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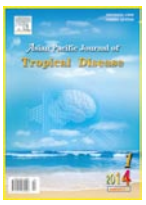

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Serum biochemical responses under oxidative stress of aspartame in wistar albino rats

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PEER REVIEW

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Comments

The study attempted by the authors is genuine and sincere by comprehensively carrying out related parameters to achieve their objective of confirming aspartame toxicity and its impact on the hepatic, renal and hematological parameters.
Details on Page S409

ABSTRACT

Objective: To study whether the oral administration of aspartame (40 mg/kg body weight) for 15 d, 30 d and 90 d have any effect on marker enzymes, some selective liver and kidney function parameter, lipid peroxidation and antioxidant status in serum. To mimic human methanol metabolism, folate deficient animals were used.

Method: Animal weight, complete hemogram, marker enzyme in serum, some selected serum profile reflect liver and kidney function, plasma corticosterone level, and in serum, lipid peroxidation, nitric oxide, enzymatic and non-enzymatic antioxidant level was measured .

Result: After 15 d of aspartame administration animals showed a significant change in marker enzymes, and antioxidant level. However, after repeated long term administration (30 d and 90 d) showed a significant change in some selected serum profile reflects liver and kidney function, along with marker enzymes, and antioxidant level.

Conclusions: This study concludes that oral administration of aspartame (40 mg/kg body weight) causes oxidative stress in Wistar albino rats by altering their oxidant/antioxidant balance.

KEYWORDS

Aspartame, Serum, Marker enzymes, Oxidative stress, Antioxidant

1. Introduction

Aspartame is widely consumed by humans who are diabetic and who are under weight loss regime. Aspartame (L-aspartyl-L-phenylalanine methyl ester) also known as NutraSweet, after oral administration to humans and experimental animals, aspartame is rapidly and completely metabolized to 50% phenylalanine, 40% aspartic acid and 10% methanol[1]. Methanol is being increasingly recognized as a substance that damages the liver cells where it is oxidized to formaldehyde and later to formate[1]. These processes are accompanied by elevation of NADH level and the formation of superoxide anion, which may be involved in lipid peroxidation[2]. Also, methanol intoxication is associated with mitochondrial damage and increased microsomal proliferation resulting in increased production of oxygen radicals[3]. And these factors together with the excess of formaldehyde formed during acute methanol intoxication cause significant increase in lipid peroxidation[2]. After aspartame consumption, the concentration of its metabolites is increased in blood[4]. There are many enzymes found in the serum that did not originate from the extracellular fluid. During tissue damage, some of these enzymes find their way into the serum through leakage arising from altered membrane permeability. Serum enzymes [alkaline phosphatase (ALP), acid phosphatase (ACP), aspartate

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aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH) and γ-glutamyl transpeptidase (γGT)] measurements are therefore a valuable tool in clinical diagnosis, providing information on the effect and nature of pathological damage to any tissue. The measurement of the activities of these marker enzymes in tissues and body fluids can be used in assessing the degree of assault and the toxicity of a chemical compound on organ/tissues[5,6]. The metabolism of xenobiotics to a large extent takes place in the liver. The byproduct of such metabolism sometimes is more toxic than the initial substance. This can cause hepatic damage and the emergency of hepatic disorders. The kidney is one of the organs responsible for the maintenance of constant extracellular environment through its in environment in the excretion of such purine catabolite as urea, creatinine, blood urea nitrogen (BUN) and uric acid as well as electrolyte balance. Abnormal concentration of this catabolite and some electrolyte in plasma or serum is a clear indication of renal function impairment[7]. The kidney function may be assessed from the level of some electrolyte (such as Na^+, and K^+,) and metabolite (such as creatinine, urea, uric acid and BUN) in the serum[8,9].

Oxidative stress was originally defined as the disequilibrium between prooxidants and antioxidants in biological systems[10]. 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[10]. The status of lipid peroxidation as well as altered levels of certain endogenous radical scavengers is taken as direct evidence for oxidative stress[14]. Therefore, serum of Wistar albino male rats was monitored for the above described parameters on exposure of aspartame (40 mg/kg body weight).

2. Materials and methods

2.1. 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.

2.2. Animal model

Animal experiments were carried out after obtaining 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. The experimental animals were healthy, inbreed adult male Wistar albino rats, weighing approximately 200-220 g (12 weeks of age). 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 making rat folate deficient were given special folate deficient diet for 37 d[11] and methotrexate(0.1 mg/100 g body weight) *i.p.* every other day for two weeks[12]. Methotrexate folate deficiency was confirmed by estimating the urinary excretion of formamino-glutamic acid (FIGLU)[13] prior to the experiment. Rats on a folate deficient diet excreted an average of 70 mg FIGLU/kg body weight per day (Range 25-125 mg) while animals on the control diet excreted an average of 0.29 mg/kg body weight per day (Range 0.15-0.55 mg). These folate deficient animals showed a significant increase in FIGLU excretion when compared to the control animals ($P<0.05$). After that animals were daily administered aspartame (40 mg/kg body weight)[14] dissolved in normal saline orally (by means of needle lavage) for 15, 30 and 90 d. All the rats were housed under condition of controlled temperature (26±2) °C with 12 h light and 12 h dark exposure.

2.3. Experimental design

Every groups consisting of 6 animals each. Group I were the control animals which were administered normal saline orally (by means of needle lavage) thought out the experimental protocol. Since human beings have very low hepatic folate content compare to rats[15]. In methanol metabolism conversion of formate to carbon dioxide is folate dependent. Hence in the deficiency of folic acid, methanol metablism could take the alternate pathway (microsomal pathway) in human[16]. Hence to mimic human methanol metabolism, folate deficient rats were used. The folate deficient animals were further divided into 4 groups. Group II was folate deficient diet fed control, Group III was folate deficient animals treated with aspartame for 15 d (40 mg/kg body weight), Group IV was folate deficient animals treated with aspartame (40 mg/kg body weight) for 30 d. Group V was folate deficient animals treated with aspartame (40 mg/kg body weight) for 90 d.

2.4. Sample collection

Blood samples were collected between 8 and 10 a.m. to avoid circadian rhythm induced changes. Stress-free blood samples were collected as per the technique described by Feldman S, *et al*[17]. At the end of experimental period all the animals were exposed to mild anesthesia and blood was collected from internal jugular vein, and used for doing complete hemograms, plasma and serum was separated respectively from anti-coagulated and coagulated blood by centrifugation at 3000 r/min at 4 °C for 15 min and kept at −80 °C until biochemical estimations.

2.5. Biochemical determinations

All assays were performed in serum. Nitric oxide levels were measured as total nitrite + nitrate levels with the use

of the Griess reagent by the method of Bradford[18]. Protein was estimated as per the method described by Lowry[19].

ALP activity (EC. 3.1.3.1), ACP activity (EC. 3.1.3.2) and LDH (EC. 1.1.1.27) was assayed by the method of King[20]. Aspartate and alanine aminotransferases (EC. 2.6.1.1, EC. 2.6.1.2) were assayed by the method of King[20]. γGT (EC. 2.3.2.2) was assayed by the method of Orlowski and Meister[21]. Total protein, creatinine, urea, uric acid, BUN, bilirubin, and electrolytes levels in serum were done by auto Analyzer (Beckman Coulter India, Ltd., Mumbai, India).

Lipid peroxidation was determined as described by Ohkawa[22]. Superoxide dismutase (SOD) (EC.1.15.1.1) according to (Marklund and Marklund[23] and catalase (CAT)(EC. 1.11.1.6) according to the method of Sinha[24]. The activity of glutathione peroxidase (GPx) (EC.1.11.1.9) was estimated by the methods of Rotruck[25]. Reduced glutathione (GSH) in the lymphoid organs was estimated by the method of Moron[26]. The vitamin-C (ascorbic acid) content in the liver tissue was determined according to the method of Omaye[27].

2.6. 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, North Carolina). Statistical significance between the different groups was determined by One way-analysis of variance (ANOVA). When the groups showed significant difference then Tukey's multiple comparison tests was followed and the significance level was fixed at $P<0.05$.

3. Result

3.1. Effect of aspartame on complete hemograms

The data are are summarized in Table 1 with mean± SD. Routine hematological parameters were performed on hematology analyzer (Beckman Coulter India, Ltd., Mumbai. Parameters like hemoglobin, bleeding time, clotting time, erythrocyte sedimentation rate, red blood cell, reticulocyte count, hemotocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelets count, eosinophil and monocyte were studied. All the parameters was similar in folate deficient group when compare to control group and also all the parameters was with-in the normal range and no significant changes were observed in all the folate deficient diet fed aspartame treated (15 d, 30 d as well as 90 d) groups as compared to respective control as well as folate deficient diet fed animals. While there was decrease in white blood cell, and neutrophil while increase in lymphocyte in all the folate deficient diet fed aspartame treated (15 d, 30 d as well as 90 d) groups with compare to control as well as folate deficient diet fed animals.

Table 1

Effect of aspartame on complete hemogram.

Parameters	Control	FD	15 d	30 d	90 d
Hemoglobin (g%)	13.78±0.60	13.50±0.50	13.30±0.39	14.16±0.40	14.33±0.58
Bleeding time (second)	70.72±1.67	70.37±2.13	71.66±1.16	72.25±2.40	70.50±1.22
Clotting time (second)	49.05±1.54	50.82±1.13	50.39±1.79	51.07±0.90	49.39±1.06
ESR (mm/h)	6.20±0.72	6.23±0.75	6.94±0.91	7.37±0.71	6.87±0.76
RBC ($\times10^6$/mm^3)	6.93±0.46	7.00±0.30	7.46±0.33	7.38±0.39	7.60±0.49
Reticulocyte count (%)	1.17±0.16	1.27±0.21	1.40±0.16	1.43±0.29	1.45±0.20
Hemotocrit (%)	42.85±0.23	42.79±0.24	42.98±0.17	42.87±0.39	42.94±0.32
MCV (μm^3)	52.90±0.60	53.33±0.56	53.54±0.87	53.16±0.69	53.46±0.56
MCH (pg)	16.87±0.54	16.78±0.43	16.85±0.71	17.21±0.68	17.59±0.40
MCHC (%)	33.17±0.36	32.92±0.48	32.90±0.42	32.95±0.45	32.99±0.43
Platelets ($\times10^5$/mm^3)	3.19±0.16	3.20±0.24	3.28±0.23	3.30±0.17	3.19±0.19
WBC($\times10^3$/mm^3)	7.89±0.54	7.59±0.55	6.66±0.37^{*a*b}	5.52±0.39^{*a*b*c}	3.57±0.44^{*a*b*c*d}
Differential count					
Neutrophil (%)	23.78±1.05	23.35±0.89	21.30±0.64^{*a*b}	18.92±0.50^{*a*b*c}	14.36±1.43^{*a*b*c*d}
Lymphocyte (%)	74.35±0.83	74.69±0.52	76.97±0.35^{*a*b}	78.15±0.60^{*a*b*c}	85.70±0.82^{*a*b*c*d}
Eosinophil (%)	3.89±0.26	3.90±0.29	3.87±0.30	4.01±0.30	3.98±0.32
Monocyte (%)	2.25±0.26	2.23±0.20	2.40±0.23	2.38±0.32	2.37±0.35

ESR: erythrocyte sedimentation rate; RBC: red blood cell; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; WBC: white blood cell.
Significance at $^*P<0.05$; ^{*a}-compared with control; ^{*b}-compared with FD group; ^{*c}-compared with 15 d group; ^{*d}-compared with 30 d; CONT: Control; FD: Folate deficient.

3.2. Effect of aspartame on marker enzymes in serum

The data are presented as bar diagram (Figure 1) with mean±SD. The marker enzymes (ALP, ACP, AST, ALT, LDH and γGT) level in serum of folate deficient diet fed animals was similar to control animals. But in folate deficient diet fed rat treated with aspartame for 15 d, 30 d and 90 d, the marker enzymes (ALP, AST, ALT, LDH and γGT) levels were consequently increased in serum when compare to controls as well as folate deficient diet fed animals and ACP level was consequently decreased, when compare to control, as well as folate deficient diet fed animals.

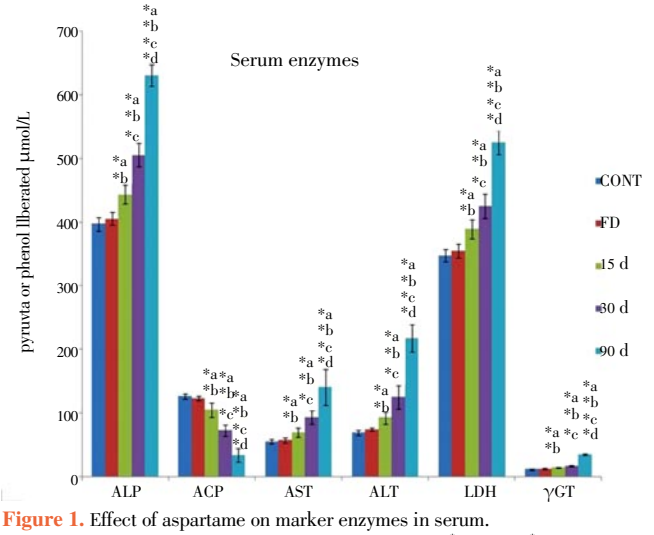

Figure 1. Effect of aspartame on marker enzymes in serum.
Each value represents mean±SD. Significance at $^*P<0.05$; ^{*a}-compared with control; ^{*b}-compared with FD group; ^{*c}-compared with 15 d group; ^{*d}-compared with 30 d; CONT: Control; FD: Folate deficient.

3.3. Effect of aspartame on serum profile reflects liver and kidney function

The data are presented in Table 2 with mean±SD. The total protein, albumin/globulin ratio and bilirubin in serum of folate deficient diet fed animal were similar to control animals and the rat serum of folate deficient diet fed 15-d aspartame treated animals also not showed any marked variation from these groups. But in rat treated with aspartame for 30 d and 90 d, the total protein and bilirubin was significantly increased, and albumin/globulin ratio was significantly decreased in serum when compare to control, folate deficient diet fed, as well as 15-d treated animals. However the blood glucose remained unchanged in all the groups.

Table 2

Effect of aspartame on serum profile reflects liver and kidney function.

Parameter	Control	FD	15 d	30 d	90 d
Total Protein(gm/dL)	6.42±0.50	6.47±0.40	6.50±0.40	8.00±0.70 $^{*a, *b, *c}$	10.39±0.65 $^{*a, *b, *c,*d}$
Total bilirubin (mg/dL)	0.80±0.07	0.84±0.10	0.90±0.20	1.82±0.22 $^{*a, *b, *c}$	3.52±0.48 $^{*a, *b, *c,*d}$
Albumin/Globulin	1.40±0.09	1.32±0.08	1.24±0.13	0.83±0.09 $^{*a, *b, *c}$	0.43±0.21 $^{*a, *b, *c,*d}$
Glucose (mg/dL)	95.30±8.96	94.20±7.80	96.40±6.20	98.00±3.10	100.00±2.16
Creatinine (mg/dL)	0.80±0.07	0.90±0.15	1.20±0.20	1.72±0.44 $^{*a, *b, *c}$	2.41±0.35 $^{*a, *b, *c,*d}$
Urea (mg/dL)	30.80±0.34	30.93±0.30	31.23±0.43	35.00±1.33 $^{*a, *b, *c}$	54.67±1.75 $^{*a, *b, *c,*d}$
BUN (mg/dL)	42.50±2.00	43.20±1.80	44.20±2.50	54.33±2.73 $^{*a, *b, *c}$	64.16±2.31 $^{*a, *b, *c,*d}$
Uric acid (mg/dL)	2.74±0.31	2.51±0.21	2.22±0.29	1.61±0.38 $^{*a, *b, *c}$	0.74±0.29 $^{*a, *b, *c,*d}$
Na^+ (mEq/L)	125.00±1.90	123.00±2.00	120.00±2.31	112.00±2.60 $^{*a, *b, *c}$	84.00±6.12 $^{*a, *b, *c,*d}$
K^+ (mEq/L)	4.14±1.90	4.41±0.45	4.74±0.44	6.11±0.50 $^{*a, *b, *c}$	7.95±0.65 $^{*a, *b, *c,*d}$
Ca^{2+} (mg/dL)	9.50±0.58	9.00±0.75	8.50±0.55	5.40±0.60 $^{*a, *b, *c}$	2.6±0.50 $^{*a, *b, *c,*d}$

Significance at $^*P<0.05$; ^{*a}-compared with control; ^{*b}-compared with FD group; ^{*c}-compared with 15 d group; ^{*d}-compared with 30 d; CONT: Control; FD: Folate deficient.

The creatinine, urea, uric acid, BUN and electrolytes (Na^+, K^+, Ca^{2+}) in serum of folate deficient diet fed animal as well as folate deficient diet fed 15 d aspartame treated animals was similar to control animals. However, in rat treated with aspartame for 30 d and 90 d, the creatinine, urea, BUN and potassium level were significantly increased, while uric acid, sodium, and calcium levels were significantly decreased in serum when compared to control, folate deficient as well as folate deficient diet fed 15 d aspartame treated animals.

3.4. Effect of aspartame on animal weight

The data are presented in Figure 2 with mean±SD. The animal weights of folate deficient diet fed animals were similar to control animals. Even folate deficient diet fed 15 d aspartame treated animals also not showed any significant change when compare to control as well as folate deficient animals. But in rat treated for 30 d and in consequent treatment of aspartame during whole study period, the animal weight was significantly decreases when compare to control, folate deficient diet fed animals as well as folate deficient diet fed 15-d aspartame treated animals.

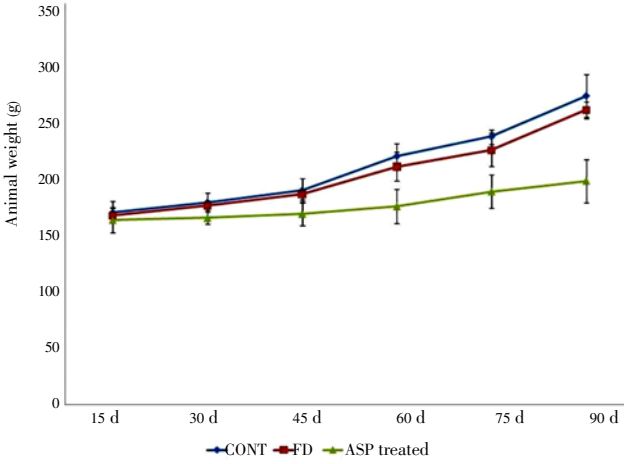

Figure 2. Effect of aspartame on animal weight.

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 30 d; CONT: Control; FD: Folate deficient.

3.5. Effect of aspartame on plasma corticosterone level

The data are presented in Table 3 with 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 d, 30 d as well as 90 d) when compared to the control as well as folate deficient groups. There was marked increases in the corticosterone level of 30 d and 90 d aspartame treated animals when compare to control, folate deficient animals as well as 15 d treated animals, moreover this increase was more marked in 90 d aspartame exposed rats than the 30 d exposed rats indicate that aspartame may act as a chemical

Table 3

Effect of aspartame on serum antioxidant parameter.

Parameters	Control	FD	15 d	30 d	90 d
Plasma corticosterone (μg/dL of plasma)	40.82±1.72	42.89±2.31	48.71±1.77 ^{*a,*b}	54.99±2.20 ^{*a,*b*c}	92.96±1.9 ^{*a,*b,*c,*d}
Serum					
Nitric oxide (μmol/L)	26.33±2.58	29.50±1.87	39.68±3.74 ^{*a,*b}	50.12±2.92 ^{*a,*b*c}	81.00±3.25 ^{*a,*b,*c,*d}
LPO [MDA(mmol/L)]	2.57±0.14	2.61±0.14	3.42±0.24 ^{*a,*b}	4.08±0.18 ^{*a,*b*c}	7.29±0.22 ^{*a,*b,*c,*d}
SOD (units/mL)	0.60±0.07	0.59±0.08	1.43±0.13 ^{*a,*b}	0.93±0.10 ^{*a,*b*c}	0.33±0.10 ^{*a,*b,*c,*d}
Catalase (μmol of H_2O_2 consumed /mL)	10.85±0.50	11.03±0.71	15.86±0.68 ^{*a,*b}	9.39±0.60 ^{*a,*b*c}	5.82±0.54 ^{*a,*b,*c,*d}
GPx (μg of GSH consumed/mL)	13.85±0.66	14.00±0.63	20.25±0.71 ^{*a,*b}	12.18±0.52 ^{*a,*b*c}	7.90±0.63 ^{*a,*b,*c,*d}
GSH (μg/mL)	7.13±0.44	7.21±0.53	12.65±0.46 ^{*a,*b}	5.64±0.43 ^{*a,*b*c}	2.59±0.49 ^{*a,*b,*c,*d}
Vitamin C (μg/mL)	10.97±0.79	11.20±0.63	15.36±0.69 ^{*a,*b}	9.33±0.58 ^{*a,*b*c}	4.84±0.56 ^{*a,*b,*c,*d}

Significance at $^*P<0.05$; ^{*a}-compared with control; ^{*b}-compared with FD group; ^{*c}-compared with 15 d group; ^{*d}-compared with 30 d; CONT: Control; FD: Folate deficient.

stressor.

3.6. Effect of aspartame on nitric oxide level

The data are presented in Table 3 with mean±SD. The nitric oxide level was similar in folate deficient group when compare to control group. While folate deficient diet fed rat treated with aspartame showed a marked increase in nitric oxide level irrespective of the duration of exposure (15 d, 30 d as well as 90 d) when compared to the control as well as folate deficient groups. There were marked increases in the nitric oxide level of 30 d and 90 d aspartame treated animals when compare to control, folate deficient animals as well as 15 d treated animals, moreover this increase was more marked in 90 d aspartame exposed rats than 30 d exposed rats clearly indicates the generation of free radicals by aspartame.

3.7. Effect of aspartame on lipid peroxidation level

The data are presented in Table 3 with mean±SD. The lipid peroxidation level was similar in folate deficient group when compare to control group. While folate deficient diet fed animals treated with aspartame showed a marked increase in lipid peroxidation level irrespective of the duration of exposure (15 d, 30 d as well as 90 d) when compared to the control as well as folate deficient groups. There were marked increases in the lipid peroxidation level of 30 d and 90 d aspartame treated animals when compare to control, folate deficient animals as well as 15 d treated animals, moreover this increase was more marked in 90 d aspartame exposed rats than the 30 d exposed animals clearly indicates the generation of free radicals by aspartame.

3.8. Effect of aspartame on enzymatic and non-enzymatic antioxidant level

The results of enzymatic and non-enzymatic antioxidant level in serum are summarized in Table 3 with mean±SD. All enzymatic (SOD, CAT and GPx) and non-enzymatic (GSH and vitamin C) antioxidants level didn't get altered in the folate deficient animal when compare to control animal. Though the rat treated with aspartame for 15 d showed a significant increase in all enzymatic (SOD, CAT and GPx) and non-enzymatic (GSH and vitamin 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 d and 90 d aspartame treated animal when compared to the control, folate deficient, as well as 15 d aspartame treated animals. Furthermore, this enzymatic and non-enzymatic level decrease was more marked decrease in 90 d aspartame treated animals when compared to the 30 d aspartame treated animals.

4. Discussion

To mimic human methanol metabolism, folate deficient rats were used. However the folate deficient animals did not showed any significant changes in the parameters studied and remained similar to controls. Lipid peroxidation leads to generation of free radicals (such as peroxyl, alkoxyl and aldehyde) which cause cell damage and leading to the release of marker enzymes. When liver and kidney cell plasma membrane is damaged, a variety of enzymes normally located in the cytosol are released in to the blood stream. Their estimation in the serum is a useful quantitative marker of the extent and type of hepatic and renal cellular damage[28]. The consequent increase in level of these enzymes ALP, ACP, AST, ALT, LDH and γGT and decrease of ACP (marker enzyme of the lysosomal membrane) in serum confirmed that damage has been inflicted on the plasma membrane, which might have resulted in the compromise of its integrity[29]. Disruption of the ordered lipid-bilayer of the membrane structure probably due to the presence of reactive oxygen species produced due to oxidative stress leading to escape of detectable quantity of these enzymes out of the cell into the extracellular fluid. The reactive oxygen species might have oxidized the polyunsaturated fatty acids which make up the lipid bilayer resulting in its disruption. The elevated levels of serum globulin also suggest adverse effect of reactive oxygen species on the secretory ability of the liver and hence it also effects the normal functioning of the organ. Increased bilirubin production, enhanced hepatic conjugation, and biliary excretion of the pigment present in aspartame treated animals may be as a result of decreased uptake, conjugation, or increased bilirubin production. A increase in the level of plasma total protein, decrease in A/G ratio with significant increase in total bilirubin observed in aspartame treated rats could be due to increased free radical production by methanol metabolite of aspartame. The elevated level of nitrite was positively increased with total bilirubin and negatively decreased with uric acid. Lower value of serum sodium indicated inability of kidney to conserve sodium. Haemo dilution too may be involved in the fall of sodium value via excess of water intake and or increased production of endogenous water increased in plasma volume and extracellular fluid volume would increase the circulating filling pressure, resulting in an increase in cardiac output and better blood flow to the kidneys. Increase of Potassium may be due to reduced excretion of K aggravated by leakage of intracellular potassium into blood stream as a result of methanol metabolite of aspartame induced lesions in renal tubular epithelium.

The present study clearly confirms that aspartame can be 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[30] it may be due to methanol, a metabolite of aspartame which stimulates nor epinephrine to act on corticotrophin releasing factor neurons in the paraventicular nucleus of hypothalamus to directly stimulate corticotrophin releasing factor release to acts on pituitary to release ACTH. Then ACTH acts on adrenal gland to release corticosterone secretion. Parthasarathy[2] reported a similar increase in plasma corticosterone level in rats after methanol administration for 1-day and 15-d. However after 30-d of methanol intoxication it showed considerable decrease in corticosterone level. This difference may be due to the higher dosage of methanol they have used.

In aspartame treated animals, irrespective of duration of exposure, there was decrease in total wbc count. This decrease appears to have a linear relationship as duration of oxidative stress increased. This reduced blood leucocyte numbers during stress reflect a dynamic redistribution of cells rather than loss of cells. glucorticoids mediate the trafficking of leucocyte out of the blood and among tissue during stress[31]. Based on these the redistribution of the leucocyte as suggested by seyle[32] possibly by circulating corticosteroids level. In current study an increase in lymphocyte percentage with a decrease in neutrophil percentage was observed in aspartame treated rats. The neutrophils and lymphocyte varies in opposite direction. Decrease in neutrophil can be attributed due to margination of neutrophils[33]. The decrease in neutrophil count may result from its abnormal distribution, due to local chemotaxis that causes the cell retention in several organs.

The increase level of lipid peroxidation, nitric oxide level is taken as direct evidence for oxidative stress[34]. The modified enzymatic and non-enzymatic free radical scavenging system with an elevated lipid peroxidation level after aspartame administration clearly indicated the generation of free radicals in present study. 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. Llipid peroxidation in cellular membranes damages polyunsaturated fatty acids tending to reduce membrane fluidity, which is essential for proper functioning of the cell. 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[2] who observed an increase lipid peroxidation level after methanol administration in the lymphoid organs. Similarly, Zararsiz[35] recorded a significant increase in lipid peroxidation level in the kidney of rats after treatment with formaldehyde.

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[36]. Catalase further detoxifies H_2O_2 into H_2O and O_2[36]. Glutathione peroxidase also functions in detoxifying H_2O_2 similar to CAT. Thus, SOD, CAT and GPx act mutually and constitute the enzymatic anti oxidative defense mechanism against reactive oxygen species[37]. In this study there as a marked increase of SOD, CAT and GPx enzyme activity after aspartame 15 d 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[38] and justifying the findings of this study. However, repeated administration for 30 d and 90 d 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[3]. Zararsiz[35] demonstrated that the renal tissue activity of SOD was significantly decreased in rats treated with formaldehyde when compared with the control animals. The decline in the activities of these enzymes might be due to their inactivation caused by excess reactive oxygen species production[39] normally, the antioxidant enzymes CAT and GPx protect SOD against inactivation by H_2O_2. Reciprocally, SOD protects CAT and GPx against superoxide anion. However, over load of free radical could have been upset 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[40] 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 recorded a decrease in SOD activity and there was a dose-response relationship between formaldehyde concentration and SOD activity[41].

Usually, GSH non-enzymatically reacts with superoxide, nitric oxide, hydroxyl radical and peroxynitrite radicals. Though during 15 d of aspartame administration were elevate GSH level, but after 30 d and 90 d 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[2]. The decrease in cellular GSH content increases cell vulnerability to oxidative stress[42].

GSH reduction can also explain the decreased concentration of vitamin C, which enters the cell mainly in its oxidized form where it is reduced by GSH[43]. 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[43].

The effect of aspartame on body weight ratio in this study suggests that the reactive oxygen species cause inflammation or constriction at the cellular levels of the rat organs. The decrease in body weight observed might be the result of protein wasting due to unavailability of carbohydrate for utilization as an energy source. The significant reduction in organ weight may be due to oxidative damage which

studies by Skrzydlewksa and Szynaka[44] who reported that oxidative damage caused marked organ weight loss in albino rats upon methanol intoxication. This is also reported by Parthasarathy[2]. Formaldehyde the first metabolite of methanol increases the population of shrunken cells, dead cells and hydolipid cells, Nakao[45] which might be the reason for decreased cellularity (reduction in organ weight).

The results of this study thus indicate that oral administration of aspartame (40 mg/kg body weight) lead to labialization of the cell plasma membrane, Such as disruption of the ordered lipid bilayer of the plasma membrane, has resulting in leakage of the enzymes to the the serum and induced oxidative stress. As the days of administration increased induces more oxidative stress and imbalancing the homeostasis in the whole body. These alterations may be due to the production of high number of free radical by methanol metabolite of aspartame.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

In spite of controversies report on aspartame, it is consumed by diabetic and weight loss regime people and also common people as it is easily available in the market as diet coke, table sugar chewing gum and in all sugar free products. Hence it is mandatory to have scientific validation to understand whether aspartame is toxic or not.

Research frontiers

To determine the macromolecular level changes following prolonged use of aspartame in animal model.

Related reports

Earlier reports of the same group [Ashok I and Dr. R. Sheeladevi, 2012] confirmed aspartame toxicity (75 mg/kg body weight) in discrete region of brain. They have also shown in 2011 that short term ingestion of aspartame (40 mg/kg body weight) resulted with the oxidative stress in cerebral cortex. Similarly in kidney and liver aspartame (40 mg/kg body weight) induced oxidative stress have been reported by Mourad (2011).

Innovations & breakthroughs

This study has made an attempt to elucidate the enzyme levels in the blood which could affect the homeostasis in the whole body and affect other systems too. Influence of these on hematological parameters is another highlight of this manuscript.

Applications

Creating awareness can be done to people who consumed aspartame if the toxicity is clearly scientifically proved.

Peer review

The study attempted by the authors is genuine and sincere by comprehensively carrying out related parameters to achieve their objective of confirming aspartame toxicity and its impact on the hepatic, renal and hematological parameters.

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