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Research Article

ANTINEURODEGENERATIVE ACTIVITY OF MICROALGAE *DUNALIELLA SALINA* IN RATS WITH ALZHEIMER'S DISEASE

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ABSTRACT

Objective: The present study is aimed to investigate the promising action of *Dunaliella salina* extract as a natural protector against Alzheimer's disease (AD) and reported to possess a variety of activities, including antioxidant effects due to its ability to create large amount of carotenoids.

Methods: *D. salina* is a type of halophile green microalgae was used in the present study. 50 male rats were used in this study, where aluminum chloride was orally administered to induce AD in a dose of 100 mg/kg, daily for 6 weeks. Al-intoxicated rats treated orally daily with *D. salina* ethanolic extract for 6 weeks in a dose of 150 mg/kg b.wt., whereas standard anti-Alzheimer drug donepezil tartrate was administered at the dose of 10 mg/kg b.wt./day for 6 consecutive weeks. The anti-Alzheimer properties of *D. salina* extract were achieved through measuring the calmodulin (CaM) level, paraoxonase 1 (PON1) activity, the antiapoptotic marker (Bcl2), brain-derived neurotrophic factor (BDNF), the generation of the DNA adducts (8-hydroxy-2-deoxyguanosine [8-OHdG]/2-deoxy guanosine [2-dG]), and alteration in the expression of amyloid precursor protein, β-site APP-cleaving enzyme 1 (BACE1), and β-site APP-cleaving enzyme 2 (BACE2) in AD rats.

Results: The current results demonstrated that supplementation of AD rats with *D. salina* extract-enhanced CaM level, and increased PON1 activity, upregulated Bcl2 and BDNF, decreased the levels of DNA adducts (8-OHdG/2-dG), and suppressed the alterations of the expression levels of APP, BACE1, and BACE2-m RNAs as compared with those in AD rats.

Conclusion: It could be concluded that the biological activity of *D. salina* extract might be regulated by 9-cis b-carotene protecting the brain cells from the oxidative stress in AD rats.

Keywords: *Dunaliella salina*, Calmodulin, Paraoxonase 1, Bcl2, Brain-derived neurotrophic factor, Alzheimer's disease, DNA adduct, Amyloid precursor protein.

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INTRODUCTION

Alzheimer's disease (AD) is characterized by extracellular deposition of β-amyloid plaques, neurofibrillary tangles, loss of synapses, neurons, and vacuolar degeneration. AD, the fourth leading cause of death in the USA, also characterized by neuropsychiatric symptoms including apathy, depression, aggression, agitation, sleep disruption, and psychosis [1]. The amyloid precursor protein and the microtubule-associated protein, tau, are two major proteins involved in AD pathology. The β-amyloid protein is produced from the amyloid β-protein precursor (APP) by two sequential proteolytic cleavages enzymes called secretases [2]. Studies also showed that the products of oxidative stress were found in neurofibrillary tangles and senile plaques in AD [3,4]. Therefore, special interest has been focused in establishing the therapeutic role of antioxidants in neurodegenerative disease such as AD. The natural antioxidants vitamin E, C, and β-carotene considerably decreased the symptoms associated with AD [5].

Among all natural sources studied to date, *Dunaliella* possesses the highest content of 9-cis β-carotene reaching levels up to 100 g/kg b.wt. β-carotene-rich *Dunaliella* powder has been commercially exploited in many countries since the 1980s [6-10]. *Dunaliella salina* is a type of halophile green microalgae especially contains potential bioactive compounds for recent biomedical and pharmaceutical values [11]. It has been reported that due to the abundance of β-carotene, which is an antioxidant as well as a vitamin A precursor, *D. salina* is a popular pro-vitamin A food supplement and cosmetic additive [12]. It was demonstrated that *D. salina* exhibits strong protective effects on ultraviolet B radiation-induced corneal oxidative damage in mice, which seems to be caused by the increase of antioxidant enzyme activity and the inhibition of lipid peroxidation. Research performed at the Cancer Research Centre of Hawaii showed that *D. salina* contains a certain type of β-carotene called 9-cis β-carotene, which is up to ten times stronger at preventing cancer than ordinary β-carotene [13]. Clinical studies showed that whole dried *D. salina* cells are an effective source of bioavailable carotenoids and 14 weeks of supplementation with *D. salina* can produce favorable shifts oxidative mutagens in humans [14].

Although several works have been focused on the AD therapy, *D. salina* alga has been not investigated yet. Therefore, the present study aimed to evaluate the regulating role of *D. salina* alga against neurodegenerative disorders in rats.

METHODS

Chemicals

Donepezil and all chemicals were purchased from Sigma Co. (USA) and aluminum chloride from BDH Laboratory Supplies, Poole (UK). TRIzol reagent was bought from Invitrogen (Germany). The reverse transcription (RT) and polymerase chain reaction (PCR) kits were obtained from Fermentas (USA). SYBR Green Mix was purchased from Stratagene (USA).

Collection of *D. salina* and ethanolic extract preparation

D. salina (Strain No. NIES-2257) was isolated by spreading 0.1 ml of water samples collected from the Egyptian Company for Salts and Mineral (EMISAL) effluent ponds using BG11 media [15] for algal isolation with addition of NaCl (100 g/l) into Petri dishes containing 1.5% agar for solidification. Single colonies of algae were then recultivated in the specified liquid media as nonaxenic

batch cultures (50 ml) at 25±2°C and 24 hr with continuous white fluorescent lamp intensity ≈2500 Lux. Cultivation was carried out on an open pond with a capacity of 70 L containing 55 L of growth media. After cultivation, *D. salina* biomass was harvested using electro flocculation method.

About 100 g of *D. salina* powder were soaked in ethanol (80%) and shacked on shaker (Heidolph UNIMAX 2010) for 48 hr at 150 rpm. The extract was filtered using a Buchner funnel and Whatman No. 4 filter paper and the algal residue was reextracted with the addition of fresh ethanol (80%) 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 [16]. The dry extract was stored at −20°C in freeze and kept for further analysis.

Experimental animals

Animals

Male Wistar rats (180-200 g) procured from Central Animal House, National Research Centre (NRC), were used. Animals were acclimatized to the laboratory conditions at room temperature before the experimentation. Animals were kept under standard conditions of a 12 hr light/dark cycle with food and water *ad-libitum* in plastic cages with soft bedding. All the experiments were carried out between 9.00 and 15.00 hr. The protocol was approved by the NRC Ethics Committee Guidelines for the use and care of animals.

Drugs and treatment schedule

Aluminum chloride ($AlCl_3$) (CDH, India) solutions were made freshly at the beginning of each experiment. For oral administration, $AlCl_3$ was dissolved in drinking water and administered in a dose of 100 mg/kg, p.o. was administered to rats daily for 6 weeks 0.5 ml/100 g body weight [17]. Donepezil tartrate (10 mg/kg b.wt./day) diluted in ultrapure water daily for 6 weeks [18]. Animals were randomized into five groups (50 adult male Sprague–Dawley rats) based on their body weight. Each group had 10 numbers of animals. The groups were as follows:

Group 1: Normal control rats.
Group 2: Normal control rats treated with *D. salina.*
Group 3: Serving as Al-intoxicated rats were orally administered with $AlCl_3$.
Group 4: Al-intoxicated rats treated orally daily with *D. salina* ethanolic extract for 6 weeks in a dose of 150 mg/kg b.wt. [19].
Group 5: Al-intoxicated rats orally administered daily with standard drug.

Brain tissue sampling and preparation

At the end of the experiment, the rats were fasted overnight, subjected to anesthesia with diethyl ether and sacrificed. The whole brain of each rat was rapidly dissected, washed with isotonic saline, and dried on filter paper. Each brain was divided sagittally into two portions. The first portion was weighed and homogenized in ice-cold medium containing 50 mMTris/HCl and 300 mM sucrose at pH 7.4 to give a 10% (w/v) homogenate [20]. This homogenate was centrifuged at 1400 ×*g* for 10 minutes at 4°C. The supernatant was stored at −80°C and used for biochemical analyses that included antioxidant enzyme CaM and PON1 level. The ethical conditions were applied such that the animals suffered no pain at any stage of the experiment, and the study was approved by the Ethics Committee of the NRC. Animals were disposed of in bags provided by the Committee of Safety and Environmental Health, NRC.

Biochemical analyses

The activity of CaM as an activator of cAMP phosphodiesterase was assayed in brain tissue homogenate by the spectrophotometric method according to Garg *et al.* [21] while, PON1 was determined in brain tissue by ELIZA technique method. Brain Bcl2 was determined by ELISA technique according to the method of Barbareschi *et al.* [22]. Brain-derived neurotrophic factor (BDNF) was determined by ELISA technique according to the method of Barakat-Walter [23].

Determination of 8-hydroxy-2-deoxyguanosine (8-OHdG)/2-deoxy guanosine (2-dG) in brain tissues by high-performance liquid chromatography (HPLC)

DNA was extracted from rat brain by homogenization in buffer containing 1% sodium dodecyl sulfate, 10 mM Tris, 1 mM EDTA (pH 7.4), and an overnight incubation in 0.5 mg/ml proteinase K at 55°C. Homogenates were incubated with RNase (0.1 mg/ml) at 50°C for 10 minutes and extracted with chloroform/isoamyl alcohol. The extracts were mixed with 3M sodium acetate and two volumes of 100% ethanol to precipitate DNA at −20°C. The samples were washed twice with 70% ethanol, air-dried for 15 minutes, and dissolved in 100 µl of 10 mM Tris/1 mM EDTA (pH 7.4) [24]. DNA was then digested and the adduct 8-OHdG was measured with HPLC equipped with a Coul Array system (Model 5600). Analytes were detected on two coulometric array modules, each containing four electrochemical sensors attached in series, which allows identification targets based on reduction potential. The UV detection was set to 260 nm. The HPLC was controlled and the data acquired and analyzed using Coul Array software. The mobile phase was composed of 50 mM sodium acetate 5% methanol at pH 5.2. Electrochemical detector potentials for 8- OHdG and 2-dG were 120/230/280/420/600/750/840/900 mV, and the flow rate was 1 ml/minute.

Gene expression analysis

Extraction of total RNA and complementary DNA (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. Afterward, total RNA was dissolved and preserved in diethylpyrocarbonate-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 RT reaction or otherwise for storing at −80°C up to use.

To synthesize the 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 TM First Strand cDNA Synthesis Kit (MBI Fermentas, Germany). The RT reaction was performed for 10 minutes at 25°C. Afterward, the tubes of the reaction were put in thermocycler machine for 60 minutes at 42°C, and then, the reaction was terminated for 5 minutes at 99°C. The PCR products containing the cDNA were kept at −20°C up to use for DNA amplification [25,26].

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 were incubated at 95°C for 3 minutes. In the second step, the reaction program consisted of 50 cycles. Each cycle of them consisted of 3 substeps: (a) 15 seconds at 95°C; (b) 30 seconds at 60°C; and (c) 30 seconds 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 seconds, and then, the followed cycles increased about 0.5°C every 10 seconds up to 95°C. A melting curve of the reaction was performed for each qRT-PCR termination at 95°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 nontemplate control were used. The sequences of the 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 using the $2^{-\Delta\Delta CT}$ method.

Statistical analyses

Data were analyzed by one-way analysis of variance using the Statistical Package for the Social Sciences program, version 11 followed by least significant difference to compare significance between groups. The difference was considered significant when $p\leq0.05$.

RESULTS AND DISCUSSION

Effect of *D. salina* extract on CaM level and PON1 activity

Table 2 showed the effect of *D. salina* extract on AD-induced rats, through measuring CaM and PON1 levels in rats' brain tissue. The results showed an insignificant change in CaM level in normal rats treated with *D. salina* extract comparing with normal control rats. While a significant increase in PON1 activity as compared to normal untreated one was observed with percentage 15.32%. AD-induced rats showed a significant decrease in CaM level (62.41%), and PON1 activity (27.36%) comparing with normal control. Treatment of intoxicated rats with *D. salina* extract demonstrated a significant increase in CaM with improvement percentage 32.62%, whereas an insignificant change in PON1 activity (34.47%) as compared to normal control rats was detected. Standard drug recorded a significant decrease in CaM level with amelioration percentage 25.53%, whereas insignificant change in PON1 activity (17.73%) comparing with normal control rats was recorded.

The present results demonstrated a significant reduction in both CaM and PON1 activity in AD rats. In accordance with the present result McLachlan *et al.* [30] declared, the calcium binding proteins in the Alzheimer-induced rats, frontal, temporal, parietal cortex, and subjacent white matter CaM content was significantly reduced (66%). The author added that CaM extracted from temporal cortex also demonstrated reduced efficacy as an activator of 3',5' cyclic nucleotide phosphodiesterase. Reduced concentrations of these important proteins may affect calcium homeostasis and the regulation of a large number of calcium-mediated brain functions. It was suggested that an imbalance of calcium levels in cells precedes the signaling pathway malfunctions and neuronal deterioration observed in neurodegenerative diseases [31].

Calmodulin binding proteins linked to the formation of amyloid plaques. The "amyloid hypothesis" is arguably the predominant hypothesis for the symptoms and progression of AD. CaM is significantly decreased in the brains of AD individuals [30]. In spite of this, the existing CaM can interact with several proteins in the amyloid pathway. It is based on the aggregation of Aβ peptides plus a multitude of other components to form extracellular amyloid plaques in the brains of AD sufferers [31].

Regarding PON1, it exerts a potent protective role *in vivo* against oxidative (OxS)-induced damage [32,33]. Only relatively few studies have examined the relationship between biochemical determinants of this HDL-associated protein and dementia, the decrease in antioxidant protection by PON-1 might be one of the reasons for the exacerbation of OxS observed in dementia patients [34-37]. In addition, PON1 is an enzyme with multiple physiological functions and roles. Relevant to this notion, studies conducted in knockout mice have shown that PON-1 may serve as a key determinant in the detoxification of organophosphate pesticides that, in turn, are regarded as strong risk factors for neurological diseases, such as ate onset Alzheimer's disease (LOAD) and Parkinson's disease [38].

Effect of *D. salina* extract treatment on brain antiapoptotic marker (Bcl2) and BDNF in Al-intoxicated rats

Table 3 declared in a significant change in the antiapoptotic marker (Bcl-2) and BDNF in normal rats treated with *D. salina* as compared to normal untreated rats. AD rats demonstrated a significant decrease in Bcl-2 and BDNF with percentages of 57.46 and 43.11%, respectively. Treatment of AD-induced rats with *D. salina* extract recorded significant reduction in both Bcl2 and BDNF levels with amelioration 33.65% and 30.26%, respectively, compared with 27.20% and 24.70%, respectively, for standard drug.

Respecting to the Bcl2 and BDNF levels, the present data showed a significant decrease in brain levels of Bcl2 and BDNF in Al-intoxicated rats. $AlCl_3$ decreased Bcl2 expression and increased proapoptotic marker (Bax) expression in the rat hippocampus. Altered Bax/Bcl2 ratio

Table 1: Primer sequences used for qPCR

Gene	Primer sequence (5'-3')	References
APP	F: ACT GGC TGA AGA AAG TGA CAA T R: AGA GGT GGT TCG AGT TCC TAC A	Stein and Johnson [27]
BACE1	F: GCG CTT GCC ATG TGC AC R: TGC CGT AAC AAA CGG ACC TT	Luo *et al*. [28]
BACE2	F: AAA TTT CTG GGC CCT TTT CC R: GGG CTC ATT CAG AGC CTG TG	Luo *et al*. [28]
β-actin	F: GGAGATTACTGCCCTGGCTCCTA R: GACTCATCGTACTCCTGCTGCTG	Deng *et al*. [29]

F: Forward primer, R: Reverse primer, APP: Amyloid β-protein precursor, BACE1: β-site APP-cleaving enzyme 1, BACE2: β-site APP-cleaving enzyme 2, qPCR: Quantitative polymerase chain reaction

Table 2: Effect of *D. salina* extract on CaM level and PON1 activity

Groups	Parameters	
	CaM (ng/ml)	PON1 (U/L)
Normal control	2.80±0.13[a]	210.90±10.20[a]
D. salina treated normal control	2.76±0.10[a]	243.20±10.50[c]
% change	3.16	15.32
AD	1.06±0.95[b]	153.20±12.13[d]
% change	62.41	27.36
D. salina treated AD rats	1.98±0.52[c]	225.90±14.20[a]
% change	29.78	7.11
% of improvement	32.62	34.47
Standard drug treated AD rats	1.78±0.42[d]	190.59±11.50[a]
% change	36.87	9.63
% of improvement	25.53	17.73

Data are means±SD of 10 rats in each group. Unshared letters between groups are the significance value at P≤0.05. SD: Standard deviation, *D. salina*: *Dunaliella salina*, AD: Alzheimer's disease, PON1: Paraoxonase 1, CaM: Calmodulin

Table 3: Effect of *D. salina* extract treatment on brain antiapoptotic marker (Bcl-2) and BDNF in Al-intoxicated rats

Groups	Parameters	
	BCl2 (pg/mg protein)	BDNF (pg/mg protein)
Normal control	118.00±6.94[a]	90.00±4.00[a]
D. salina treated normal control	119.00±7.96[a]	89.23±3.22[a]
% change	0.85	0.86
AD	50.20±4.10[b]	51.20±5.20[b]
% change	57.46	43.11
D. salina treated AD rats	89.90±3.17[c]	78.43±2.11[c]
% change	23.81	12.86
% of improvement	33.65	30.26
Standard drug treated AD rats	82.30±3.17[c]	73.43±2.11[c]
% change	30.25	18.41
% of improvement	27.20	24.70

Data are means±SD of 10 rats in each group. Unshared letters between groups are the significance value at p≤0.05. SD: Standard deviation, *D. salina*: *Dunaliella salina*, AD: Alzheimer's disease, BDNF: Brain-derived neurotrophic factor

is critical to Al-induced apoptosis leading to activation of caspase-3 and release of cytochromec [39,40]. Kumar *et al.* [17] reported that Al increases p53 protein expression by activating p38 MAPK to initiate apoptosis and this is accompanied by a marked inhibition of Bcl-2 and increased Bax expression. Takuma *et al.* [41] showed marked decrease in the BDNF mRNA level in the hippocampus due to ovariectomy in mice. Disruption of the pro-inflammatory cytokine/neurotrophin balance by Al plays an important role in the neurodegenerative disease [42].

Effect of *D. salina* extract on the DNA adducts

Determination of the 8-OHdG generation in brain tissues of AD-induced rats' genome following *D. salina* extract treatment is summarized in Fig. 1. Fig. 1 showed that AD-induced rats revealed a significant increase in the 8-OHdG/2-dG ratio compared with those in control rats. However, the results showed that 8-OHdG/2-dG ratio following treatment of AD-induced rats with *D. salina* extract decreased significantly compared with those in AD-induced rats and reached relatively similar to that of the control group. Moreover, the ratio of 8-OHdG/2-dG generation in AD rats treated with donepezil (10 mg/kg), as reference drug for AD treatment, was decreased significantly compared with those in AD rats (Fig. 1).

The results of the present study revealed that AD rats exhibited significantly high levels of DNA adducts in the form of the ratio of 8-OHdG/2-dG and high expression levels of AD-related genes (APP, BACE1, and BACE2). These results are in the same line of Guix *et al.* [43], who reported that levels of brain nitric oxide (BNO) which is responsible for increase the oxidation and DNA damage are increasing in neurodegenerative diseases such as AD, stroke, and Parkinson's diseases due to the formation of highly reactive peroxynitrite. Moreover, Dorheim *et al.* [44]reported that the increase in the levels of BNO in AD patients may result from the activation of NO synthesis, in which it is also increasing in the brain tissue of AD patients, suggesting that BNO may play a role in neuronal cell degeneration in the AD disease.

Effect of the *D. salina* extract on the expression of AD genes

The expression levels of the genes encoding AD enzymes including APP and β-site APP-cleaving enzyme 1 and 2 (BACE1 and BACE2) in brain tissues of AD rats were quantified by real-time RT-PCR (Figs. 2-4). The results revealed that AD-induced rats revealed a significant increase in the expression of APP, BACE1, and BACE2 genes compared with those in control rats. The percentages of the mRNA expression of APP, BACE1, and BACE2 genes in AD rats were 513.5%, 602.8%, and 332.5%, respectively, compared with those in control rats (Figs. 2-4). In contrary, the expression levels of APP, BACE1, and BACE2 genes decreased significantly in AD rats treated with *D. salina* extract compared with those in AD rats. The percentage of the mRNA expression of APP, BACE1, and BACE2 genes in AD rats treated with *D. salina* extract decreased to 234.5%, 311.1%, and 183.1%, respectively, compared with those in AD rats (Figs. 2-4). Moreover, the percentage of the mRNA expression of APP, BACE1, and BACE2 genes decreased to 226.9%, 255.6%, and 166.2%, respectively, in AD rats treated with donepezil compared with those in AD rats.

Some reports suggested that oxidative stress and DNA damage are associated with signal transduction pathways of N-methyl-D-aspartate receptors (NMDA), in which they are glutamate receptors and ion channel protein found in nerve cells receptors. In this pathway, the activation of NMDA receptors leads to increased intracellular calcium in the postsynaptic neuron, which, in turn, binds to CaM and triggers the activation of the NOS enzyme opening a gate for the electron flux into the active center of the NOS in brain tissue [45,46]. Thus, it gives rise to the elevated levels of BNO that are apparently involved in neurodegeneration by different mechanisms, including oxidative stress and activation of intracellular signaling mechanisms [47].

The present study exhibited that treatment of AD rats with *D. salina* extract decreased significantly the levels of DNA adducts and the expression levels APP, BACE1, and BACE2 genes. The formation of

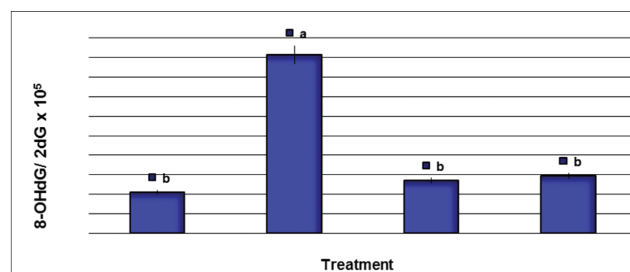

Fig. 1: Generation of 8-hydroxy-2-deoxyguanosine (8-OHdG) in the brain tissues of Alzheimer's disease-induced rats treated with *Dunaliella salina* extract. DNA damage was expressed as the ratio of oxidized DNA base (8-OHdG) to nonoxidized base (2-deoxy guanosine) in brain DNA. Data are presented as mean±standard error of mean, [a,b,c]followed by different superscripts are significantly different (p≤0.05)

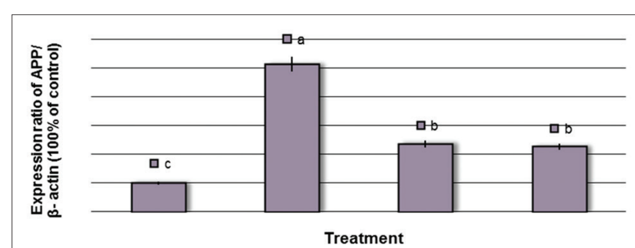

Fig. 2: Expression levels of amyloid β-protein precursor gene in brain tissues of Alzheimer's disease-induced rats treated with *Dunaliella salina* extract. Data are presented as mean±standard error of mean, [a,b,c]followed by different superscripts are significantly different (p≤0.05)

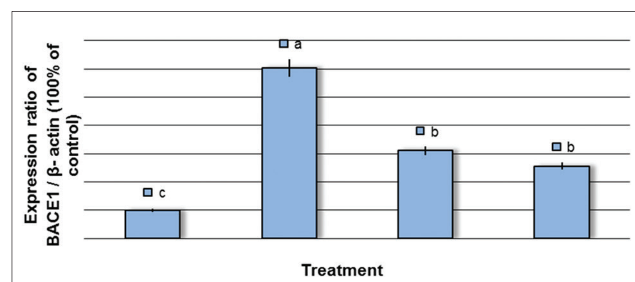

Fig. 3: Expression levels of BACE1 gene in brain tissues of Alzheimer's disease-induced rats treated with *Dunaliella salina* extract. Data are presented as mean±standard error of mean, [a,b,c]followed by different superscripts are significantly different (p≤0.05)

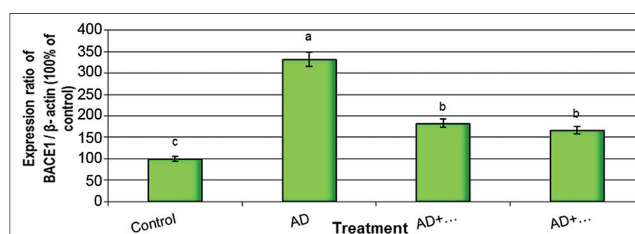

Fig. 4: Expression levels of BACE2 gene in brain tissues of Alzheimer's disease-induced rats treated with *Dunaliella salina* extract. Data are presented as mean±standard error of mean, [a,b,c]followed by different superscripts are significantly different (p≤0.05)

Alzheimer's Aβ peptide is initiated when APP is cleaved by the BACE1 and BACE2 enzymes [48]. It has been reported that *D. salina* is known to have high carotenoid content [19]. Moreover, *D. salina* extracts are shown to contain high levels of antioxidant activity in the both *in vitro* and *in vivo* studies [49]. These studies demonstrated that the ameliorative effect of *D. salina* is attributed to the 9-cis β-carotene content [49].

Several studies revealed that 9-cis β-carotene inhibited the chromosomal breaks (micronucleus formation) in the lymphocytes of human *in vitro* [49]. Moreover, other reports indicating that the therapeutic role of 9-cis β-carotene is attributed to its antioxidant properties and inhibition of the metabolic pathway of the most pro-mutagens [50,51]. Thus, the results of the current work could be suggested that *D. salina* extract is able to DAN adducts and alterations of AD-related genes through the protective pathway of 9-cis β-carotene which protect the cells from the oxidative stress occurred in the degenerative cells of AD rats.

Moreover, several reports revealed that β-carotene extracted from *Dunaliella* sp., which contain high levels of bioavailable 9-cis, have in fact provided verification of a lower incidence of several kinds of cancer and degenerative disorders [50].

CONCLUSION

D. salina extract is capable to suppress the DNA adducts and decrease the alterations in the AD-related genes in DA rats. The biological activity of *D. salina* extract is might be regulated by 9-cis β-carotene which it is coinciding with degenerative protection in the oxidative stressed cells in AD patients.

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