improvement	7.66^{b}±0.19 24.30 15.90

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

Groups	Parameters	TGF-β (ρg/ml)
Normal control	Mean±S.D.	250.23^{e}±27.74
Diabetic rats	Mean±S.D. % Change to control	474.42^{a}±18.87 89.59
Diabetic + *D. salina* extract	Mean±S.D. % Change to control % of improvement	379.74^{c}±9.14 51.75 37.83
Diabetic + glibenclamide	Mean±S.D. % Change to control % of improvement	356.30^{d}±5.78 42.38 47.20

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.

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.

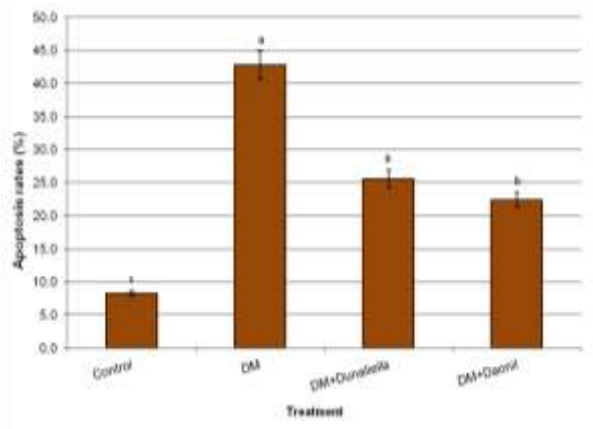

Figure 1: Levels of apoptosis in liver tissues of different groups. Data are presented as mean ± S.D. [a,b,c] followed by different superscripts are significantly different (P≤0.05).

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 -treaded glibenclamide compared with STZ-injected.

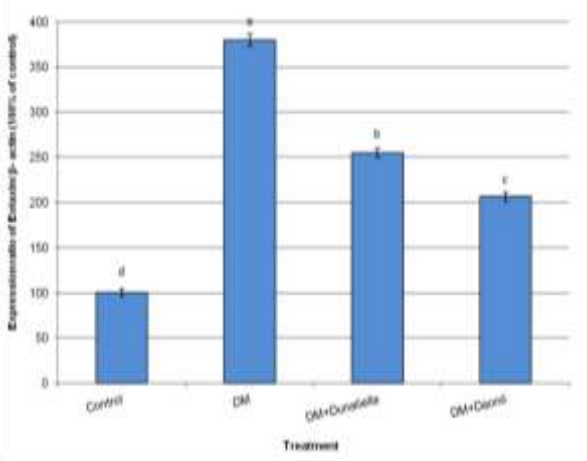

Figure 2: Expression levels of Eotaxin mRNA in liver tissues of different groups. Data are presented as mean ± S.D.[a,b,c,d] followed by different superscripts are significantly different (P≤0.05).

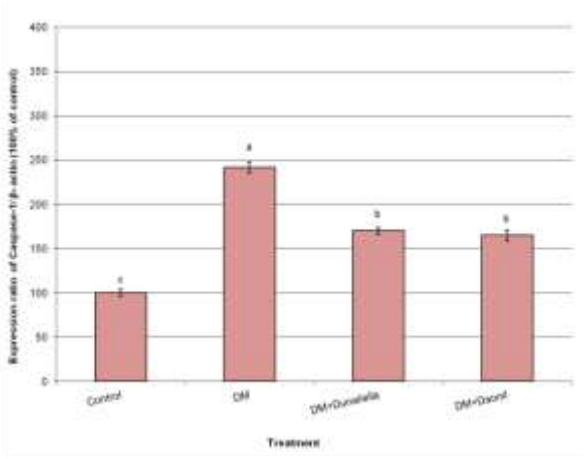

Figure 3: Expression levels of Caspase-1mRNA in liver tissues of different groups. Data are presented as mean ± S.D.[a,b,c] followed by different superscripts are significantly different (P≤0.05).

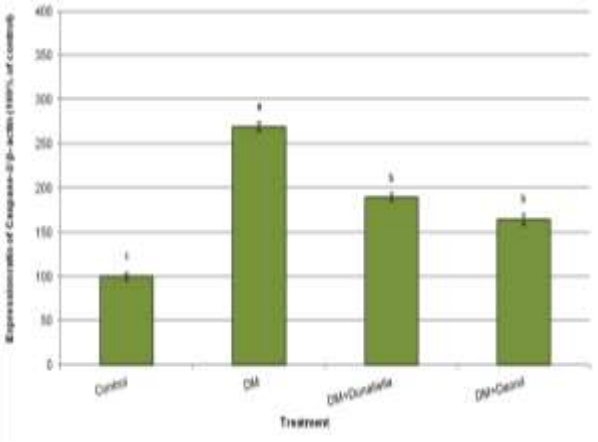

Figure 4: Expression levels of Caspase-2 mRNA in liver tissues of different groups. Data are presented as mean ± S.D.[a,b,c] followed by different superscripts are significantly different (P≤0.05).

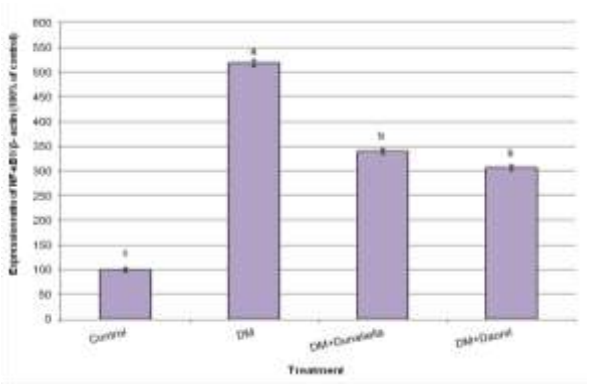

Figure 5: Expression levels of NF-κB1mRNA in liver tissues of different groups. Data are presented as mean ± S.D.[a,b,c] followed by different superscripts are significantly different (P≤0.05).

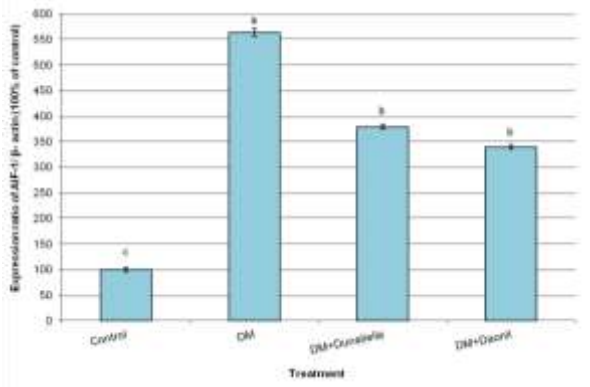

Figure 6: Expression levels of AIF-1 mRNA in liver tissues of different groups. Data are presented as mean ± S.D.[a,b,c] followed by different superscripts are significantly different (P≤0.05).

DISCUSSION

Diabetes mellitus is regarding as a progressive and complex metabolic disorder described with inveterate hyperglycemia 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 Karabina[26], 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 to 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, Yener[30] 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, hyperglycemia 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 hyperglycemia 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 Saini[32] 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 Muniandy[23] and Ferretti[25] 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 microalga was selected due to its high carotenoid content and was submitted to an environmentally friendly extraction process with pressurized liquids.[37,38] Its extract showed highest antioxidant activity *in vitro* and *in vivo*.[39] It has been found that the protective effect of Dunaliella alga is attributed to the 9-cis β-carotene content.[40] It has been found that 9-cis β-carotene inhibited the micronucleus formation in human lymphocytes *in vitro*.[40] Additionally, several authors considered that the 9-cis β-carotene protection is due to its antioxidant properties and inhibition of the metabolic activation of promutagens. The 9-cis β-carotene anti-mutagenic and promotional effects might result from its pro-oxidant activity under conditions of high oxidative stress and high concentration.[41,42] Therefore, we can suggest that *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 β-carotene which protect the cells from the oxidative stress induced by STZ.

CONCLUSION

From the obvious results it can be concluded that, *D. salina* extract is able to improve the activity of PON1 and reduce the elevated level of TGF-1β 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 *D. salina* could be attributed to its high level of 9-cis β-carotene which protect the cells from the oxidative stress.

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REFERENCES

1. Navarro JF, Mora C, Diabetes, inflammation, proinflammatory cytokines, and diabetic nephropathy. The Scientific World Journal, 6, 2006, 908-917.
2. Namitha D, Nusrath A, Rajeshwari A, Rani NA, Serum paraoxonase levels in type 2 diabetes mellitus patients: a case control study. Journal of Medical Sciences & Health, 1(2), 2015, 14-18.
3. Maladkar M, Awatramani M, Bhong K, Resveratrol-The "Chateau hormone" for cardio diabetic protection. Journal of Diabetes Mellitus, 4, 2014, 371-378.
4. Gomes KB, Rodrigues KF, Fernandes AP, The role of transforming growth factor-beta in diabetic nephropathy. International Journal of Medical Genetics, 2014, 2014, 1-6.
5. Herder C, Brunner EJ, Rathmann W, Strassburger K, Tabak AG, Schloot NC, Witte DR, Elevated levels of the anti-inflammatory interleukin-1 receptor antagonist precede the onset of type 2 diabetes: the Whitehall II study. Diabetes Care, 32, 3, 2009, 421-423.
6. Krijnen PAJ, Simsek S, Niessen HWM, Apoptosis in diabetes. Apoptosis, 14(12), 2009, 1387-1388.
7. France´s DE, Ronco MT, Monti JA, Ingaramo PI, Pisani GB, Parody JP, Pellegrino JM, Sanz PM, Carrillo MC, Carnovale CE, Hyperglycemia induces apoptosis in rat liver through the increase of hydroxyl radical: new insights into the insulin effect. Journal of Endocrinology, 205, 2010, 187-200.
8. Ogilvie P, Bardi G, Clark-Lewis I, Baggiolini M, Uguccioni M, Eotaxin is a natural antagonist for CCR2 and an agonist for CCR5. Blood, 1, 2001, 1920-1924.
9. Creagh EM, Conroy H, Martin SJ. Caspase-activation pathways in apoptosis and immunity. Immunological Review, 193, 2003, 10-21.
10. Grant RW, Dixit VD, Mechanisms of disease: inflammasome activation and the development of type 2 diabetes. frontiers in Immunology, 4, 2013, 1-10.
11. Indira M, Abhilash PA, Role of NF-Kappa B (NF-κB) in diabetes. Forum on Immunopathological Diseases and Therapeutics, 4(2), 2013.
12. Ruperez FJ, Garcia-Martinez D, Baena B, Maeso N, Cifuentes A, Barbas C, Herrera E, Journal of Pharmacy and Pharmacology, 60, 2008, 871-878.
13. Stanier RY, Kunisawa R, Mandel M, Cohen-Bazire, G, Purification and properties of unicellular blue-green algae (order Chroococcales). Bacteriological Reviews, 35, 1971, 171-205.
14. Milani E, Nikfar S, Khorasani R, Zamani MJ, Abdollahi M, Reduction of diabetes-induced oxidative stress by phosphodiestrase inhibitors in rats. Comparative Biochemistry & Physiology, 140C, 2005, 251-255.
15. Rajesh RK, Aparna HS, Manjunatha HB, Comparative analysis of silk gland proteins of both heat shocked and normal silkworm larvae of NB4D2 strain by 2-DE. International Journal of Applied Agricultural Research, 4, 2009, 125-130.
16. Shalaby NMM, Abd-Alla HI, Aly HF, Albalawy MA, Shaker KH, Bouajila J, Preliminary *in vitro* and *in vivo* evaluation of antidiabetic activity of *Ducrosia anethifolia* Boiss. and its linear furanocoumarins. BioMed Research International, 2014, 2014, 1-13.
17. Dachicourt N, Bailbé D, Gangnerau MN, Serradas P, Ravel D, Portha B, Effect of gliclazide treatment on insulin secretion and beta-cell mass in non insulin dependent diabetic Goto-kakisaki rats. European Journal of Pharmacology, 361, 1998, 243-251.
18. Villalba M, Martinez-Serrano A, Borner C, Blanco P, Satrustegui J, NMDA-induced increase in [Ca2+](i) and 45Ca2+ uptake in acutely dissociated brain cells derived from adult rats. Brain Research, 570(1-2), 1992, 347-353.
19. Deng Y, Xu ZF, Liu W, Xu B, Yang HB, Wei YG, Riluzole-triggered GSH synthesis via activation of glutamate transporters to antagonize methylmercury-induced oxidative stress in rat cerebral cortex. Oxidative Medicine and Cellular Longevity, 2012, 2012, 1-12.
20. Steel RGD, Torrie JH, Principles and procedures of statistics, 2nd Edition, New York: McGraw-Hill, 1980.
21. Mahfouz MH, Emara IA, Shouman MS, Ezz MK, Asymmetrical dimethylarginine (ADMA) and nitric oxide as potential cardiovascular risk factors in type 2 diabetes

mellitus. African Journal of Biochemistry Research, 3(8), 2009, 293-301.

22. Litvinov D, Mahini H, Garelnabi M, Antioxidant and anti-inflammatory role of paraoxonase 1: implication in arteriosclerosis diseases. North American journal of medicine & science, 4, 2012, 523-532.

23. Poh R, Muniandy S, Paraoxonase 1 activity as a predictor of cardiovascular disease in type 2 diabetes. Southeast Asian Journal of Tropical Medicine and Public Health, 41(5), 2010, 1231-1246.

24. Rani AJ, Mythili SV, Study on total antioxidant status in relation to oxidative stress in type 2 diabetes mellitus. Journal of Clinical and Diagnostic Research, 8(3), 2014, 108-110.

25. Ferretti G, Bacchetti T, Busni D, Rabini RA, Curatola G, Protective effect of paraoxonase activity in high-density lipoproteins against erythrocyte membranes peroxidation: a comparison between healthy subjects and type 1 diabetic patients. Journal of Clinical Endocrinology and Metabolism, 89, 2004, 2957-2962.

26. Karabina SA, Lehner AN, Frank E, Parthasarathy S, Santanam N. Oxidative inactivation of paraoxonase-implications in diabetes mellitus and atherosclerosis. Biochim Biophys Acta, 1725(2), 2005, 213-221.

27. Mackness B, Mackness M, Anti-inflammatory properties of paraoxonase-1 in atherosclerosis. Advances in Experimental Medicine and Biology, 660, 2010, 143-151.

28. Levy Y, Zaltsberg H, Ben-Amotz A, Kanter Y, Aviram M, Dietary supplementation of a natural isomer mixture of β-carotene inhibits oxidation of LDL derived from patients with diabetes mellitus. Annals of Nutrition and Metabolism, 44, 2000, 54-60.

29. McGill CR, Green NR, Meadows MC, Gropper SS, Beta-carotene supplementation decreases leukocyte superoxide dismutase activity and serum glutathione peroxidase concentration in humans. J Nutr Biochem, 14(11), 2003, 656-662.

30. Yener S, Comlekci A, Akinci B, Akan P, Demir T, Bayraktar F, Yesil S, Serum transforming growth factor-beta 1 levels in normoalbuminuric and normotensive patients with type 2 diabetes. Effect of metformin and rosiglitazone. Hormones, 7(1), 2008, 70-76.

31. El-Sherbini SM, Shahen SM, Mosaad YM, Abdelgawad MS, Talaat RM, Gene polymorphism of transforming growth factor-β 1 in Egyptian patients with type 2 diabetes and diabetic nephropathy. Acta Biochimica et Biophysica Sinica, 45(4), 2013, 330-338.

32. Saini KS, Thompson C, Winterford CM, Walker NI, Cameron DP, Streptozotocin at low doses induces apoptosis and at high doses causes necrosis in a murine pancreatic beta cell line, INS-1. Biochemistry and Molecular Biology International, 39(6), 1996, 1229-1236.

33. Turk J, Corbett JA, Romanadham S, Bohrer A, McDaniel ML, Biochemical evidence for nitric oxide formation from streptozotocin in isolated pancreatic islets. Biochemical and Biophysical Research Communications, 197, 1993, 1458-1464.

34. Eizirik DL, Strandell E, Sandler S, Culture of mouse pancreatic islets in different glucose concentrations modifies B cell sensitivity to streptozotocin. Diabetologia, 31, 1988, 168-174.

35. Soon YY, Tan BK, Evaluation of the hypoglycemic and antioxidant activities of Morinda officinalis in streptoztocin-induced diabetic rats. Singapore Medical Journal, 43, 2002, 77-85.

36. Mak DH, Ip SP, Li PC, Poon MK, Ko KM, Alterations in tissue glutathione antioxidant system in streptozotocin-induced diabetic rats. Molecular and Cellular Biochemistry, 162, 1996, 153-158.

37. Zhu Y H, Jiang JG, Continuous cultivation of *Dunaliella salina* in photobioreactor for the production of β-carotene. European Food Research and Technology, 227, 2008, 953-959.

38. Perkins ND, Integrating cell-signalling pathways with NF-kappaB and IKK function. Nature Reviews Molecular Cell Biology, 8(1), 2007, 49-62.

39. Ruperez FJ, Garcia-Martinez D, Baena B, Maeso N, Cifuentes A, Barbas C, Herrera E, Journal of Pharmacy and Pharmacology, 60, 2008, 871-878.

40. Xue KX, Wu JZ, Ma GJ, Yuan S, Qin HL, Comparative studies on genotoxicity and antigenotoxicity of natural and synthetic b-carotene stereoisomers. Mutation Research, 418, 1998, 73-78.

41. Omenn GS, Goodman GE, Thornquist MD, Balmes J, Cullen MR, Gluss A, Keogh JP, Meyskens FL, Hammar S, Risk factors for lung cancer and for intervention effects in CARET, the beta-carotene and retinol efficacy trial, Journal of the National Cancer Institute, 88, 1998, 1550-1559.

42. Polozza P, Calviello G, Bartoli, Prooxidant activity of beta-carotene under 100% oxygen pressure in rat liver microsomes. Free Radical Biology and Medicine, 19, 1995, 887-892.

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