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Original Article

Effects of aspartame on hsp70, bcl-2 and bax expression in immune organs of Wistar albino rats

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Abstract

Aspartame, a "first generation sweetener", is widely used in a variety of foods, beverages, and medicine. The FDA has determined the acceptable daily intake (ADI) value of aspartame to be 50 mg/kg·day, while the JECFA (Joint FAO/WHO Expert Committee on Food Additives) has set this value at 40 mg/kg of body weight/day. Safety issues have been raised about aspartame due to its metabolites, specifically toxicity from methanol and/or its systemic metabolites formaldehyde and formic acid. The immune system is now recognized as a target organ for many xenobiotics, such as drugs and chemicals, which are able to trigger unwanted apoptosis or to alter the regulation of apoptosis. Our previous studies has shown that oral administration of aspartame [40 mg/(kg·day)] or its metabolites for 90 days increased oxidative stress in immune organs of Wistar albino rats. In this present study, we aimed to clarify whether aspartame consumption over a longer period (90-days) has any effect on the expression of hsp70, bcl-2 and bax at both mRNA transcript and protein expression levels in immune organs. We observed that oral administration of aspartame for 90 days did not cause any apparent DNA fragmentation in immune organs of aspartame treated animals; however, there was a significant increase in hsp70 expression, apart from significant alteration in bcl-2 and bax at both mRNA transcript and protein expression level in the immune organs of aspartame treated animals compared to controls. Hence, the results indicated that hsp70 levels increased in response to oxidative injury induced by aspartame metabolites; however, these metabolites did not induce apoptosis in the immune organs. Furthermore, detailed analyses are needed to elucidate the precise molecular mechanisms involved in these changes.

Keywords: aspartame, immune organs, hsp70, bcl2, bax

Introduction

Aspartame, an artificial sweetener in low-calorie foods and soft drinks, is a low calorie option for people who should or need to limit their sugar intake. Unlike sugar, aspartame does not contribute to tooth decay and is approximately 200 times sweeter than sugar. Aspartame is currently used in over 6,000 products in over 120 countries worldwide. It consists of 2 amino acids, aspartic acid and phenylalanine, in addition to methanol. Phenylal1anine is believed to mediate or exacerbate hepatic encephalopathy[1]. Safety issues that have been raised include possible toxicity from methanol and/or its systemic metabolites, formaldehyde and formic acid, which are corrosive to mucous membranes and can result in liver and kidney injury

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when ingested[2]. Formic acid is an established human toxin. Emerging evidence supports an association between formaldehyde exposure and multiple adverse health effects[3]. The European Food Safety Authority established acceptable daily intake levels of aspartame at 40 mg/kg(body weight)·day[4]. The LD_{50} of aspartame in mice and rats is > 5 g/kg[5]. As aspartame is an artificial sweetener used in food products, many safety tests have been carried out, but its safety for long term consumption still remains controversial. Although many tests on the safety and toxicity of aspartame have been performed, the results are conflicting. The toxicity of a substance is based on the premise that toxicity increases in a dose-dependent manner. Thus, it is not surprising that markedly high doses of aspartame induce deleterious effects in sensitive animal species. The critical question is whether aspartame is potentially harmful at common or abuse usage levels[6].

Immune cells are particularly sensitive to oxidative stress due to a high percentage of polyunsaturated fatty acids in their plasma membranes and they generally produce more oxidative products[7]. The immune system is now recognized as a target organ for many xenobiotics, such as drugs and chemicals, which are able to trigger unwanted apoptosis or to alter the regulation of apoptosis. Reducing the number of immune-competent cells after xenobiotic treatment can lead to immunosuppressive effects, resulting in an increased susceptibility to tumors or infectious diseases. Many experimental works have dealt with the influence of xenobiotics on the immune system. The mammalian immune system is a complex network of organs, tissues, cells, and cell products which function in an orderly manner to generate and sustain proper defense responses and homeostasis[8]. The immune system carries out effective immune surveillance by connecting immune cell production centers with peripheral components of the body[9]. To maintain the homeostasis under oxidative stress, cells produce high levels of stress proteins, which protect against the damage[10]. Heat shock proteins (hsps) play a key role in cellular protection against multiple stressors, including oxidative stress. Among different classes of hsps which vary in molecular mass, hsp70 seems to be the most abundant and important[11].

There are 2 major apoptotic pathways in cells: the death receptor-mediated pathway (extrinsic)[12] and the mitochondria-mediated pathway (intrinsic)[13]. The mitochondrial pathway can be divided into caspase-dependent and caspase-independent pathways. The former is regulated by members of the bcl-2 family, which have either anti-apoptotic (e.g., bcl-2) or pro-apoptotic (e.g., bax) properties[14]. Family members of bcl-2 effectively increase or decrease apoptotic activity[15]. The balance between pro- and anti-apoptotic proteins plays a crucial role in apoptosis.

Recent studies have investigated the ability of aspartame and its metabolites to alter the oxidative status of the cells via reactive oxygen species (ROS) generation and modulation of intracellular antioxidant enzymes levels. The previous results suggested that oral aspartame 40 mg/(kg·day) consumption caused oxidative stress in immune organs[16–19]. The carcinogenic effects of oral aspartame 40 mg/(kg·day) has reported in the immune organs of mice[20]. However, little is known about the effects of aspartame on apoptotic gene expression in immune organs. Therefore, the present study aimed to clarify whether aspartame consumption over a longer period had any effect on hsp70, bcl-2 and bax expression in immune organs of Wistar albino rats.

Materials and methods

Materials

Total RNA isolation reagent (TRI) and primers were purchased from Sigma (St. Louis, MO, USA). Superscript-III reverse transcriptase was purchased from Invitrogen, (Carlsbad, CA, USA) and PCR Ready Mix DNA polymerase was purchased from KAPA Biosystem (USA). Antibodies were purchased from Sigma.

Animals

Animal experiments were carried out after obtaining clearance from the Institutional Animal Ethical Committee (IAEC No: 01/21/2014) and the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). All the rats were housed under the conditions of controlled temperature (26±2) °C with 12-hour light and 12-hour dark exposure. Healthy adult male Wistar albino rats, weighing approximately 200-220 g were maintained under standard laboratory conditions and allowed *ad libitum* access to food and water (M/s. Hindustan Lever Ltd., India). Rats were made folate deficient by feeding them on a special folate deficient diet[21] for 37 days and thereafter was given methotrexate (MTX) in sterile saline by every other day for two weeks[22]. MTX folate deficiency was confirmed by estimating the urinary excretion of formaminoglutamic acid[23]. Rats fed a normal diet (group I, n = 6) received normal saline or aspartame by lavage for 90 days [40 mg/(kg·day)][4] (group III, n = 6). Rats fed a folate deficient diet received normal saline (group II, n = 6) or aspartame by gavage for 90 days [40 mg/kg·day)] (group IV, n = 6).

Sample collection

Blood sample collection and isolation of spleen, thymus and lymph nodes was performed between 8 and 10 a.m. to avoid circadian rhythm induced changes. Stress-free blood samples were collected as described by Feldman and Conforti[24]. At the end of the experiment, all rats were exposed to mild anesthesia and blood was collected from the internal jugular vein; plasma and serum was separated by centrifugation at 1,000 *g* at 4°C for 15 minutes. Then, the animals were sacrificed under deep anesthesia using pentothal sodium (40 mg/kg). The spleen, thymus and lymph nodes were excised, washed in ice cold saline and blotted dry. The spleen, thymus and lymph nodes were weighed and then homogenized by using Teflon glass homogenizers.

DNA fragmentation

One hundred mg of spleen, thymus and lymph node tissue was homogenized in 1 mL 1x suspension buffer in 2 mL microcentrifuge tube. After homogenization, 5 µL RNase solution (10 mg/mL) was added, mixed 5–6 times by inverting the vial and incubated at 65°C for 10 minutes with intermittent mixing. After incubation, 1 mL lysis buffer was added, mixed, incubated at 65°C for 15 minutes and then cooled at room temperature (RT). After incubation, the lysate was centrifuged at 13,000 rpm for 1 minute at RT. To the supernatant, an equal volume of isopropanol was added to each vial, mixed well and centrifuged at 13, 000 *g* for 15 minutes at RT. To the pellet, 0.5–1 mL of 70% ethanol was added and centrifuged at 13,000 *g* for 15 minutes at RT and this was repeated again. The pellet was dried at 37°C for 10 minutes. 50 µL of autoclaved milli-Q water was added and the DNA was suspended by placing the vial at 4°C overnight. The isolated DNA were resolved by electrophoresis through a 1% agarose gel and stained with ethidium bromide. The resolved fragments of DNA in the agarose gel were scanned with a Gel Doc image scanner (Bio-Rad, USA).

Reverse transcriptase-polymerase chain reaction

Total RNA was isolated from spleen, thymus and lymph node using TRI reagent following the method of Chomczynski and Sacchi[25]. One µg of total RNA was subjected to two steps RT–PCR. For first strand synthesis, complementary DNA (cDNA) was made from mRNA template using dNTPs and reverse transcriptase (Superscript-III Reverse Transcriptase, Invitrogen). PCR was run using PCR Ready Mix DNA polymerase (KAPA Biosystem) using primers and conditions described in (***Table 1***). The amplified products were separated by electrophoresis on 2% agarose gel and visualized by ethidium bromide staining. Specificity was confirmed by the size of the amplified products with reference of 100 bp DNA ladder (Chromus Biotech, Chennai) and the band intensities were quantified by Quantity One Software (Bio-Rad, Hercules, CA, USA).

Western blotting assays

Western blotting assays were carried out as per the standard protocol. Spleen and lymph node tissues were lysed with RIPA containing protease inhibitors (1 mmol/L PMSF and 0.5 mg/mL each of leupeptin and apoprotinin), and protein concentrations were determined by the Lowry Method. The samples were solubilized in a reducing loading buffer (62.5 mmol/L Tris, pH 6.8, 6 mol/L urea) 10% glycerol, 2% sodium dodecyl sulfate (SDS), 0.003% bromophenol blue, 5% 2-mercaptoethanol), electrophoresed in 10% SDS polyacrylamide gels and then transferred onto PVDF membranes. The membranes were incubated with primary antibodies at appropriate dilutions, bcl2 (1:2,000), bax (1:2,000) and hsp70 (1:1,000) followed by the incubation with horseradish peroxidase conjugated anti-mouse IgG or anti-rabbit IgG secondary antibody which were diluted 1:10,000. Finally, protein bands were visualized on the Chemi Doc Imaging System, Bio-Rad (USA) using an enhanced chemiluminescence system (ECL, Pierce, USA).

Immunohistochemical analysis

Rats were sacrificed by perfusion with normal saline. The spleen and lymph nodes were excised and 4% paraformaldehyde was used for fixation. Immunohistochemistry was performed on 5 µm paraffin embedded

Table 1 **Sequences of primers used in the study**

Gene	Sequence	Gene accession No	Amplified product (b.p)	Annealing temp/cycles
Hsp70	Sense: 5′-TGAACGTATGGTTAATGATG-3′ Antisense : 3′CGAGCCAGTCAAGAGCATCC5′	XM_625373.1	290	55°C/35
Bcl2	Sense: 5′-TCTCATGCCAAGGGGGAAAC-3′ Antisense: 3′CGGTAGCGACGAGAGAAGTC5′	NM_016993.1	282	55°C/35
Bax	Sense: 5′-TGGGATGGCCTCCTTTCCTA-3′ Antisense: 3′GAAGCCTCAGCCCATCTTCT5′	NM_017059.2	172	55°C/35

tissue sections on gelatin coated glass slides. The tissue sections were deparaffinized by placing the slides in an oven at 60°C for 10 minutes and then rinsed twice in xylene for 10 minute each time. The slides were then hydrated in gradient ethanol (100%, 90%, 70%, 50%, and 30%) for 10 minute each and then rinsed in double-distilled water for 10 min. The sections were incubated with (citrate buffer plus Tween 20) for 15 minutes in a water bath at 80°c for antigen retrieval. The slides were then rinsed in double-distilled water for 8 min and twice with TBS (Tris-HCl containing 150 mmol/L NaCl, pH 7.4). Then, the sections were rinsed with 1% H_2O_2 in methanol for 15 minutes at 22°C to quench endogenous peroxidase activity. The sections were then rinsed again twice with TBS and blocked in blocking buffer (1% BSA in TBS,) for 15 minutes at 22°C. The sections were incubated with primary antibodies against hsp70, bcl2 or bax(Sigma-Aldrich, USA) and at a dilution of 1:500 in PBS for overnight at 4°C. After incubation, the sections were rinsed twice with TBS and incubated with secondary goat anti-rabbit IgG-HRP conjugate antibody for 1 hour at 4°C. After wash with TBS, immunoreactivity was developed with 0.05% DAB (DAB Kit, GeNei biotech) and 0.01% H_2O_2 for 1–3 minutes, rinsed twice with TBS and counterstained with hematoxylin. The sections were mounted in DPX and observed under light microscope (40x; Nikon Corporation, Tokyo, Japan) with appropriate settings and photographed.

Statistical analysis

Data are expressed as mean±standard deviation (SD). All data were analyzed with the SPSS for Windows statistical package (version 20.0, SPSS Institute Inc., Cary, NC, USA). Statistical significance between the different groups was determined by one way-analysis of variance (ANOVA). When the groups showed significant differences, Tukey's multiple comparison tests was followed and the significance level was fixed at $P < 0.05$.

Results

Effects of aspartame on DNA fragmentation in immune organs

DNA fragmentation study images are shown in ***Fig. 1***. We found no DNA fragmentation in the spleen, thymus and lymph nodes of the control rats fed a normal diet as well as in the folate deficient diet fed rats. Treatment with aspartame for 90 days also did not cause apparent DNA fragmentation in the spleen, thymus and lymph nodes of rats fed a normal diet and the folate deficient diet fed rats with aspartame in this study duration.

Effects of aspartame on the RNA expression of hsp70 in the immune organs

The mRNA transcript levels of hsp70 in the spleen,

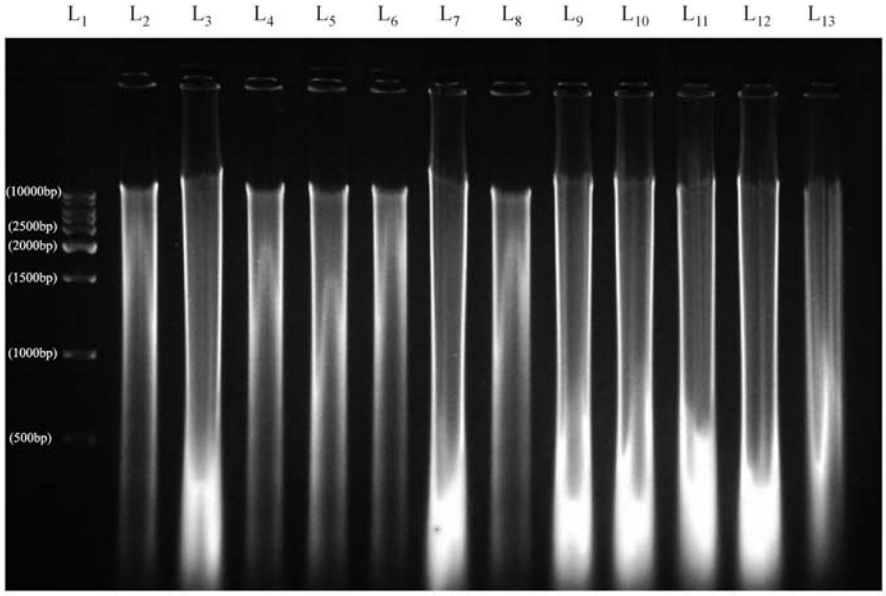

Fig. 1 **DNA fragmentation.** DNA fragmentation analysis in spleen, thymus and lymph nodes treated with aspartame for 90-days. Lane 1 DNA molecular marker, Lane 2, 3, 4 Group-1 (spleen, thymus and lymph nodes) resp; Lane 5, 6, 7, Group-2 (spleen, thymus and lymph nodes) resp; lane 8, 9, 10 Group-3 (spleen, thymus and lymph nodes) respectively; Lane 11, 12, 13 Group-4 (spleen, thymus and lymph nodes) respectively. Group I- Control animals, Group 2- Folate deficient diet fed animals, Group 3- Control animals + aspartame, Group 4- Folate deficient diet fed animals + aspartame.

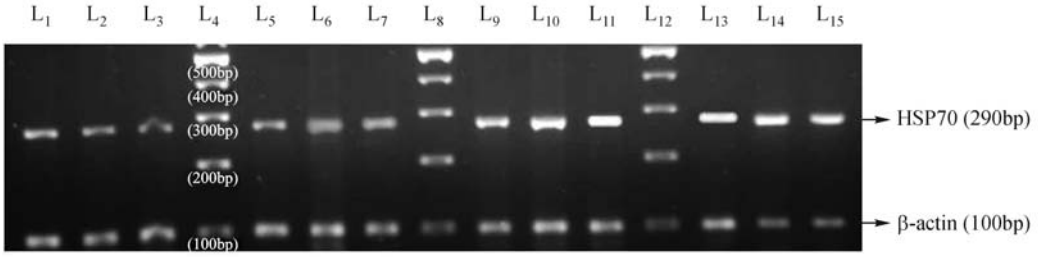

Fig. 2 **RT-PCR analysis of hsp70 mRNA expression level, (a).** Lane 4,8,12, DNA molecular marker; lane 1, 2, 3, Group-1 (spleen, thymus, lymph node) resp; lane 5, 6, 7, Group-2 (spleen, thymus, lymph node) respectively; lane 9, 10, 11, Group-3 (spleen, thymus, lymph node) respectively; Lane 13, 14, 15, Group-4 (spleen, thymus, lymph node) resp. Group I- Control animals, Group II- Folate deficient diet fed animals, Group III- Control animals + aspartame, Group IV- Folate deficient diet fed animals + aspartame.

thymus and lymph node of rats fed with the folate deficient diet were comparable to rats fed a normal diet (***Fig. 2, Table 2***). Aspartame for 90 days caused a significant increase in the mRNA transcript levels of hsp70 in the spleen, thymus and lymph node of both the rats fed a normal diet and rats fed with the folate deficient diet compared to their control counterparts.

Effects of aspartame on the RNA expression of the bcl-2 family in the immune organs

We examined the RNA expression of anti-apoptotic (*bcl-2*) and pro-apoptotic (*bax*) genes in the spleen, thymus and lymph node of rats. We found that folate deficient diet fed rats exhibited similar mRNA transcript levels of *bcl-2* and *bax* to those of rats fed a normal diet

Table 2 **RT-PCR mRNA expression and protein analysis of Hsp70, Bcl-2, and Bax.**

Organs	Group-1	Group-2	Group-3	Group-4
Hsp 70/ β-actin mRNA (Relative intensity)				
Spleen	146.66±21.60	165.00±19.70	231.66±22.28^{*ab}	250.66±25.81^{*ab}
Thymus	93.33±18.60	107.50±21.38	163.33±28.84^{*ab}	178.33±22.50^{*ab}
Lymph node	126.66±17.79	138.33±20.16	195.55±18.70^{*ab}	206.66±21.60^{*ab}
Bcl_2 / β-actin mRNA (Relative intensity)				
Spleen	81.66±10.90	82.83±12.41	87.16±11.19	90.33±12.66
Thymus	53.50±11.05	57.16±12.78	60.83±10.24	63016±11.32
Lymph node	75.00±9.91	77.66±10.49	80.16±10.44	83.00±11.01
Bax/ β-actin mRNA(Relative intensity)				
Spleen	99.16±13.28	101.00±14.12	110.00±17.08	108.00±18.70
Thymus	61.00±10.58	62.50±11.12	70.66±10.42	68.16±12.77
Lymph node	83.33±11.80	87.66±10.91	91.33±13.82	88.16±12.81
Hsp70 / β -actin				
Spleen	206.66±24.60	228.50±28.30	299.16±22.70^{*ab}	318.00±30.42^{*ab}
Lymph node	159.83±20.10	173.00±16.69	231.50±24.44^{*ab}	248.66±19.72^{*ab}
Bcl_2 / β-actin				
Spleen	125.83±15.00	128.00±17.40	134.66±20.80	138.16±18.58
Lymph node	107.50±12.41	105.83±13.19	112.66±16.14	117.83±18.20
Bax / β-actin				
Spleen	166.66±17.60	±170.33±19.79	182.00±20.70	178.33±21.22
Lymph node	145.00±15.70	150.00±16.16	159.50±18.14	155.83±17.21

Note: RT-PCR mRNA expression and protein analysis of Hsp70, Bcl-2, and Bax level, in Spleen, Thymus and Lymph node treated with aspartame for 90-days. The intensity of the signals was quantified by densitometry. Each value represents mean±SD. Significance at $^{*}P < 0.05$, *a- compared with Group-1, *b- compared with Group-2. Group 1- Control animals, Group 2- Folate deficient animals, Group 3- Control animals + aspartame, Group 4- Folate deficient animals + aspartame.

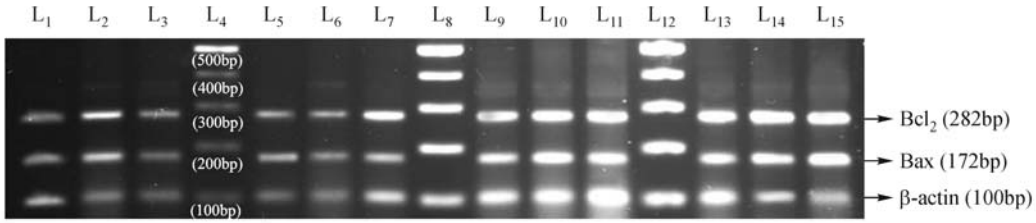

Fig. 3 **RT-PCR analysis of bcl-2 and bax mRNA expression level, (a).** Lane 4,8,12, DNA molecular marker; lane 1, 2, 3, Group-1 (spleen, thymus, lymph node) resp; Lane 5, 6, 7, Group-2 (spleen, thymus, lymph node) resp; Lane 9, 10, 11 Group-3 (spleen, thymus, lymph node) resp; Lane 13, 14, 15 Group-4 (spleen, thymus, lymph node) resp. Group I- Control animals, Group 2- Folate deficient diet fed animals, Group 3- Control animals + aspartame, Group 4- Folate deficient diet fed animals + aspartame.

(***Fig. 3, Table 2***) Aspartame for 90 days did not cause any significant change in the mRNA transcript levels of *bcl-2* and *bax* in the immune organs compared to their control counterparts.

Effects of aspartame on protein expression of hsp70 and the bcl-2 family in the spleen and lymph node of rats

Furthermore, the expression of hsp70, anti-apoptotic (bcl-2) and pro-apoptotic (bax) proteins in the spleen and lymph nodes of rats fed a folate deficient diet was similar to rats fed a normal diet (***Fig. 4, Table 2***). However, treatment with aspartame for 90 days, significantly increase in the expression of hsp70 and did not significantly alter bcl-2 and bax expression when compared to their control counterparts.

Effects of aspartame on immunohistochemical expression of hsp70 and the bcl-2 family proteins in the spleen and lymph node.

The results are summarized in ***Fig. 5a-e, 6a-e, and 7a-e***). The protein expression (hsp70, bcl-2 and bax) in the spleen and lymph node of folate deficient diet fed animals was similar to control animals. However, in animals treated with aspartame for 90-days, there was a significant increase in the expression of hsp70 while the change in expression of bcl-2 and bax was not significant when compared to their control counterparts.

Discussion

Imbalance or perturbations in immune function might result upon exposure to various xenobiotics/chemicals because of altered physiological, biochemical or cellular parameters. These alterations may lead to increased susceptibility to infection, prolonged recovery periods, ineffective immune surveillance and decreased response to antibiotics or vaccines[8]. In this study, the folate deficient diet fed animals were used to mimic human methanol metabolism. However, the folate deficient diet fed animals did not show any significant changes in the parameters studied and remained similar to control animals. Our previous studies showed that oral consumption of aspartame (40 mg /kg. bw/ day) for 90-days acts as a chemical stressor induced oxidative stress due to its metabolites[16-17]. During the course of the present study, we observed that there was up regulation of hsp70 gene expression in immune organs at both the RNA expression level and the protein expression level (Western blot and immunohistochemistry), which may be a primary biological response to the oxidative stress caused by aspartame and its metabolites. The increased hsp70 expression showed greater susceptibility of immune organs to oxidative stress, since hsp70 has immuno-regulatory functions which were associated with oxidative stress[26]. Heat shock (or stress) proteins (hSPs), particularly hsp70,

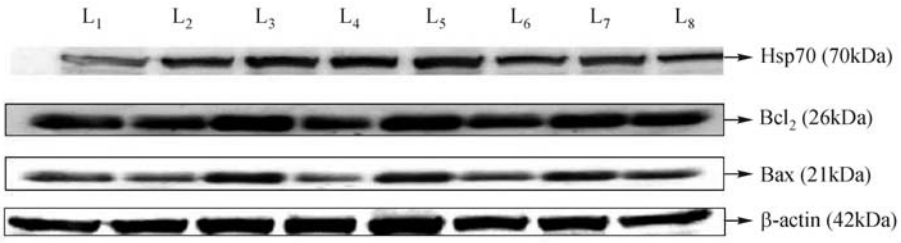

Fig. 4 **Protein analysis of hsp70, bcl-2, and bax protein expression.** Lane 1, 2, Group-1 (spleen, lymph node) resp; lane 3, 4, Group-2 (spleen, lymph node) resp; lane 5, 6, Group-3 (spleen, lymph node) resp; Lane7, 8, Group-4 (spleen, lymph node) resp. Group 1- Control animals, Group 2- Folate deficient animals, Group 3- Control animals + aspartame, Group 4- Folate deficient animals + aspartame.

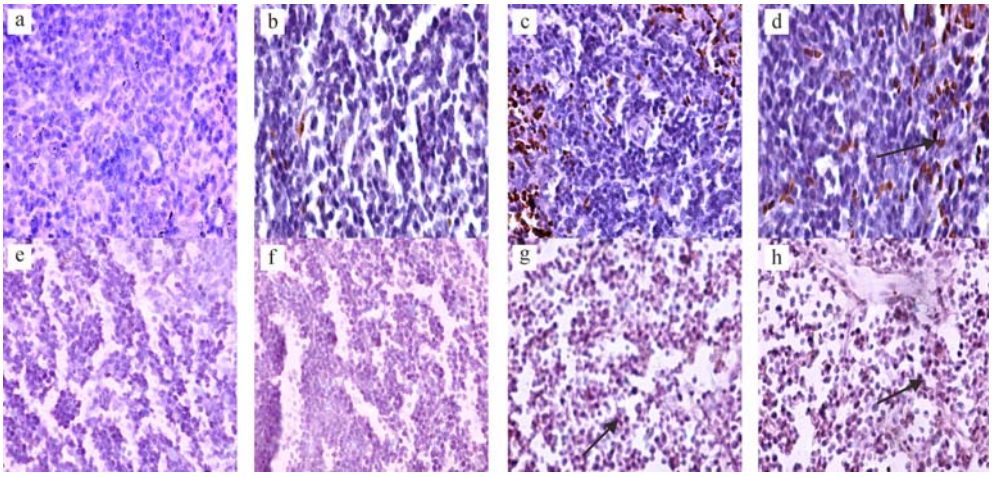

Fig. 5 **Immunohistochemical analysis of hsp70 protein expression in spleen and lymph node treated with aspartame for 90-days.** a-d represents group-1,2,3,4 (spleen) respectively. and e-h, represents group-1,2,3,4 (Lymph node) respectively. Arrow indicate positive expression of hsp70 protein. Group 1- Control animals, Group 2- Folate deficient diet fed animals, Group 3- Control animals + aspartame, Group 4- Folate deficient diet fed animals + aspartame.

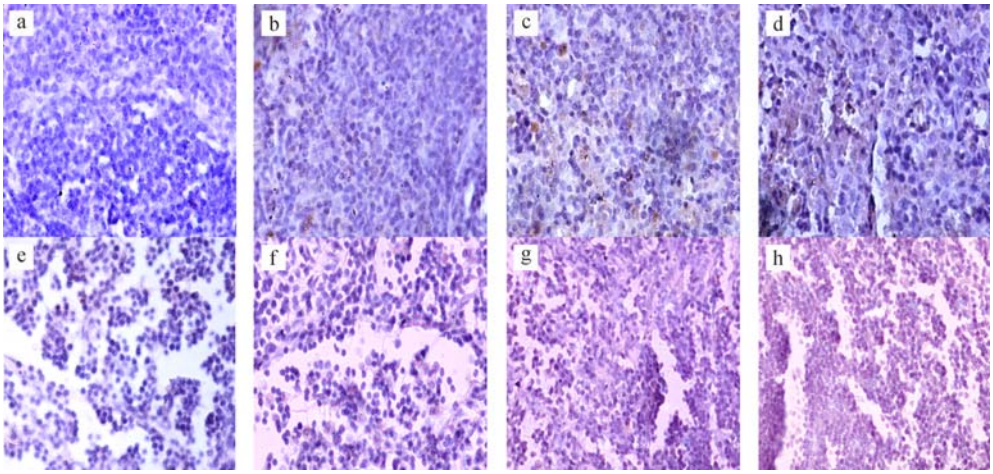

Fig. 6 **Immunohistochemical analysis of anti-apoptotic (bcl-2) protein expression in spleen and lymphnode treated with aspartame for 90-days.** a-d represent group-1,2,3, and 4 (spleen), respectively. and e-h, represent group-1,2,3, and 4 (lymph node), respectively. Group 1- Control animals, Group 2- Folate deficient diet fed animals, Group 3- Control animals + aspartame, Group 4- Folate deficient diet fed animals + aspartame.

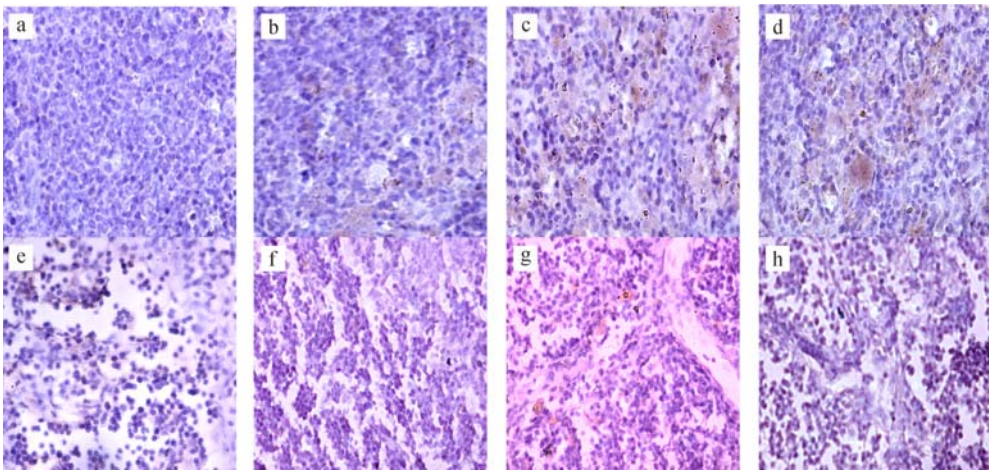

Fig. 7 **Immunohistochemical analysis of pro-apoptotic (bax) protein expression in spleen and lymphnode treated with aspartame for 90-days.** and a-d represent group-1,2,3,4 (spleen) resp and e-h represent group-1,2,3,4 (lymph node) resp. Group 1- Control animals, Group 2- Folate deficient diet fed animals, Group 3- Control animals + aspartame, Group 4- Folate deficient diet fed animals + aspartame.

function as molecular chaperones, assisting in the folding and transport of proteins and their assembly into complexes. They protect cells from stress or nocive conditions, and provide cytoprotection against a wide number of stressors and stress hormones such as corticosterone[27]. It appears that the role of hsp is to protect the oxyradical induced changes because oxygen radical induced synthesis of stress proteins leads to oxidative stress tolerance. Furthermore, hsps are also recognized regulators of apoptosis[28]. Toxic insult to the cell can result in necrosis or apoptosis. Apoptosis is a paramount mechanism contributing to the maintenance of immunological homeostasis. It occurs whenever the body is exposed to non-lethal doses of xenobiotic chemicals[29] and it succeeds to eliminate the damaged cells without inducing an inflammatory reaction. It is a process involved in the removal of potentially harmful cells which are phagocytized prior to the release of pro-inflammatory intracellular contents. It is a highly coordinated mechanism of cell death adopted to control tissue cell status (by eliminating unwanted or superfluous cells) in normal and pathological conditions. Stimulated cells respond by activating genetically controlled cell suicide machinery that leads to apoptosis[30]. Several pro- and anti-apoptotic intracellular elements able to control the apoptotic process. A protein such as bax are promoters of apoptosis, and is often found in the cytoplasm, while bcl-2 is mostly membrane bound[31]. In response to specific signals instructing a cell to undergo apoptosis, a number of distinctive biochemical and morphological changes occur within the cell. Cell apoptosis is characterized by a series of biochemical and morphological changes: cell volume loss, chromatin condensation, cytoplasmic shrinking, and dilation of the endoplasmic reticulum, and nucleosomal DNA fragmentation. In many of the systems studied, this cell death is programmed and occurs via apoptosis. Nonetheless, apoptosis is also associated with several pathological disorders in addition to normal physiology. For example, neurodegenerative diseases may be a result of excessive apoptosis whereas cancer may be due to lack of apoptosis[32]. Our present studies showed that there was no significant change in bcl-2 and bax expression in aspartame treated groups compared with controls groups, at both the RNA expression level and the Protein expression level (western blot and immunohistochemistry). There was no DNA fragmentation observed in immune organs of aspartame treated groups, which indicates that aspartame consumption of this dose 40 mg /kg bw/day for 90-days in Wistar albino rats induces free radical production[16,17], but this free radical production induces cancer[20] but not apoptosis because cancer may be due to lack of apoptosis[32]. However, recent studies suggested that hsp70 is a general anti-apoptotic protein that protects tissue from cytotoxicity induced by oxidative stress; although the mechanism of action of its antiapoptotic effects is still obscure[33]. Hsp70 inhibits apoptosis by preventing recruitment of procaspase-9 to the Apaf-1 apoptosome[34]. Therefore, the present study showed that increased hsp70 reaction was a cell response to damaging oxidative stress induced by aspartame metabolites.

In summary, the present study showed that 90-days of oral administration of aspartame (40 mg/kg.bw/day) or its metabolites may increase oxidative stress in immune organs of Wistar albino rats. An increase in the amount of hsp70 in the immune organs was a protective response to oxidative injury due to aspartame metabolites; however, it does not induce apoptosis in the immune organs in the duration of this study. Furthermore, detailed analyses are needed to elucidate the precise molecular mechanisms involved in these changes.

Acknowledgements

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