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Imbalance of the oxidant - antioxidant status by aspartame in the organs of immune system of Wistar albino rats

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Aspartame (L- aspartyl- L-phenylalanine methyl ester) is one of the most widely artificial sweeteners consumed in so many products worldwide in various countries which added to a large variety of food, most commonly found in low calorie beverages. On metabolism in humans and experimental animals, aspartame is rapidly and completely metabolized to aspartic acid (40%), phenylalanine (50%) and methanol (10%). Methanol, a toxic metabolite is primarily metabolized by oxidation to formaldehyde and then to formate; these processes are accompanied by the formation of superoxide anion and hydrogen peroxide. This study focus is to understand whether the oral administration of aspartame (40 mg/kg b.w) for 15, 30 and 90 days have any effect on the antioxidant status (enzymatic and non-enzymatic) in immune organs such as the spleen, thymus, lymph nodes and bone marrow of rats. To mimic human methanol metabolism, folate deficient rats were used. After 15 days of aspartame administration, animals showed a significant increase in free radical production as indicated by the increase in both enzymatic (superoxide dismutase, catalase, glutathione peroxidase) and non-enzymatic (reduced glutathione and vitamin C) antioxidant level along with the marked increase in lipid peroxidation and nitric oxide level. However, after repeated long term administration (30 and 90 days), the generation of reactive free radicals overwhelmed the antioxidant defense as indicated by an increase in lipid peroxidation with the decrease in antioxidants level. This study concludes that administration of aspartame even at the Food and Drug Administration permitted level its repeated exposure causes oxidative stress by altering the oxidant/antioxidant balance in immune organs of the rats and its effects also reflected in the histology of the spleen and lymph nodes

Key words: Aspartame, folate- deficient, immune organs, oxidative stress.

INTRODUCTION

Aspartame also marketed as nutra sweet, candrelel or equal was serendipitously discovered in 1965 by James Schlatter, a chemist working for G.D. Searle & company

(Garriga and Metcalfe, 1988). Aspartame (L- aspartyl- L-phenylalanine methyl ester) is one of the most widely artificial sweeteners consumed in so many products

worldwide in various countries (Magnuson et al., 2007) which added to a large variety of food most commonly found in low calorie beverages, desserts and table top sweeteners added to tea or coffee (Butchko and Stargel, 2001). After oral administration to humans and experimental animals, aspartame is rapidly and completely metabolized to 40% aspartic acid, 50% phenylalanine and 10% methanol (Karim and Burns, 1996; Steginik and Filer, 1996). It forms methanol when the methyl group of aspartame encounters the enzyme chymotrypsin in the small intestine (Steginik et al., 1981). A relatively small amount of aspartame can significantly increase plasma methanol levels (Davoli, 1986). In an update on the safety in use of aspartame, the European Union Scientific Committee on Food maintained the established acceptable daily intake (ADI) of aspartame in humans at 40 mg/kg of body weight (European Food Safety Authority (EFSA), 2006).

According to Garriga (1988), consumption of 40 mg aspartame (ASP)/kg body weight would result in ingestion of 4 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. Based on these acceptable dosages the aspartame dosage was fixed at 40 mg/kg. Oxidative stress is defined as an imbalance between higher cellular levels of reactive oxygen species (like superoxide, hydrogen peroxide, hydroxyl radicals, singlet oxygen, nitric oxide, peroxynitrite) and the cellular antioxidant defense (Ilhan et al., 2005). Methanol is being increasingly recognized as a substance that damages the liver cells where it is oxidized to formaldehyde and later to formate (Oppermann et al., 1984). These processes are accompanied by elevation of nicotinamide adenine dinucleotide (NADH) level and the formation of superoxide anion, which may be involved in lipid peroxidation (Parthasarathy et al., 2006). However, accumulating evidence has implied that the production of free radicals plays a critical role in oxidative stress (Liu and Mori, 1999).

Cells of the immune system are particularly susceptible to changes in the antioxidant status because they carry out essential functions through the generation of a high number of oxygen free radicals (Pieri et al., 1993). This antioxidant-oxidant balance is an important determinant of immune cell function, including maintenance of the integrity and functionality of membrane lipids, cellular proteins, and nucleic acids and control of signal transduction of gene expression in immune. The secondary lymphoid organ (spleen and lymph node) is considered the draining site of toxic substance and is therefore considered an important organ to evaluate for any immune toxic and immune modulatory compounds treatment.

Due to the presence of B and T lymphocytes, the immune toxic effects of xenobiotic or their metabolites on these cell populations may be reflected in the spleen and lymph nodes and the routine histology of lymphoid organs such as spleen and lymph nodes could reveal the modifications occurring at cellular level. Hence the focus of the study is to investigate lipid peroxidation and antioxidant status and histology of the immune organ of wistar albino male rats on exposure of aspartame (40 mg/kg bw).

MATERIALS AND METHODS

Chemicals

Pure aspartame powder and methotrexate was purchased from sigma Aldrich chemical, st.louis, USA and all other chemical used were of analytical grade obtained from sisco research laboratory, Mumbai, India.

Animal model

Animal experiments were carried out only after getting clearance from the Institutional Animal Ethical Committee (IAEC No: 02/03/11) and the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The experimental animals were healthy; inbreed adult male Wistar albino rats, weighing approximately 200 to 220 g. The animals were maintained under standard laboratory conditions and were allowed to have food and water *ad libitum* (standard rat feed pellets supplied by M/s. Hindustan Lever Ltd., India) for control animals and for folate deficient, and aspartame treated rat had given special folate deficient diet (Andrew and Rosemaryl, 1988) for 37 days. All the rats were housed under condition of controlled temperature (26 ± 2°C) with 12 h light and 12 h dark exposure.

Experimental design

Group-I was saline control animals, in order to make animals folate deficient the folate deficient diet was given to them on a special dietary regime for 37 days and after that methotrexate (MTX) in sterile saline were administered by intra peritoneal every other day for two weeks (Ming et al., 1989) before euthanasia. After that, MTX folate deficiency was confirmed by estimating the urinary excretion of formaminoglutamic acid (FIGLU) (Rabinowitz and pricer, 1956) prior to the experiment and in these confirmed animals the rest of the study were conducted, and these folate deficient animals were further divided into 4 groups consisting of 6 animals each. Group-II is folate deficient control, Group-III is folate deficient rats treated with aspartame for 15 days, Group-IV is folate deficient rats treated with aspartame for 30 days and Group-V is folate deficient rats treated with aspartame for 90 days. Animals of control and folate deficient animals (Groups I and II) received daily normal saline orally (by means of lavarge needle) throughout the experimental protocol, prior to the experiment. Animals of aspartame treated group were daily administered aspartame (40 mg/kg) (EFSA, 2006) dissolved in normal saline orally (by means of lavarge needle) for 15, 30 and 90 days.

Sample collection

At the end of the experimental period (15, 30 and 90 days) all the animals were sacrificed under deep anesthesia using pentothal sodium (40 mg/kg). Blood samples and isolation of immune organs were performed between 8 and 10 a.m. to avoid circadian rhythm induced changes. Stress-free blood samples were collected as per the technique described earlier (Feldman and Conforti, 1980).

Table 1. Effect of aspartame on plasma corticosterone level (µg of corticosterone/dl of plasma).

Control	FD	15 days	30 days	90 days
40.82±1.72	42.89±2.31	48.71±1.77[*a,*b]	54.99±2.29[*a,*b]	92.96±1.9[*a,*b,*c,*d]

Each value represents mean ± SD. Significance at $*p < 0.05$, *a - compared with control, *b - compared with FD group, *c- compared with 15D group, *d compared with 30D group. FD – Folate deficient, D – Number of days.

Table 2. Effect of aspartame on lipid peroxidation level [MDA (n moles/mg protein)].

Organs	Control	FD	15 days	30 days	90 days
Spleen	2.55±0.37	2.82±0.23	5.91±0.58[*a,*b]	8.84±0.49[*a,*b,*c]	12.16±1.00[*a,*b,*c,*d,]
Thymus	3.58±0.36	3.85±0.22	7.93±0.26[*a,*b]	10.87±0.50[*a,*b,*c]	14.94±0.53[*a,*b,*c,*d]
Lymphnode	2.93±0.333	3.09±0.21	6.70±0.45[*a,*b]	8.78±0.53[*a, *b,*c]	12.86±0.45[*a,*b,*c,*d]
Bonemarrow	3.71±0.42	3.89±0.37	5.68±0.52[*a,*b]	7.82±0.59[*a, *b,*c]	13.94±0.73[*a,*b,*c,*d]

Each value represents mean ± SD. Significance at $*p < 0.05$, *a - compared with control, *b - compared with FD group,   *c- compared with 15 D group, *d compared with 30D group. FD – Folate deficient, D – Number of days.

Handling of sample

After administrating long acting anesthesia (penrtathol sodium), the animal was then perfused with ice-cold phosphate buffered saline (PBS), the immune organs (spleen, thymus, lymph nodes and bone marrow) were removed immediately, washed with the PBS solution to remove blood cells, blotted on filter paper, quickly weighed and homogenized by using Teflon glass homogenizers in (1/10 weight per volume) ice cold phosphate buffer (0.1 M, pH 7.0) and centrifuged. The supernatant was used for estimation of lipid peroxidation and various enzymatic and non-enzymatic antioxidants. A separate set of animals were used for the histological study.

Biochemical determinations

Estimation of plasma cortisol was determined by the procedure of Clark (1955). Lipid peroxidation (LPO) was determined by the method of Ohkawa et al. (1979). Protein estimations were carried out according to the method of Lowry et al. (1951). Nitric oxide (NO) levels were measured as total nitrite + nitrate levels with the use of the Griess reagent as previously described by Bradford (1976).

Determination of the activities of enzymatic antioxidants

Superoxide dismutase ((EC 1.15.1.1) (SOD) was assayed according to the method of Marklund and Marklund (1974). The activity of Catalase (EC.1.11.1.6) (CAT) was assayed by the method of Sinha (1972). Glutathione peroxidase (EC.1.11.1.9) (GPx) activity was estimated by the method of Rotruck et al. (1973).

Estimation of non-enzymatic antioxidants

Reduced glutathione (GSH) in the immune organs were estimated by the method of Moron et al. (1979). Ascorbic acid was assayed by the method of Omaye et al. (1979).

Histology

For light microscopic study, spleen and lymph nodes were

processed for routine paraffin sectioning and stained with Haematoxylin and Eosin (Bancroft and Gamble, 2002).

Statistical analysis

All data were analyzed with the statistical package for social sciences (SPSS) statistical package for Windows (version 20.0, SPSS Institute Inc., Cary, North Carolina). Data are expressed as mean ± standard deviation (SD) and was analyzed by one way-analysis of variance (ANOVA). The significance was fixed at $p < 0.05$. If the data showed a significant difference, it was followed by Tukey's multiple comparison tests.

RESULT

Effect of aspartame on plasma corticosterone level

The data are presented in Table 1 as mean ± SD. The corticosterone level was similar in folate deficient group when compare to control group. The rat treated with aspartame showed a marked increase in corticosterone level irrespective of the duration of exposure (15, 30 as well as 90 days) when compared to the control as well as folate deficient groups. There was marked increases in the corticosterone level of 30 and 90 days aspartame treated animals when compare to control, folate deficient animals as well as 15 days treated animals, moreover this increase was more marked in 90 days aspartame exposed rats than the 30 days exposed rats indicating that aspartame may act as a chemical stressor.

Effect of aspartame on LPO Level

The result of lipid peroxidation in the spleen, thymus, popliteal lymph nodes and bone marrow are summarized in Table 2 as mean ± SD. The LPO level of folate

Table 3. Effect of aspartame on Nitric oxide (µmoles /L).

Organs	Control	FD	15 days	30 days	90 days
Spleen	8.68±0.43	9.02±0.35	10.64±0.37*a,*b	12.08±0.54*a,*b,*c	16.84±0.41*a,*b,*c,*d
Thymus	4.93±0.41	5.09±0.64	6.41±0.49* a, *b	8.07±0.58*a, *b,*c	12.76±0.66*a,*b,*c,*d
Lymphnode	7.08±0.35	7.12±0.53	8.70±0.37* a, *b	9.80±0.34*a, *b,*c	13.90±0.60*a,*b,*c,*d
Bonemarrow	9.22±0.46	9.65±0.53	11.69±0.50*a,*b	13.67±0.39*a,*b,*c	17.75±0.84*a,*b,*c*d,

Each value represents mean ± SD. Significance at *$p < 0.05$, *a - compared with control, *b - compared with FD group, *c- compared with 15D group, *d compared with 30D group. FD – Folate deficient, D – Number of days.

Table 4. Effect of aspartame on (organ weight/animal weight ratio)

Organs	Control	FD	15 days	30 days	90 days
Spleen	3.82±0.23	3.79±0.22	3.73±0.19*a,*b	3.19±0.38*a,*b,*c	1.69±0.10*a,*b,*c,*d
Thymus	1.53±0.07	1.52±0.08	1.50±0.06* a, *b	1.06±0.19*a,*b,*c	0.46±0.06*a,*b,*c,*d
Lymphnode	0.10±0.02	0.09±0.01	0.08±0.04*a,*b	0.06±0.007*a,*b,*c	0.02±.009*a,*b,*c,*d

Each value represents mean ± SD. Significance at *$p < 0.05$, *a - compared with control, *b - compared with FD group, *c- compared with 15D group, *d compared with 30D group. CONT – Control, F.D – Folate deficient, D – Number of days.

deficient animals was similar to the control animals. In animals treated with aspartame, the LPO levels were significantly elevated irrespective of the duration of exposure (15, 30 as well as 90 days). This increase was more marked in 30 days as well as 90 days aspartame exposed rats when compare to control, folate deficient as well as 15 days treated animals. Moreover, this increase was more marked in 90 days aspartame exposed rats than the 30 days exposed rats clearly indicating the generation of free radicals by aspartame.

Effect of aspartame on nitric oxide level

The results of nitric oxide level in spleen, thymus, popliteal lymph nodes and bone marrow are summarized in Table 3 as mean ± SD. The nitric oxide level was similar in control animals as well as in the folate deficient animals. In rat treated with aspartame, the nitric oxide level was significantly increased irrespective of duration of exposure (15, 30, as well as 90 days) from control as well as folate deficient animals. Moreover the nitric oxide level was found to be more elevated in the 30 days, as well as 90 days aspartame treated animals when compare to control, folate deficient, as well as 15 days treated animals. Moreover this increase was more marked in 90 days aspartame exposed rats than the 30 days exposed rats clearly indicating the generation of free radicals by aspartame.

Effect of aspartame on enzymatic and non-enzymatic antioxidant level

The result of enzymatic and non-enzymatic antioxidant

level in spleen, thymus, popliteal lymph nodes and bone marrow are summarized in Figures 1 to 5, as bar diagram with mean ± SD. All enzymatic (SOD, CAT and GPx) and non-enzymatic (GSH and Vit C) antioxidants level did not get altered in the folate deficient animal when compare to control animal. Though the rat treated with aspartame for 15 days showed a significant increase in all enzymatic (SOD, CAT and GPx) and non-enzymatic (GSH and Vit C) antioxidants level, when compare with control as well as folate deficient animals. However, all the enzymatic and non-enzymatic antioxidant levels were significantly decreased in 30 and 90 days aspartame treated animal when compared to the control, folate deficient, as well as 15 days aspartame treated animals. Furthermore, this enzymatic and non-enzymatic level decrease showed more marked decrease in 90 days aspartame treated animals when compared to the 30 days aspartame treated animals.

Effect of aspartame on organ-weight to animal-weight ratio

The results of organ weight ratio are summarized in Table 4 as mean ± SD. The organ weight ratio of folate deficient animal did not deviate from the control animals. Though the organ weight ratio of the 15 days aspartame treated animals remained similar to controls as well as folate deficient animals. The 30 and 90 days aspartame treated animals showed a marked decrease from control, folate deficient animals and also 15 days aspartame treated animals. This decrease was more marked in 90 days aspartame treated animals when compared to 30 days treated animals.
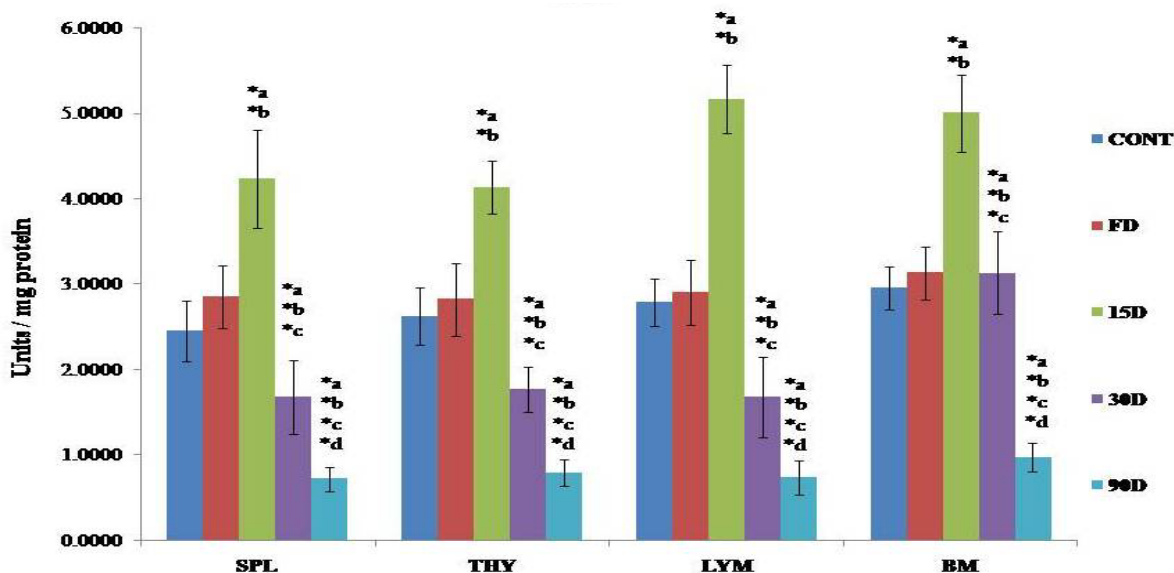
Figure 1. Effect of aspartame on SOD level in spleen, thymus , lymph node, and bone marrow of Wistar albino rats . Each value represents mean ± SD. Significance at   *$p < 0.05$, *a - compared with control, *b - compared with FD group,   *c- compared with 15D group, *d compared with 30D group.
CONT – Control, F.D – Folate deficient, D – Number of days. SPL- Spleen, THY – Thymus, LYM – Lymphnode, BM – Bone Marrow.
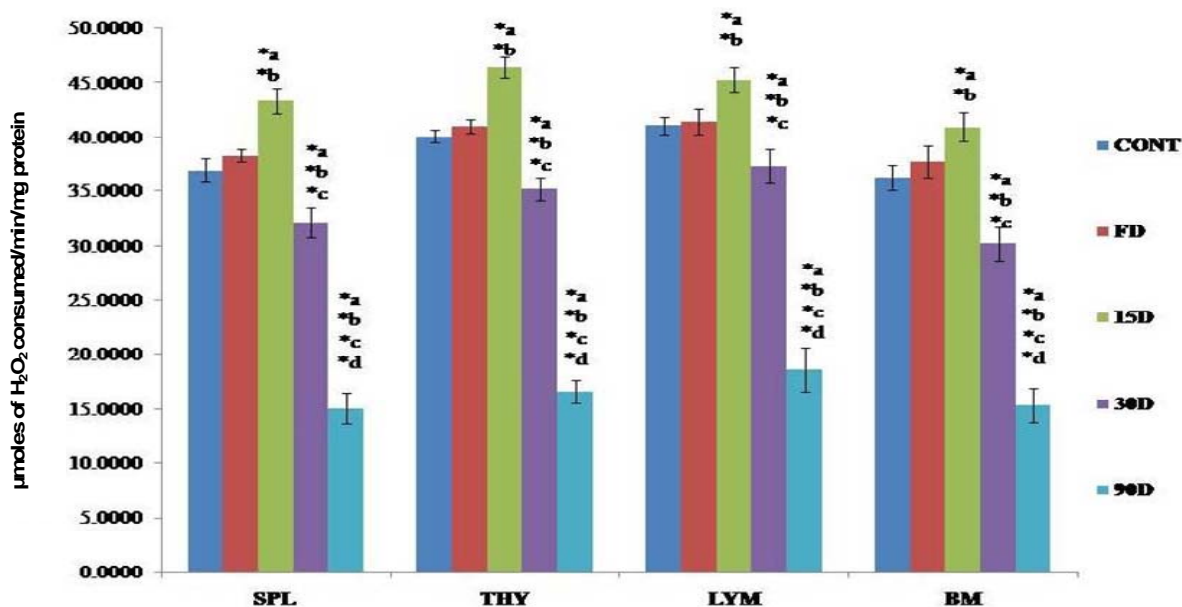

Figure 2. Effect of aspartame on Catalase level in spleen, thymus, lymphnode, and bone marrow of Wistar albino rats. Each value represents mean ± SD. Significance at   *$p < 0.05$, *a - compared with control, *b - compared with FD group,   *c- compared with 15D group, *d compared with 30D group.
CONT – Control, F.D – Folate deficient, D – Number of days. SPL- Spleen, THY – Thymus, LYM – Lymph node, BM – Bone Marrow.

Effect of aspartame on histology of Spleen and Lymph node

The histology of spleen and lymph nodes are given in Figures 6a, b, c and 7a, b, c. There was no variation in the histology of spleen and lymph node of folate deficient animal observed when compared to control animals. In90 days aspartame treated animal's spleen, the boundary between white and red pulp started to disappear. There were cellular disruption and degeneration of the white
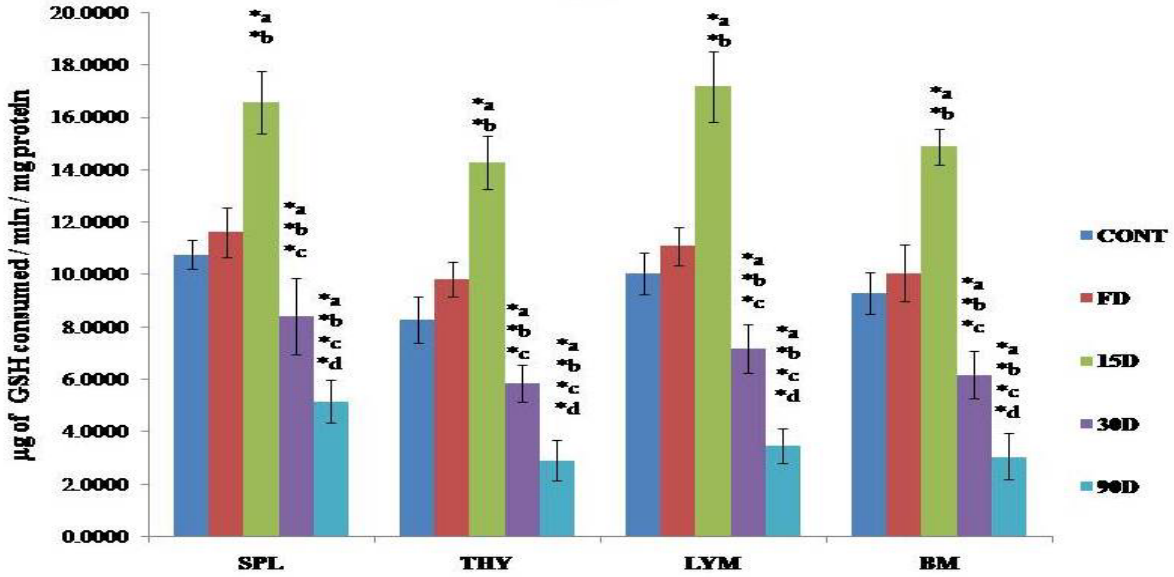
Figure 3. Effect of aspartame on GPX level in spleen, thymus, lymph node, and bone marrow of wistar albino rats. Each value represents mean ± SD. Significance at *p < 0.05*, *a - compared with control, *b - compared with FD group, *c- compared with 15D group, *d compared with 30D group.
CONT – Control, F.D – Folate deficient, D – Number of days. SPL- Spleen, THY – Thymus, LYM – Lymph node, BM – Bone Marrow.
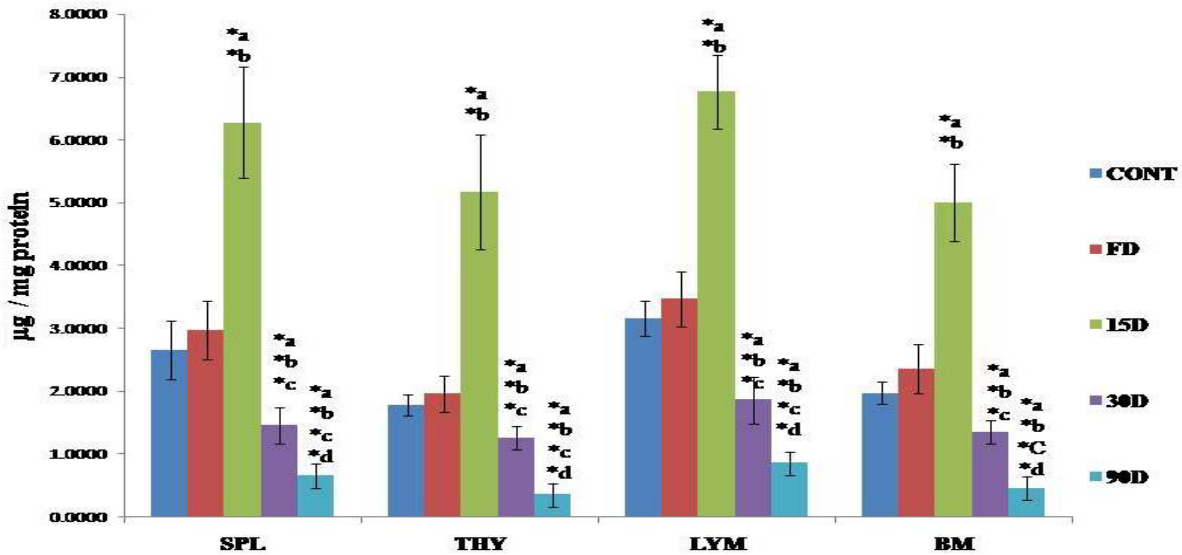

Figure 4. Effect of aspartame on GSH level in spleen, thymus, lymph node, and bone marrow of wistar albino rats. Each value represents mean ± SD. Significance at *p < 0.05*, *a - compared with control, *b - compared with FD group, *c- compared with 15D group, *d compared with 30D group.
CONT – Control, F.D – Folate deficient, D – Number of days. SPL- Spleen, THY – Thymus, LYM – Lymphnode , BM – Bone Marrow .

pulp and there was little absence of germinal centers. This was also associated with a slight increase in the thickness of the capsule and trabeculae. Moreover, there was also infiltration of neutrophils and lymphocytes observed with a significant number of giant cells increase in the parenchyma of spleen. In 90 days aspartame treated animal's lymphnode, there was little absence of germinate centers in cortex. There was infiltration of neutrophils and lymphocytes, and a proliferation of macrophages with vacuolated cytoplasm within the medullary region was observed in the 90 days aspartame treated animals.
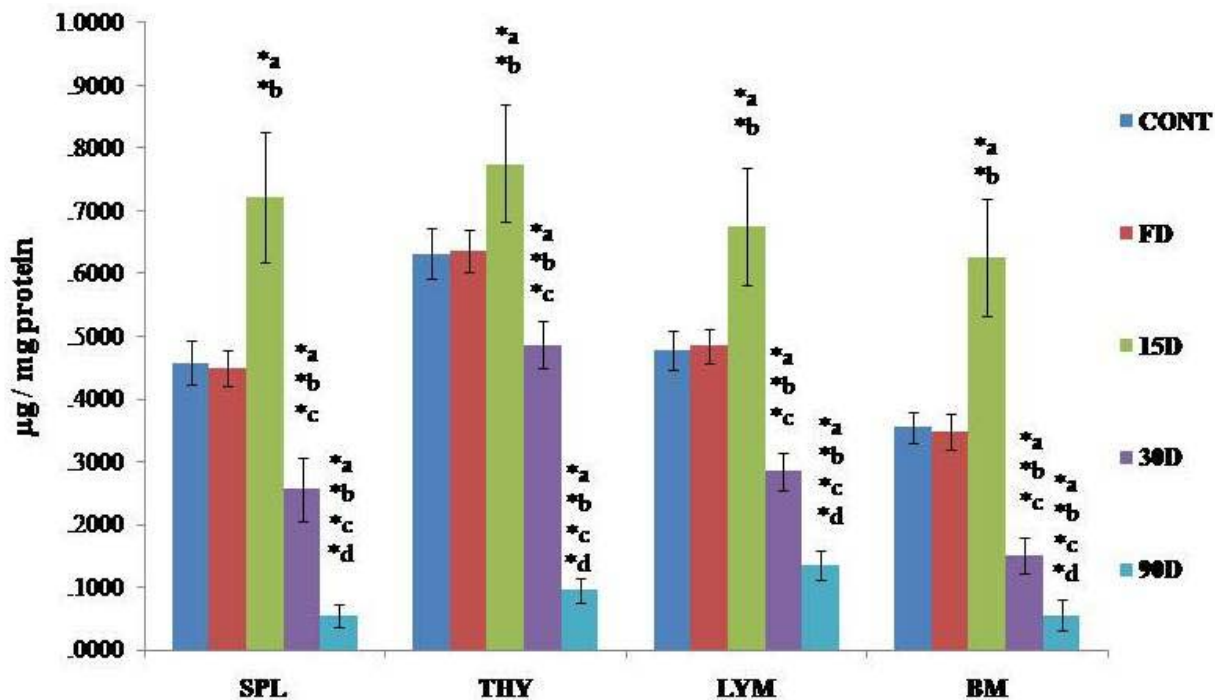
Figure 5. Effect of aspartame on Vitamin- C   level in spleen, thymus, lymph node, and bone marrow of wistar albino rats. Each value represents mean ± SD. Significance at   *$p < 0.05$, *a - compared with control, *b - compared with FD group, *c- compared with 15D group, *d compared with 30D group.
CONT – Control, F.D – Folate deficient, D – Number of days. SPL- Spleen, THY – Thymus, LYM – Lymph node, BM – Bone Marrow.
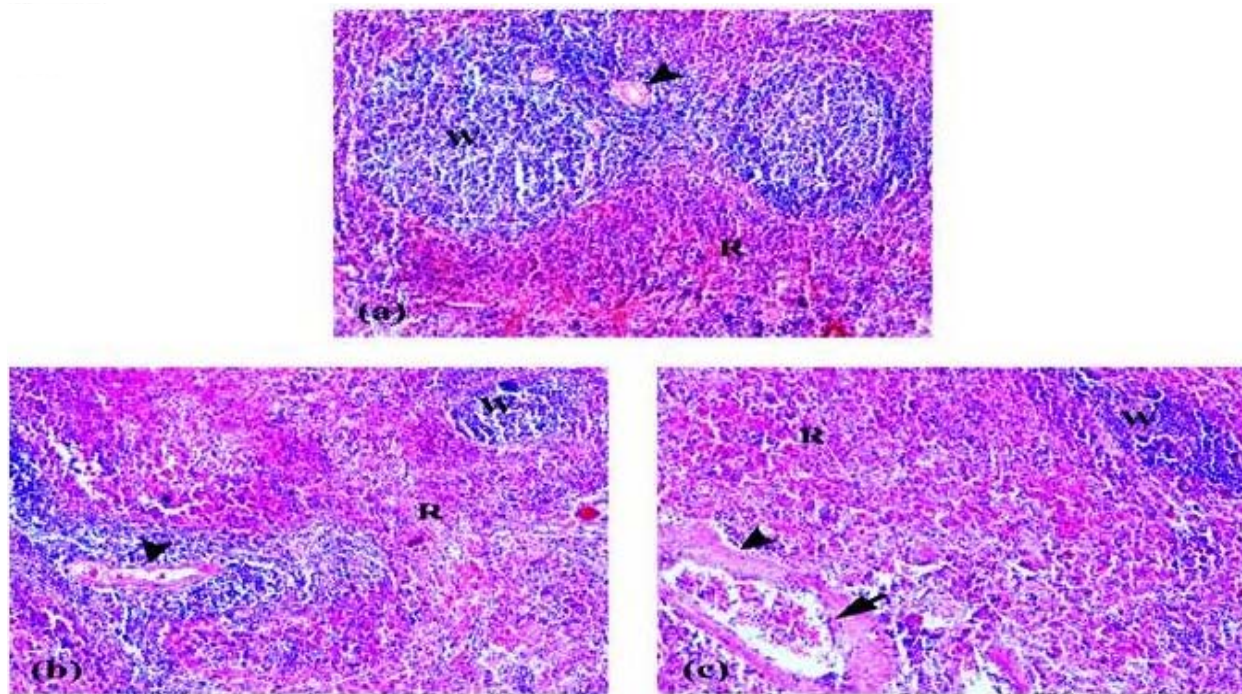

Figure 6. Photomicrograph showing histological changes in spleen of (a) Control, (b) Folate deficient and (c) Aspartame treated (90D) groups, stained with H&E depicting the central arteriole (arrow heads), white pulp (W), red pulp (R). Arrow indicating leukocytes infiltration in the arteriole of Aspartame treated group. Magnification: ×10.
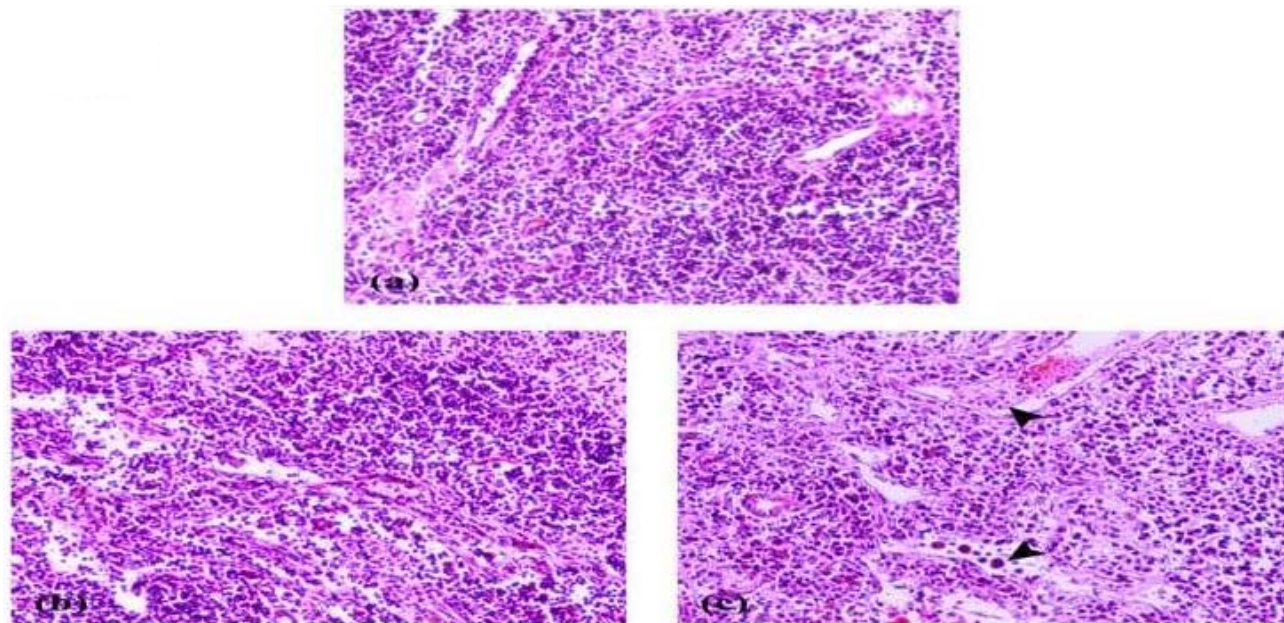
Figure 7. Photomicrograph showing histological changes in lymph nodes of (a)Control, (b) Folate deficient and (c) Aspartame treated (90D) groups, stained with H&E. Arrow heads indicating leucocytes infiltration. Magnification: ×10.

DISCUSSION

In this study, the folate deficient animals were used to mimic the human methanol metabolism. However the folate deficient animals did not show any significant changes in the parameters studied and remained similar to controls. The present study clearly confirms that aspartame can act as chemical stressor as indicated by the elevated corticosteroid level in the entire aspartame group studied, irrespective of duration of exposure. However it is not clear at what level the aspartame/its products are interfering with the HPA axis. According to Britton et al. (1992), it may be due to methanol, a metabolite of aspartame which stimulates norepinephrine to act on corticotrophin releasing factor (CRF) neurons in the paraventicular nucleus of hypothalamus to directly stimulate CRF release to act on pituitary to release adrenocorticotropic hormone (ACTH). Then ACTH acts on adrenal gland to release corticosterone secretion. Parthasarathy et al. (2006) reported a similar increase in plasma corticosterone level in rats after methanol administration for day 1 and 15 days. However after 30 days of methanol intoxication it showed considerable decrease in corticosterone level. This difference may be due to the higher dosage of methanol they have used.

The modified enzymatic and non-enzymatic free radical scavenging system with an elevated LPO level after aspartame administration clearly indicated the generation of free radicals in present study. The status of lipid per-oxidation as well as altered levels of certain endogenous radical scavengers is taken as direct evidence for oxide-tive stress (Khan, 2006). This alteration after aspartame

administration may be attributed to its metabolite methanol. Methanol is primarily metabolized by oxidation to formaldehyde and then to formate, these processes are accompanied by the formation of superoxide anion and hydrogen peroxide (Parthasarathy et al., 2006). Oxidative damage in a cell or tissue occurs when the concentration of reactive oxygen species (O_2^-, H_2O_2, and OH^-) generated exceeds the antioxidant capability of the cell (Sies, 1991). Lipid peroxidation is one of the main manifestations of oxidative damage and has been found to play an important role in the toxicity of many xenobiotic (Hernanz et al., 1990).

LPO is initiated by the abstraction of a hydrogen atom from the side chain of polyunsaturated fatty acids in the membrane (Bergendi et al., 1999). LPO is an auto catalytic mechanism leading to oxidative destruction of cellular membranes (Cheese-man, 1993). LPO in cellular membranes damages polyunsaturated fatty acids tending to reduce membrane fluidity, which is essential for proper functioning of the cell. Hence, the elevated level of LPO in the immune organs after aspartame administration in this study could not be ignored as it could affect the organ functions. This alteration could have been due to the methanol released during aspartame metabolism and the formaldehyde formed during methanol metabolism. This is well supported by the report of Parthasarathy et al. (2006) who observed an increase LPO level after methanol administration in the lymphoid organs. Similarly, Zararsiz et al. (2007) recorded a significant increase in LPO level in the kidney of rats after treatment with formaldehyde.

Aspartame administration to rats induces excess free

radical generation obviously, which is also again substantiated by the elevated nitric oxide level in this study. Nitric oxide is thought to react with superoxide anion to gain a radical property, which is a potent source of oxidative injury (Jaeschke, 2003). NO in excess can cause organ damage either directly or by reacting with superoxide anion to yield per- oxy- nitrate (Blough and Zafiriou, 1995). Elevated NO during stress reported to trigger the lipid peroxidation reactions probably due to its conversion to per-oxy-nitrite radical (Matsumoto et al., 1999). One of the reasons for enhancement of free radicals during stress may be due to the elevation of nitric oxide (NO) production (Matsumoto et al., 1999).

SOD constitutes an important link in the biological defense mechanism through dismutation of endogenous cytotoxic superoxide radicals to H_2O_2 and O^{2-}, which are deleterious to polyunsaturated fatty acids and proteins (Fridovich, 1975). Catalase further detoxifies H_2O_2 into H_2O and O_2 (Murray et al., 2003). Glutathione peroxidase also functions in detoxifying H_2O_2 similar to catalase. Thus, SOD, catalase and glutathione peroxidase act mutually and constitute the enzymatic anti oxidative defense mechanism against reactive oxygen species (Bhattacharjee and Sil, 2006).

In this study, there was a marked increase of SOD, catalase and GpX enzyme activity after aspartame 15 days administration. The free radical slowly increases due to methanol metabolite of aspartame. To remove the free radical there is increase in both enzymatic and non-enzymatic level initially in order to prevent oxidative cell damage (Vidyasagaret et al., 2004) and justifying the findings of this study. However, repeated administration for 30 and 90 days could markedly inhibit these enzyme activities, and methanol may be the cause behind this. This is in agreement with earlier report that methanol administration could decrease the enzymatic antioxidant (SOD, CAT and GPx) in the lymphoid organs (Parthasarathy et al., 2006).

Zararsiz et al. (2007) demonstrated that in renal tissue of formaldehyde treated rats and Mourad (2011), in liver tissue of aspartame treated rats, SOD was significantly decreased when compared with the control animals. The decline in the activities of these enzymes might be due to their inactivation caused by excess ROS production (Pigeolet et al., 1990). Normally, the antioxidant enzymes catalase and GPx protect SOD against inactivation by H_2O_2. Reciprocally, SOD protects catalase and GPx against superoxide anion. However, over load of free radical could have been these regulations. Furthermore, the decrease in SOD and CAT activities may be due to the formation of formaldehyde from the methanol. This is in accordance with Gulec et al. (2006) who indicated that formaldehyde exposure led to a decrease in SOD and CAT activities in the liver tissue compared to the control. Also, Chang and Xu (2006) recorded a decrease in SOD activity and there was a dose-response relationship between formaldehyde concentration and SOD activity.

Usually, GSH non-enzymatically reacts with superoxide, NO (Clancy et al., 1994), hydroxyl radical (Bains and Shaw, 1997) and per-oxy-nitrite radicals (Koppal et al., 1999). Though during 15 days of aspartame administration it could elevate GSH level, but after 30 and 90 days of administration the drastic decrease in GSH may be a contributing factor for the nitric oxide level increase. The decrease in GSH activity observed in the present study could be caused by methanol, because methanol metabolism depends upon GSH (Pankow and Jagielki, 1993). The decrease in cellular glutathione content increases cell vulnerability to oxidative stress (Oyama et al., 2002).

GSH reduction can also explain the decreased concentration of Vit C, which enters the cell mainly in its oxidized form where it is reduced by GSH (Briviba and Sies, 1994). Vitamin C is a hydrophilic reducing agent which directly reacts with superoxides, hydroxyls and various lipid hydro peroxides more effectively than any other water soluble antioxidant (Niki, 1991). Vit C is a nutrient that regulates the immune system, and because of its antiviral and antioxidant properties, it plays a role in the phagocytic function (Hernanz, 1990). Therefore the decrease in the vitamin C could not be overlooked as it is essential for the immune regulations.

In the present study, though 15 days did not alter the organ weight/animal weight ratio, but later after 30 and 90 days aspartame administered animals showed a marked decrease in organ weight/animal weight ratio. The significant reduction in organ weight may be due to oxidative damage which is studied by Skrzydlewksa and Szynaka (1996) who reported that oxidative damage caused marked organ weight loss in albino rats upon methanol intoxication. This is also reported by Parthasarathy et al. (2006). Formaldehyde, the first metabolite of methanol, increases the population of shrunken cells, dead cells and hydolipid cells (Nakao et al., 2003). This reduction was also associated with the histological changes in the 90 days aspartame administered animals. Normal spleen was composed of white and red pulps surrounded by a capsule of dense connective tissue. In 90 days, in aspartame treated animals spleen, the boundary between white and red pulp started to disappear and in these animals, there was little absence of germinal centers in cortex. There were cellular disruption and degeneration of the white pulp and there was little absence of germinate centers indicating the cellular loss could be the source behind the decrease in the organ weight body weight ratio.

Farshid et al. (2002) reported that peroxynitrite, a biological oxidant and cytotoxic anion, induced inflammation and injury to the lung parenchyma (Mageid, 1994). The possible causative agent behind the cellular changes includes the free radicals that are generated by aspartame. Then next possibility is the methanol metabolite formaldehyde. Menezes et al. (2005) reported that all extensive injuries were repaired with collagen fibers

(scar) irrespective of its cause. Therefore the increased thicknesses of the capsule and of trabeculae in our experiment may be due to increase in collagen fibers deposition induced by injury resultant after aspartame administration. Most et al. (1997) reported that monocytes migrated at the site of inflammation coalesce with macrophages to form multinucleated giant cells. Therefore, it could be considered that infiltration of neutrophils, lymphocytes and increase in the number of giant cells observed in the present study might be due to the inflammation in the immune organs studied. The proliferation of macrophages with vacuolated cytoplasm within the medullary region in the 90 days aspartame treated animals strongly recommend further studies with aspartame on the cells involved in immunity.

Conclusion

The present study clearly points out that aspartame can increase the excess free radicals where by it inactivates the scavenging system as the days of administration increased that results in oxidative stress. It is essential to note that the dosage used in this study is Food and Drug Administration (FDA) permitted level and humans are consuming more than this without knowing its consequences. Aspartame metabolite methanol and also its metabolite formaldehyde may be the causative factors behind the changes observed. Moreover, such induced oxidative stress and the resultant inflammation in the immune organs along with the elevated corticosteroid level could be immune suppressive.

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Conflict of Interests

The author(s) have not declared any conflict of interests.

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