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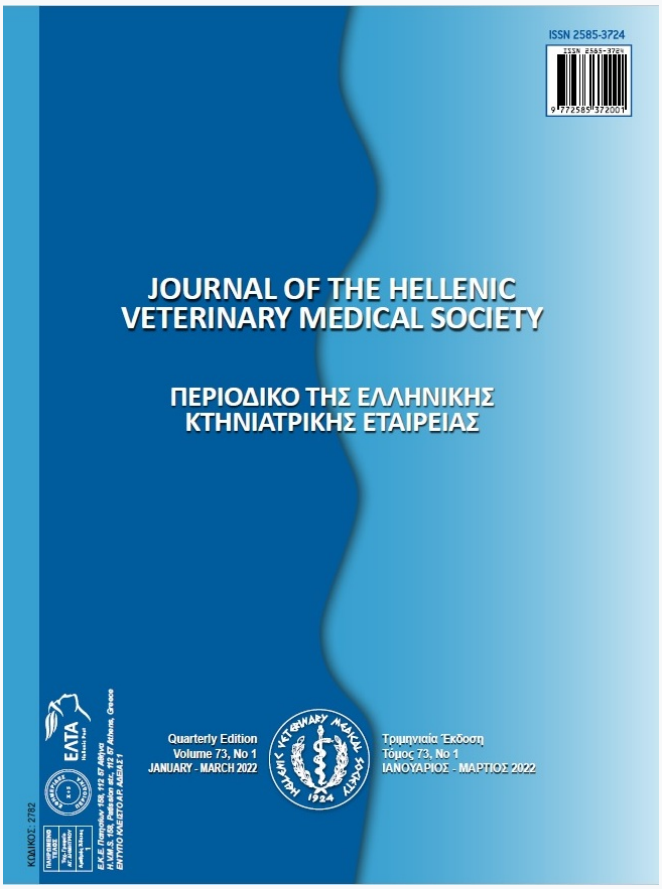

Protective Effect of Spirulina against Aspartame Induced Oxidative Stress and Molecular Gene Brain damage

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Research article
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Protective Effect of *Spirulina platensis* against Aspartame Induced Oxidative Stress and Molecular Gene Brain damage in New-Zealand rabbits

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ABSTRACT: *Spirulina* and alpha-lipoic acid have been considered as one of the most effective in antioxidative stress and anti-inflammatory *activity.* Aspartame (ASP) is one of the most widely used (artificial sweeteners) used in a variety of foods and feeds. This study was conducted to evaluate the possible protective effect of *Spirulina* and alpha-lipoic acid against aspartame induced oxidative stress and brain damage in rabbits. Forty two white male New-Zealand Rabbits were classified into seven equal groups. Group I: (Control group) received no drugs. Group II: rabbits administered with aspartame (250 mg/kg b. wt/day). Group III: rabbits received alpha- lipoic acid (100 mg/kg b. wt/day). Group IV: rabbits received *Spirulina platensis* (1500 mg/kg. b. wt/day). Group V: rabbits received aspartame (250 mg /kg b. wt) and treated with alpha- lipoic acid (100 mg/kg b. wt). Group VI: rabbits received aspartame (250 mg mg/kg b.wt) and treated with *Spirulina platensis* (1500 mg/kg b. wt). Group VII: rabbits received aspartame (250 mg mg/kg b.wt) and treated daily with alpha-lipoic acid (100 mg/kg b. wt) and *Spirulina platensis* (1500 mg/kg b. wt) for 8 weeks. At the end of experiment brain tissue was isolated and analyzed for determination of L-malondialdehyde (L-MDA), catalase (CAT) and reduced glutathione (GSH) in addition to anti-inflammatory cytokines: interleukin-10 (IL-10), Activator Protein-1 (AP-1), Bax gene expression and DNA damage. The obtained results showed a significant up-regulation of AP-1, Bax gene expression level and a significant down-regulation of IL-10 and marked increase in L-MDA and DNA damage that was indicated by an increase in tail length and tail DNA percent in brain tissue of aspartame treated rabbits. However, brain CAT activity and GSH concentration were significantly decreased when compared with control group. Co-administration of*Spirulina*and alpha-lipoic acid protected aspartame induced brain damage in rabbits *via* a significant improvement of all previous parameters and attenuate DNA changes. Conclusively, *Spirulina platensis* and alpha-lipoic acid exert a protective effect against DNA damage and oxidative stress in aspartame induced brain through free radical scavenging and anti-inflammatory activities as well as regenerating endogenous antioxidants defense system mechanisms.

Keywords: *Spirulina platensis*, Alpha-lipoic acid, Aspartame, Oxidative stress, DNA damage.

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INTRODUCTION

Aspartame (L-aspartyl-L-phenylalanine methyl ester ASP) is one of the most widely used artificial sweeteners. It is composed of substances normally found in human and animal foods, i.e. the amino acids, aspartic acid, phenylalanine, and the alcohols (i.e. methanol). The metabolism of aspartame provides approximately 4 kcal/g of energy (Gouge et al., 2004).However, this energy is negligible as the high intensity sweetening power of ASP (approximately 200 sweeter than sucrose by weight means that little is needed to be added to foods to achieves wetness (Magnuson et al., 2007) after oral administration to humans and experimental animals, ASP is rapidly and completely metabolized to aspartic acid, phenylalanine and methanol. Aspartic acid is a highly excitatory neurotransmitter (Krebs, 1992). However, the blood-brain barrier precludes influx of aspartate into the brain (Pardridge, 1979). The neutral amino acid phenylalanine is the precursor of the two brain catecholamine neurotransmitters, dopamine and norepinephrine. When phenylalanine concentration in blood plasma is elevated, the uptake into the brain increases at the expense of that of the other neutral amino acids. Thus, an increased phenylalanine level may affect brain levels of dopamine and norepinephrine and thus influence brain functions (Harper, 1984).

In addition, the increased uptake of phenylalanine might reduce the uptake of tryptophan (precursor of serotonin) and hence indirectly influence the biosynthesis level of serotonin in the brain, and thus affect brain function (Pardridge, 1986).The methanol is oxidized in the liver to formaldehyde which is further oxidized to formic acid. Formic acid is converted to CO_2 and water, via formation of 10-formyl tetra hydro folate (Barceloux et al., 2000). However, consumption of 50 mg ASP/ kg body weight would result in ingestion of 5 mg methanol/kg body weight (10% of ASP by weight is methanol), which is less than the amount of methanol formed during consumption of many foods including fruits and vegetables (Garriga, Metcalfe, 1988). Additionally, consumption of aspartame has been reported to be responsible for neurological and behavioral disturbances in sensitive individuals. The adverse neurological effects such as headaches, insomnia and seizures may be attributed to the alterations in regional brain concentrations of catecholamine (Coulombe and Sharma, 1986).Oxidative stress is very important pathophysiology of the central nervous system (CNS) and via regulating of mitochondrial activities, mediation of inflammation mediators, and others (Halliwell and Gutteridge 1999).

Compared to other organs, brain is especially vulnerable to oxidative stress due to the high utilization of oxygen, the large amount of easily oxidizable polyunsaturated fatty acids, the abundance of redox-active transition metal ions and the relative reduction of antioxidant defense systems (Butterfield and Stadtman, 1997). Antioxidant system is involved in the defense system against free radical mediated tissue or cellular damage. An enzymatic antioxidant system included glutathione-dependent enzymes, superoxide dismutase and catalase (Chaudiere and Ferrari-iliou, 1999), in addition to the non-enzymatic antioxidant (glutathione and uric acid). Moreover, GSH acts as the non-enzymatic anti-oxidative defense because it reacts with nitric oxide and protects the cellular system against the toxic effects of lipid peroxidation which produced from induced aspartame (Chaudiere and Ferrari-Iliou, 1999). Kono and Fridorich (1989) suggested that CAT is the main scavenger of H_2O_2 at high concentration. It catalyzed the conversion of H_2O_2 to water and molecular oxygen. Hence, the increase in CAT activity after 6 weeks of ASP treatment could be expected to converted toH_2O_2, which produced, as a result of the enzymatic activity of SOD, to H_2O and molecular oxygen. Also, AP-1 is a pivotal transcription factor that regulates a wide range of cellular processes including proliferation, apoptosis, differentiation, survival, cell migration, and transformation. Accumulating evidence supports that AP-1 plays an important role in several severe disorders including cancer, fibrosis, and organ injury, as well as inflammatory disorders such as asthma, psoriasis, and rheumatoid arthritis. AP-1 has emerged as an actively pursued drug discovery target over the past decade. Excitingly, a selective AP-1 inhibitor T-5224 (Shen et al., 2008).

Additionally, it was demonstrated that chronic ASP consumption (75 mg/kg b.wt/day) for 90 days significantly increased the brain damage revealed to brain markers (BDNF, COX-2 andPGE2) and elevated the production of cerebral cortex cytokines, IL-6 and TNF-a, respectively (Soffritti et al., 2007; Soffritti et al., 2010). Moreover, the same exposure reduced GSH levels, enhanced TNO production as well as ROS generation and LPO of ASP-result in apoptosis. ASP can be triggered by signals arising from the activation of death receptor-mediated (extrinsic) or mitochondrial-mediated (intrinsic) signaling pathways

(Itoh and Nagata, 1993).Extrinsic apoptotic signaling involves the activation of cell surface death receptors belonging to the protein family of tumor necrosis factor receptors (Itoh and Nagata, 1993). The binding of Fas receptor with its cognate ligand, FasL, can result in activation of caspase 8, activating downstream effector caspases (e.g., caspases 3, 6, and 7), resulting in apoptosis (Boldin et al., 1995). Cytochrome c accelerates the activation of caspase 9, initiating a downstream caspase cascade, which ultimately leads to cell death. AIF induces apoptosis via a caspase-independent pathway when cells experience serious oxidative stress. Beg et al., (1993) demonstrated that, cytokine, TNF-α, mediates early-stage responses of inflammation by regulating the production of other cytokines, including IL-1, IL-10 and IL-6. Because TNF-α is the main mediator of several inflammatory toxic responses to chemicals, it represent promising target for the prevention of uncontrolled inflammation. TNF-α has also been reported to induce nuclear factor-kappa beta (NF-$\kappa\beta$) production and this protein is inhibited by the presence of antioxidants.

Alphalipoic acid acts as coenzyme of pyruvate, free radical scavenger, metal chelator, and it protects against oxidative stress both in peripheral tissues and central nervous system (Winiarska et al., 2008).Additionally, *Spirulina* is a widely used microalgae in aqua feeds due to the richness with vitamins, proteins, fatty acids, minerals, amino acids, and carotenoids and it documented as potent antioxidant and antiinflammatory agent. Also, it strongly induces antioxidant enzyme activity, helps to prevent lipid peroxidation and DNA damage, and scavenges free radicals (Abdelkhalek et al., 2015).Accordingly, the present study aims to investigate the harmful effect of aspartame exposure on brain of rabbits, and the potential ameliorating role of *Spirulina platensis* and alpha-lipoic acid against aspartame induced oxidative stress and brain damage in rabbits.

MATERIAL AND METHODS

Experimental animals

Forty two white males New-Zealand rabbits of 4-6 weeks old age of average body weight 800-1200 g were used in the experimental investigation of this study. Rabbits were obtained from Laboratory Animals Research Center Faculty of Veterinary Medicine, Benha University. Rabbits were housed in separated metal cages (6per cage) with well-balanced ration and fresh clean drinking water ad-libitum. Rabbits were kept at a constant environmental and nutritional condition throughout the whole period of experiment. All rabbits were left for 15 days for acclimatization before the beginning of the experiment.

Chemicals and antioxidants

All chemicals were of analytical grade are obtained from standard commercial suppliers. Aspartame was purchased from Al-Ameriya pharma company, Egypt. *Spirulina* microalgae green powder was obtained from National Research Center, Dokki-Egypt.

Experimental design

Alpha-lipoic acid (Thioctic acid) was manufactured by Eva pharma, Egypt. *Spirulina* was freshly prepared by dissolved in distilled H_2O and administered orally using gastric tube in a daily dose of 1500 mg/kg body weight (Collaet al., 2008). DL-α-Lipoic acid was given orally in a daily dose of 100 mg/kg body weight of rabbits according to Şehirliet al., 2008.

After acclimatization to the laboratory conditions, the animals were randomly classified into seven groups, 6 rabbits each, placed in individual cages and classified as following: Group I: (Control group) received no drugs. Group II: rabbits administered with aspartame (250 mg/kg b. wt/day). Group III: rabbits received alpha- lipoic acid (100 mg/kg b. wt/day). Group IV: rabbits received *Spirulina platensis* (1500 mg/kg. b. wt/day). Group V: rabbits received aspartame (250 mg /kg b. wt) and treated with alpha-lipoic acid (100 mg/kg b. wt). Group VI: rabbits received aspartame (250 mg mg/kg b.wt) and treated with *Spirulina platensis* (1500 mg/kg b. wt). Group VII: rabbits received aspartame (250 mg mg/kg b.wt) and treated daily with alpha-lipoic acid (100 mg/kg b. wt) and *Spirulina platensis* (1500 mg/kg b. wt) for 8 weeks.

Tissue samples for biochemical analysis

At the end of the experiment, the brain tissues of rabbits were isolated immediately and weighed. Brain tissue was divided into 2 parts. One part was cleaned by rinsing with cold saline and stored at -20 ºC for subsequent biochemical analysis. All brain samples were analyzed for the determination of reduced Glutathione (GSH), catalase (CAT) activity and L-malondialdehyde (L-MDA) and the second part was kept at - 80°Cfor molecular analysis.

Brain tissue preparation for biochemical analysis

Brain tissues were cut, weighed and minced into

small pieces, homogenized with a glass homogenizer in 9 volume of ice-cold 0.05 mM potassium phosphate buffer (pH7.4) to make 10% homogenates. The homogenates were centrifuged at 6000 r.p.m for 15 minutes at 4 ºC then the resultant supernatant was used for the determination of the following parameters: L-MDA and CAT. About 0.2 g of brain tissues were minced into small pieces homogenized with a glass homogenizer in 0.4 ml of 25% metaphosphoric acid (MPA), (ref. No.: 253-433- 4, Sigma-Aldrich, Germany), then 1.4 mL of distilled water was added, mixed, incubated for 1 hour and centrifuged for 10 min at 3, 000 r.p.m then the clear supernatant was removed and used for determination of GSH concentration.

Brain tissue for molecular and biochemical analysis

Brain tissue was immediately excised and frozen in liquid nitrogen and then in -80°C until used for determination of DNA damage using comet assay, Baxgene andIL-10, AP-1, gene expression analysis by qPCR. Brain tissue L-MDA, SOD and GSH were determined according to the method adapted by (Ohkawa et al., 1979), (Nishikimi et al., 1972) and (Beutler et al., 1963), respectively**.** Total RNA was isolated from brain tissue of rabbits using RNA easy Mini Kit (Thermo Scientific, Fermentas, #K0731) according to the manufacturer's protocol. Following determination of RNA concentration and purity by Qwell nanodrop Q5000 (USA), 5 mg of total RNA from each sample was reverse transcribed using Quant script reverse transcriptase. The produced cDNA was used as a template to determine the relative expression ofBax, IL-10), AP-1, gene using StepOnePlus real time PCR system (Applied Biossystem, USA) and gene specific primers. The reference gene, β actin, was used to calculate fold change in target genes expression. The thermal cycling conditions, melting curves temperatures, and calculation of relative expression were done. For the treated groups, assessment of 2-ΔΔCt determined the fold change in gene expression relative to the control. Also, DNA damage was determined by alkaline single-cell gel electrophoresis (comet assay) according to the protocol described by (Singh et al., 1988).

Statistical analysis

The results were expressed as mean ± SE using SAS computerized program v. 9.2 SAS. (2008) program to calculate the analysis of variance. The data were analyzed using one-way ANOVA to determine the statistical significance of differences among groups. Duncan's test was used for making a multiple comparisons with in groups for testing the inter-grouping homogeneity. Values were considered statistically significant when $p<0.05$.

RESULTS

The obtained results presented in table (2) revealed that oral aspartame administration to rabbits for 8 weeks resulted in significant increase in L-MDA concentration, decrease of CAT activity and GSH concentration when compared with control normal group. On the other hand, treatment with *Spirulina* and alpha-lipoic acid to aspartame administered rabbits for 8 weeks shown a significant decrease in serum L-MDA level, increase of CAT activity and GSH concentration when compared to aspartame group. Additionally, no significant changes were observed in L-MDA concentration, CAT activity and GSH concentration in *Spirulina* and alpha-lipoic acid treated normal rabbits groups when compared to control group.

The obtained results in (Table 3 and Figs. 1, 2, 3) revealed significant ($P\leq0.05$) up-regulation of Bax and AP-1 gene expression level in brain of Asp-treated (Asp group) rabbits as compared to the normal control group (Cnt group). In Asp administered rabbits and treated with LA and/ or Spir exhibited a significant down-regulation of Bax and AP-1 gene expression at the following order; Asp+LA+Spir (lowest expression) <Asp+Spir<Asp+LA (highest expression) as compared to Asp group. Additionally, no significant changes in the expression of Bax and AP-1 were noticed among the three groups (Cnt, LA and Spir).

Table 1. Forward and reverse primers sequence used in qPCR.

Gene	Forward primer (/5 ------ /3)	Reverse primer (/5 ------ /3)
Bax	ACACCTGAGCTGACCTTG	AGCCCATGATGGTTCTGATC
IL-10	GTTGCCAAGCCTTGTCAGAAA	TTTCTGGGCCATGGTTCTCT
AP-1	GCACATCACCACTACACCGA	TATGCAGTTCAGCTAGGGCG
β- actin	AAGTCCCTCACCCTCCCAAAAG	AAGCAATGCTGTCACCTTCCC

Table 2. Effect of alpha-Lipoic acid and/or *Spirulina* administrations on brain tissue GSH, L-MDA concentrations and CAT activity in aspartame treated male rabbits

Animal groups	GSH (ng/g. tissue)	L-MDA (nmol/g. tissue)	CAT (U/g. tissue)
Group I (Cont.)	4.63 ± 0.17^{a}	4.27± 027^{e}	1.77^{a}±0.17
Group II (Asp)	1.51± 0.10^{e}	9.40 ± 0.58^{a}	0.31^{d}±0.10
Group III (LA)	4.41± 0.19^{a}	4.36± 0.21^{e}	1. 51^{a}±0.12
Group IV (Spir)	4.75± 0.15^{a}	4.09± 0.30^{e}	1.75^{a}± 0.17
Group V (Asp+LA)	2.84± 0.12^{d}	7.51± 0.43^{b}	2.84^{c}± 0.10
Group VI (Asp+Spir)	3.37± 0.10^{c}	6.32± 0.25^{c}	3.37^{bc}± 0.10
Group VII (Asp+LA+Spir)	3.72± 0.14^{b}	5.24± 0.22^{d}	3.72^{b}± 0.14

Data are presented as (Mean ± SE). SE = Standard error. Mean values with different superscript letters in the same column are significantly different at (P≤0.05).

Table 3. Effect of alpha-lipoic acid and/or *Spirulina*administrations on the relative expression of Bax, IL-10andAp-1gene in brain tissues of Aspartame treated male rabbits

Animal Groups	(Baxgene) Fold change mean ±SEM	(IL-10 gene) Fold change ±SEM	(Ap-1 gene) Fold change ±SEM
Group I (Cont.)	1.00±0.08^{d}	1.00 ±0.06^{a}	1.00 ±0.09^{e}
Group II (Asp)	2.93±0.12^{a}	0.18±0.01^{e}	3.78±0.01^{a}
Group III (LA)	1.04±0.07^{d}	1.07±0.07^{a}	0.86±0.09^{e}
Group IV (Spir)	1.06±0.07^{d}	1.03±0.05^{a}	0.95±0. 1^{e}
Group V (Asp+LA)	2.03 ±0.1^{b}	0.39±0.02^{d}	2.91±0.09^{b}
Group VI (Asp+Spir)	1.84 ±0.01^{bc}	0.62 ±0.03^{c}	2.20 ±0.07^{c}
Group VII (Asp+LA+Spir)	1.56±0.09^{c}	0.84 ±0.02^{b}	1.74 ±0.08^{d}

Data are presented as (Mean ± SEM). SEM = Standard error of mean. Mean values with different superscript letters in the same column are significantly different (P≤0.05).

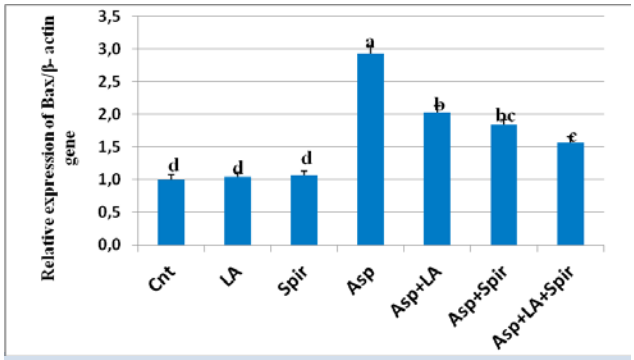

Figure 1. Graphical presentation of real-time quantitative PCR analysis of the expression of Bax gene in brain tissue of Asp administered rabbits following treatment by LA or/and Spir

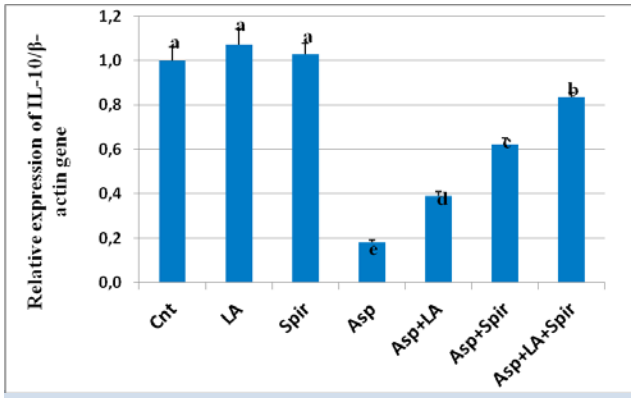

Figure 2. Graphical presentation of real-time quantitative PCR analysis of the expression of IL-10 gene in brain tissue of Asp administered rabbits following treatment by LA or/and Spir

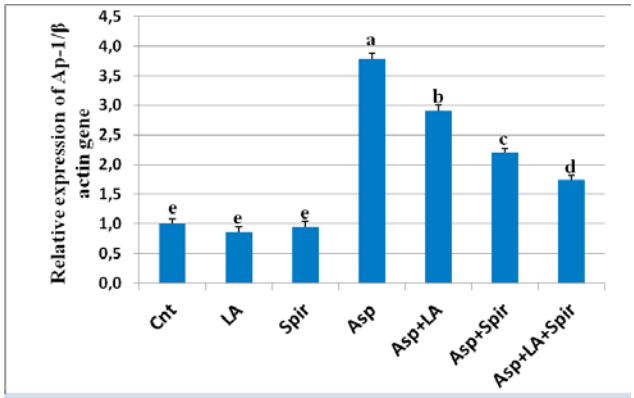

Figure 3. Graphical presentation of real-time quantitative PCR analysis of the expression of AP-1 gene in brain tissue of Asp administered rabbits following treatment by LA or/and Spir

Results of Comet assay analysis

A comet assay was performed to assess DNA damage in brain of Asp administered rabbits after treatment by *Spirulina* and/or LA as compared to the control. The results of comet assay presented in figures (3) and tables (4) shown a significant increase in DNA damage ($P < 0.05$) that was indicated by increase in tail length, tail DNA% and tail moment was observed in rabbits administrated with aspartame as compared to the control and protective groups (group administration of *Spirulina* and lipolic alone). This increased DNA damage was significantly reduced after

Table 4. Comet assay parameters obtained by image analysis in cells of all rabbits groups after treatment experiment

Animal Groups	Tailed %	Untailed %	Tails length µm	Tail DNA%	Tail moment
Group I (Cont.)	2	98	1.44±0.13^{d}	1.51	2.16
Group II (Asp)	12	88	4.58±0.15^{a}	3.67	16.81
Group III (LA)	3	97	1.72±0.15^{d}	1.30	2.23
Group IV (Spir)	1.5	98.5	1.36±0.11^{d}	1.46	1.99
Group V (Asp+LA)	11	89	4.07±0.12^{a}	3.51	15.69
Group VI (Asp+Spir)	8	92	3.26±0.20^{b}	2.39	7.78
Group VII (Asp+LA+Spir)	6.5	93.5	2.35±0.20^{c}	2.69	6.32

Different superscript letters in the same column of tail length showed significance difference at P< 0.05.

administration of *Spirulina* alone (GIII; Spir) or in combination with LA (GII; LA) with lowest damage in combined treated group (GIIIV; Asp+LA+Spir). However, no significant difference was noticed between GIV (Asp+L.A) and GV (Asp**)** or among the three normal control group (GI; control group, GII; LA and GIII; Spir).

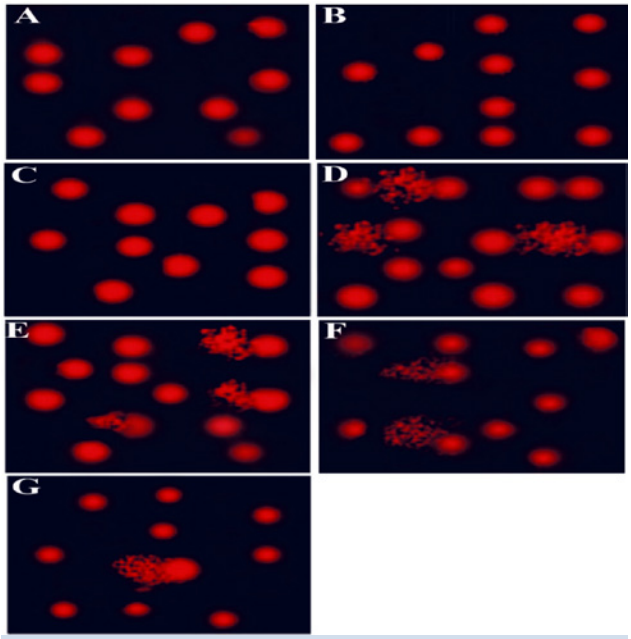

Figure 4. Photomicrographs representation of DNA damage in brain tissues using comet assay in Group I (Cont.) (A), Group II (Asp) (B), Group III (LA) (C), Group IV (Spir) (D), Group V (Asp+LA) (E), Group VI (Asp+Spir) (F) and Group VII (Asp+LA+Spir) (G)

DISCUSSION

Aspartame (E 951) is the most commonly used non-nutritive artificial sweeteners in over 100 countries in more than 6000 products pharmaceutical product and feed, drugs and including soft drinks, fruit juice, baked goods, chewing gum, candy, puddings, canned foods, ice cream, yogurt, table sweeteners and plenty of other foods and beverages (Magnuson et al., 2007).The present study was designed to investigate the possible harmful effects of aspartame induced DNA damage, inflammation, oxidative stress and molecular alterations on brain of rabbits. In the existing study oral aspartame administration to rabbits for 8 weeks resulted in significant increase in L-MDA concentration, decrease CAT activity and GSH concentration when compared with control normal group. These results are nearly similar with the recorded data of Mourad, (2011) who stated that, oral administration of ASP (40 mg/ kg b. wt) led to a significant elevation in LPO level in the liver, kidney and brain tissue and also indicated an increase in MDA level which was accompanied by a decrease in the activities of antioxidant enzymes (SOD and CAT) and GSH concentration in the liver of rat leading to degrade H_2O_2, more H_2O_2 could be converted to toxic hydroxyl radicals that may contribute to oxidative stress due to methanol metabolite from ASP. Moreover, Ashok and Sheela Devi, (2014) shown that a significant decrease in GSH concentration and glutathione reductase activity was reported in the brain of rats treated with aspartame at 100 and 500 mg/kg doses.Glutathione is decreased after repeated aspartame administration suggesting consumption of this important antioxidant defense mechanism by increased free radicals production due to aspartame administration which theoretically can further increase the vulnerability of the brain tissue to other oxidative insults (Tilsonha et al, 1991).Likewise, Feijó et al., (2013) exhibited that, oral administration of rats with aspartame (250 mg/kg b wt.) significantly increased brain tissue L-malondialdehyde concentration, LPO levels and markedly decreased GSH concentration. LPO is a free-radical-mediated process. In the entire rat brain regions after aspartame consumption, a marked increase in LPO was noted, which also supports the generation of free radicals. Generally, when the generation of reactive free radicals overwhelms the antioxidant defense, LPO of the cell membrane occurs. A marked increase in the LPO in the entire brain regions indicated this

result and possible loss of membrane integrity (Sohal et al., 2002).Meanwhile, treatment with *Spirulina* and alpha-lipoic acid to aspartame administered rabbits revealed a significant decrease in serum L-MDA level, decrease of CAT activity and GSH concentration when compared to aspartame group. Concerning to alpha lipoic acid, Li et al., (2013) exhibited that alpha-lipoic acid has been known to have positive effects on a wide variety of clinical conditions, which is completely consistent with its effect in decrease of the oxidative stress. Alpha-Lipoic Acid protects against oxidative stress both in peripheral tissues and central nervous system (Winiarska et al., 2008). Ying et al., (2004) demonstrated that alpha-lipoic acid can eliminate superoxide anions and that this process depends on both the concentration of alpha-lipoic acid and pH. Moreover, lipoic acid is a thiol containing nucleophile, reacts with endogenous electrophiles including free radicals or reactive drug metabolites and heavy metals. On the other hand, Konickova et al., (2014) reported that, *Spirulina* not only had anti-proliferative effects, but also inhibited the production of mitochondrial ROS and affected glutathione redox status. Supplementation with 1 or 5 % *Spirulina* induced GSH concentration; CAT activity and decrease L-MDA level the liver in intoxicated rats.Moreover, Abdel-Daim et al., (2013) described that the treatment with *Spirulina* significantly reduced oxidative stress and L-malondialdehyde in aspartame treated rats. Reactive oxygen species (ROS) attack and damage molecules in biological systems, leading to oxidative stress and various disorders and diseases occur. The antioxidant potential of *Spirulina* species and protective effects are mediated by phycocyanins, β-carotene, and other vitamins and minerals contained within *Spirulina*. Moreover, Phycocyanin appears to inhibit the generation of hydroxyl and peroxyl radicals, as well as lipid peroxidation. The higher lipid peroxidation observed could be due to a lower antioxidant capacity of the cells, and oxidative stress occurs in a cell or tissue when the concentration of reactive oxygen species (ROS) generated exceeds the antioxidant capability of that cell (Bermejo et al., 2008).Also, *Spirulina* is rich in β-carotene and the bioavailability is as good as the pure β-carotene, vitamin E and vitamin C and selenium, and *Spirulina* extracts could be effective against free radical induced lipid peroxidation which in turn may lead to cellular transformation (Pal et al., 2010). Furthermore, Sharoud, (2015) indicated that the protective effect of *Spirulina platensis* against paracetamol induced oxidative stress could be either direct by inhibiting lipid peroxidation and scavenging free radicals or indirect through the enhancement of the activity superoxide dismutase and the enzymatic free radicals scavengers in the cells. These properties could be attributed to the high levels of antioxidants such as c-phycocyanin, carotenoids, vitamins, minerals, lipids, proteins and carbohydrates. Lebda et al., (2017) who showed that aspartame and soft drinks induced upregulation of the relative mRNA expression levels of BAX and Casp3 and down-regulation of Bcl-2 genes in brain tissue of rats, suggesting an activation of cellular apoptosis. At the cellular level, aspartame can generate excessive reactive oxygen species (ROS) with a marked decrease in B-cell lymphoma 2 (Bcl-2), and increase in Bcl-2 like protein 4 (BAX) in rats' brain (Ashok and Sheeladevi 2014).

A significant up-regulation of BAX and AP-1 gene expression level were observed in brain of Asp-treated rabbits as compared to the normal control group. Similarly, Lebda et al., (2017) who showed that aspartame and soft drinks induced upregulation of the relative mRNA expression levels of BAX and Casp3 and down-regulation of Bcl-2 genes in brain tissue of rats suggesting an activation of cellular apoptosis. Similar results were reported earlier by El Haliem and Mohamed 2011 and Ardalan et al., 2017 who revealed that Aspartame administration at a dose of 250 mg/kg b.wt and 75 mg/kg b.wt, respectively, in rats caused significant up-regulation of the relative expression of TGF-β1, NF-κβ genes with significant down regulationin (IL-10) gene levels. ROS can trigger signal transduction pathways, primarily through nuclear factor-κB (NF-κB), promoting the production of TNF-α and increasing the production of anti- inflammatory cytokines, IL-10 (Park et al., 2008).Also Humphries and Pretorius (2008) who reported that administration of aspartame at a dose 50 mg/kg body weight for long time cause carcinogenic effect, also lead to up-regulation of AP-1 signaling and induced NF-κB signaling. Dowlati et al., (2010) reported that long term of oral administration of aspartame 75mg/kg b.wt significantly up regulated the expression of caspase-3activity and TNF-α in brain tissue of aspartame treated rat. Also, Soffritti et al., (2006) reported that the sweetener (aspartame) could increase brain TNF-α potent pro-inflammatory cytokine that is produced by glial cells involved in various physio-pathological conditions in the CNS of rats. Moreover, Mbazima et al., (2008) reported that, free radicals play a role in the initiation of apoptotic processes. The changes recognized in the expressions of genes decreasing in

Bcl-2expression and increasing of Bax and caspase-3 expression occurred. In neurons simultaneously, they give rise to apoptosis (Thomas et al., 2000). Furthermore, increase in the expression of the pro-apoptotic Bax gene lead to mitochondrial release of cytochrome c, which also triggers apoptosis. Caspases are closely associated with apoptosis. The caspase-cascade system played vital role in the induction, transduction and amplification of intracellular apoptotic signals. A depletion of intracellular GSH has been reported to occur with the onset of apoptosis) Mbazima, etal., 2008).

Additionally, both aspartate and glutamate act as neurotransmitters in the brain, carrying information from neuron to neuron. When there is an excess of neurotransmitter, certain neurons are killed by allowing too much calcium into the cells. This influx causes excessive numbers of free radicals to build up which kill the cells. The neural cell damage that is caused by excessive aspartate and glutamate is the reason they are referred to as excitotoxins. The excitotoxins are substances, usually acidic amino acids that react withspecialized receptors in the brain in such a way as to lead to destruction of certain types of neurons (Blaylock, 2002; Ho et al., 2003).In Asp administered rabbits and treated with LA and/ or Spir exhibited a significant down-regulation of caspase 3 and TNF-α gene expression as compared to Asp group. Vitamin C and E are the best antioxidants vitamins, both of which have been shown to be slightly effective in different models of neurodegeneration (Davoli et al., 1986). *Spirulina* strongly induces antioxidant enzyme activity, helps to prevent lipid peroxidation and DNA damage, and scavenges free radicals (Abdelkhalek et al., 2015).Similarly, Sun et al., (2011) reported that the expression of TNF-α, NF-κβ and IL-1b gene were significantly down-regulated following treatment by *Spirulina*. Also, *Spirulina* protected against neurotoxicity, hepatonephrotoxicity and colitis in animals by reducing oxidative stress (Abdel-Daim et al., 2015). Moreover, Juarez-Oropeza et al., (2009) reported that, *Spirulina* had both antioxidant and anti-inflammatory activities and down regulated the pro-inflammatory cytokines, which in turn might inhibit the neurodegeneration and oxidative stress thereby aids in maintaining proper brain and body health. Additionally, Ranney et al., (1976) recorded that, alpha-lipoic acid and *Spirulina* extract also extensively inhibit the inflammatory cascade by effectual modulation of inflammatory cytokines (IL-10 and IL-1), thus decrease the further exacerbation of aspartame brain injury mediated by inflammatory cytokines.

In the existing study a significant increase in DNA damage that was indicated by increase in tail length, tail DNA% and tail moment was observed in rabbits administrated with aspartame as compared to the control group. This increased DNA damage was significantly reduced after administration of *Spirulina* alone (GIII; Spir) or in combination with LA (GII; LA) with lowest damage in combined treated group (GVIIAsp+LA+Spir). Similarly, Findikli and Turkoglu (2014) showed that administration of aspartame 250 and 125mg/kg b.wt was indicated by an increasing of the tail length and tail DNA%. Consistent ingestion of food additives has been reported to induce toxic, genotoxic, and carcinogenic effects (Saad et al., 2014). The DNA damage induced by food additives depends on their transport across cellular/nuclear membranes, the activation and deactivation of intracellular enzymatic processes, the levels of radical scavengers, and the repair mechanisms in the target cell population. The comet assay has been used to determine the effects of these cellular processes on the amount of DNA damage induced. This assay is a powerful tool for determining genotoxicity, because it is simple and highly sensitive, has a short response time, and requires a relatively small number of cells and test substances (Čabarkapa et al., (2014).Aspartame caused DNA damage. Commonly used food sweeteners may be toxic at high concentrations in the long term. Lin et al., (2007) reported that DNA is a major drug target and can be damaged by harmful chemicals. The DNA damage caused by sweeteners may be associated with the generation of free radicals (reactive oxygen species), which cause DNA strand breaks and irreversible damage to proteins involved in DNA replication, repair, recombination, and transcription. Moreover, several mutagenic and genotoxic lipid peroxidation products, in particular malondialdehyde and 4-hydroxy-2-nonenal, have been shown to bind to DNA and to damage it (Eder et al., 2006). In the current study treatment with *Spirulina* platensis or and lipoic acid significantly reduced DNA damage that was indicated by comet assay in aspartame administered rabbits.These results are nearly similar to those recorded by Shirpoor et al., (2008) who reported that, alpha- lipoic acid partially alleviated the ethanol-induced DNA damage in developing hippocampus and cerebellum of rats. Hassan et al., (2012) stated that, supplementation with *Spirulina* succeed to inhibit DNA damage as indicated by the down-regulation of Fas (Fatty acid synthase) gene expression,

and decreased the percentage of DNA fragmentation and micro nucleated erythrocytes in aflatoxin intoxicated rats. Also, Saber et al., (2015) recorded that, co-treated rats with aluminum chloride and *Spirulina platensis* showed a significant decrease in all parameters of DNA damage in kidney (tail percentage, tail length, DNA tail percentage, and tail moment) when compared to the aluminium chloride -treated group. The existing results were confirmed by Ismail et al., (2009) who indicated that, polysaccharides of *Spirulina* enhanced cell nucleus enzyme activity, the process of DNA repair and the unscheduled DNA synthesis (Kajiet al., 2002).The anti-genotoxic effect of *Spirulina* may be related to its contents of phycocyanin and phycocyanobilin which also have strong anti-cyclooxygenase-2, antioxidant activity to scavenger peroxidinitrite and reduce peroxynitrite (OONO-) induced oxidative damage to DNA (Bhat and Madyastha, 2001).

CONCLUSION

In conclusion, the oral aspartame administration for 8 weeks resulted in significant increase in L-MDA concentration, decrease CAT activity and GSH concentration when compared with control normal group. The results of the present work revealed that *Spirulina* and alpha-lipoic acid alleviates the harmful effects of aspartame on brain tissue of rabbits. Also, *Spirulina* and alpha-lipoic acid has a protective antioxidant role in restoration of oxidative stress, a strong anti-inflammatory and anti-apoptotic effects on aspartame-induced brain damage. In this regard, the current study has brought a convincing indication preferring the usage of natural antioxidants like spirulina and alpha lipoic acid as a protective strategy against toxicity induced by aspartame.

CONFLICT OF INTEREST

None declared by the authors.

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