Available online at www.jbr-pub.org

Open Access at PubMed Central

JBR

The Journal of Biomedical Research, 2015, 29(5):390-396

Original Article

Acute effect of aspartame-induced oxidative stress in Wistar albino rat brain

Iyaswamy Ashok, Rathinasamy Sheeladevi ✉, Dapkupar Wankhar

Department of Physiology, Dr. ALM PG Institute of Basic Medical Sciences, University of Madras, Sekkizhar Campus, Taramani, Chennai 600 113, India.

Abstract

The present study was carried out to investigate the acute effect of aspartame on oxidative stress in the Wistar albino rat brain. We sought to investigate whether acute administration of aspartame (75 mg/kg) could release methanol and induce oxidative stress in the rat brain 24 hours after administration. To mimic human methanol metabolism, methotrexate treated rats were used to study aspartame effects. Wistar strain male albino rats were administered with aspartame orally as a single dose and studied along with controls and methotrexate treated controls. Blood methanol and formate level were estimated after 24 hours and rats were sacrificed and free radical changes were observed in discrete regions by assessing the scavenging enzymes, reduce dglutathione (GSH), lipid peroxidation and protein thiol levels. There was a significant increase in lipid peroxidation levels, superoxide dismutase activity (SOD), glutathione peroxidase levels (GPx), and catalase activity (CAT) with a significant decrease in GSH and protein thiol. Aspartame exposure resulted in detectable methanol even after 24 hours. Methanol and its metabolites may be responsible for the generation of oxidative stress in brain regions. The observed alteration in aspartame fed animals may be due to its metabolite methanol and elevated formate. The elevated free radicals due to methanol induced oxidative stress.

Keywords: aspartame, blood methanol, oxidative stress, antioxidant, free radical

Introduction

Aspartame (*L*-aspartyl-*L*-phenylalanine methyl ester), a low calorie sweetener, which was discovered in 1965[1], has biological effects at the recommended daily dose[2]. Approximately 50% of the aspartame molecule is phenylalanine, 40% is aspartic acid, and 10% is methanol[3]. Aspartic acid, a metabolite of aspartame, is an excitatory amino acid normally found in high levels in the brain. These levels are controlled by the blood-brain barrier which protects the brain from large fluctuations in plasma aspartate[4]. Phenylalanine is an amino acid essential to the production of monoamine in the brain and is found in nearly all foods that contain protein[5]. Due to high levels of phenylalanine in the blood, the consumption of aspartame may cause brain damage[6]. Certain amino acids are increased in the brain after aspartame consumption[7-9].

Among the metabolites, methanol, which is released during aspartame digestion, is a toxicant that causes systemic toxicity[10]. Large doses of aspartame and its individual metabolites have been tested in humans and other animals. It has been reported that not only the metabolites of methanol but also methanol itself

✉ Corresponding author: Dr. R Sheeladevi, Department of Physiology, ALMPG Institute of Basic Medical Sciences, University of Madras, Taramani Campus, Chennai 600 113, Tamilnadu, India. Tel/Fax: 91-44-24547162/91-44-24540709, E-mail: drsheeladeviibms@gmail.com.

Received 06 October 2012, Revised 13 November 2012, Accepted 13 April 2013, Epub 12 January 2014

CLC number: R741, Document code: A

The authors reported no conflict of interests.

doi: 10.7555/JBR.28.20120118

are toxic to the brain[11]. The primary metabolic fate of methanol is direct oxidation to formaldehyde and then into formate. The toxic effects of methanol in humans are due to the accumulation of its metabolite formate[12-13] and correlated with formate levels[14]. Formate is metabolized twice as fast in rat as in monkey[15]. The rodents do not develop metabolic acidosis during methanol poisoning, owing to their high liver folate content, and to create similar results in humans. Only folate deficient rodents are required to accumulate formate and to develop acidosis[16-17]. The aim of this study was to investigate whether acute oral administration of aspartame (75 mg/kg) can release methanol after metabolism and whether it induced oxidative stress in the rat brain regions after 24 hours of aspartame administration.

Materials and methods

Animals

Male Wistar albino rats (200–220 g) were maintained under standard laboratory conditions with water and food. For the folate deficient group, folate deficient diet was provided for 45 days before the experiment, and methotrexate was administered for a week before aspartame administration. The study was done according to the principles of laboratory care by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), government of India. The study protocol was approved by the Institutional Animal Ethical Committee (No: 01/032/2010).

Chemicals

Aspartame, methotrexate, and malondialdehyde were purchased from Sigma-Aldrich Co., (St. Luois, MO, USA). Reagents were purchased from Merck, Bangalore, India. All other chemicals were of analytical grade and obtained from Sisco Research Laboratory (Mumbai, India).

Experimental design

The rats were divided into 3 groups, including the saline control group, the methotrexate control group and the methotrexate plus aspartame group. Each group consisted of 6 animals. Aspartame mixed in sterile saline was administered orally as a single dose (75 mg/kg·body·weight) as previously described[18]. Methotrexate in sterile saline was administered (0.2 mg/kg·day) subcutaneously for 7 days to the folate deficient and the folate-deficient aspartame group[19]. One week after treatment with methotrexate, folate deficiency was confirmed by estimating urinary excretion of formamino glutamic acid (FIGLU)[20]. On day 8, only the methotrexate plus aspartame group received aspartame whereas the other two groups received equivalent volume of saline as an oral dose.

Sample collections

The blood samples and isolation of brain were performed between 8:00 am and 10:00 am to avoid circadian rhythm induced changes. Blood samples were collected as previously described[21]. The rats were sacrificed and the brain was immediately removed and washed with ice cold phosphate buffered saline (PBS). To expose the brain, the tip of curved scissors was inserted into the foramen magnum and a single lateral cut was made into the skull extending forward on the left and right side. With a bone cutter, the dorsal portion of the cranium was peeled off by using a blunt forceps. The brain was dropped onto an ice-cold glass plate leaving the olfactory bulbs behind. The whole process of brain removal took less than 2 minutes. After removal of the brain, it was blotted and chilled. Further dissection was performed on the ice-cold glass plate. The discrete regions of the brain (the cerebral cortex, cerebellum, midbrain, pons medulla, hippocampus and hypothalamus) were dissected according to a previously described method[22]. The homogenates (10%, w/v) of individual regions were prepared in a Teflon-glass tissue homogenizer, using ice-cold Tris HCl (100 mM, pH 7.4) buffer (only for SOD) and ice cold PBS, centrifuged separately in refrigerated centrifuge at 3000g for 15 minutes.

Measurement of blood methanol and formate

A 100 microliter plasma was deproteinized with an equal volume of acetonitrile and centrifuged for 7 minutes at 4°C[23]. The supernatant was analyzed for blood methanol and formate using a HPLC refractive index detector system (Shimadzu RID, Japan) (equipped with Rezex ROA-organic acid column 300 mm × 7.5 mm I.D, Phenomenex, Torrance, CA, USA) with the security guard cartridge column (AJO 4490, Phenomenex). Column oven was used to maintain the temperature at 60°C. The mobile phase was 0.026 N sulphuric acid, and the recovery of methanol (HPLC grade) from blood was found to be 92-96%. Linearity for methanol and formate was found to be 5-500 mg/100 microliter. The detector sensitivity for methanol and formate was found to be 5 mg/100 mL and reproducibility was $> 93\%$[24].

Biochemical determinations

Lipid peroxidation was measured at 532 nm by spectrophotometry and expressed as nanomole of malondialdehyde (an intermediary product of lipid peroxidation, using thiobarbituric acid) /mg protein[25]. The

activity of superoxide dismutase (SOD) was measured at 470 nm in 0, 1, 2, and 3 minute intervals. The activity was expressed as units/minute·mg protein[26]. Catalase was measured at 610 nm. The activity was expressed as the amount of H_2O_2 utilized/minute·mg protein[27]. Glutathione peroxidase (GPx), GSH and thiol were measured at 412 nm and expressed as units/minute/mg protein[28] μg of GSH/mg of protein, and μg/mg protein, respectively. Tissues were analyzed for protein and sulfhydryl concentration[29].

Statistical analysis

Statistical analysis was carried out using the SPSS statistical package version 17.0. The results were expressed as mean ± SD and the data were analyzed by the one-way analysis of variance (ANOVA) followed by Turkey's multiple comparison tests when there was a significant F test ratio. The level of significance was fixed at $P \leq 0.05$. Three replicate experiments were performed by ANOVA, the control group was compared with the methotrexate group and the aspartame methotrexate group, and the methotrexate group was compared with the aspartame methotrexate group.

Results

Blood methanol level and formate level

The data for the blood methanol level after 24 hours of aspartame administration are given in ***Table 1.*** Even after 24 hours, rats treated with aspartame and methotrexate rats (9.555 ± 0.36) showed significant increase in blood methanol level from controls as well as from the methotrexate treated group ($P<0.05$). The results of the blood formate level after 24 hours of aspartame administration are shown in ***Table 1***. Even after 24 hours, the aspartame treated methotrexate rats showed significant increase in the blood formate level from controls as well as from the methotrexate treated group. Blood formate level in the methotrexate aspartame group was 6.22 ± 0.810 μmol/L. There was significant accumulation of formates ($P<0.05$) in the methotrexate aspartame group compared to the methotrexate and saline control group.

Glutathione peroxidase, reduced glutathione and protein thiol

The results are shown in ***Table 2.*** The GSH levels in the methotrexate treated rats did not differ from the controls. However, the aspartame treated methotrexate rats showed a significant decrease in the GSH levels from control and methotrexate treated rats, with the cerebral cortex (0.023 ± 0.004), midbrain (0.037 ± 0.002), pons-medulla (0.025 ± 0.003) and hippocampus (0.021 ± 0.002). However, in the hypothalamus, the GSH level showed significant decrease from controls but not from the methotrexate treated group. Moreover, in the cerebellum (0.036 ± 0.002), the GSH level remained similar to control and methotrexate treated rats ($P<0.05$). The results are shown in ***Table 2***. The GPx activity in the methotrexate treated rats did not differ from the controls. However, the aspartame treated methotrexate rats showed a significant increase in GPx activity from control and methotrexate treated rats, with cerebral cortex (8.15 ± 0.27), cerebellum (7.78 ± 0.35), midbrain (8.11 ± 0.44), pons-medulla (9.28 ± 0.21), hippocampus (7.70 ± 0.31) and hypothalamus (8.65 ± 0.48). The results are shown in ***Table 2.*** The protein thiol levels in the methotrexate treated animals did not differ from the controls ($P<0.05$). However, the aspartame treated methotrexate rats showed a significant decrease in the protein thiol levels from control and methotrexate treated rats ($P<0.05$), with the entire brain regions such as the cerebral cortex (2.58 ± 0.51), cerebellum (2.31 ± 0.56), midbrain (2.82 ± 0.72), pons-medulla (2.45 ± 0.50), hippocampus (2.94 ± 0.27) and hypothalamus (1.7 ± 0.38).

Lipid peroxidation, superoxide dismutase and catalase

The results are shown in ***Table 3***. The SOD activity in the methotrexate treated rats did not differ from the controls. However, the aspartame treated methotrexate rats showed a significant increase in the SOD activity from control and methotrexate treated rats with the cerebral cortex (1.01 ± 0.094), cerebellum (1.03 ± 0.098), midbrain (1.25 ± 0.187), and pons-medulla (1.38 ± 0.121). However, in the hippocampus (1.52 ± 0.146) and

Table 1 **Effect of aspartame (75 mg/kg b.wt) on blood formate and methanol level (μmol/L)**

Parameter	Control	MTX	Asp+MTX
Blood methanol level	0.76 ± 0.047	1.79 ± 0.286	9.55 ± 0.361*#
Blood formate level	0.38 ± 0.064	1.13 ± 0.327	6.22 ± 0.810 *#

The data from various groups for the individual parameters are presented as table with mean ± SD (n=6). Significance fixed at $P<0.05$. The aspartame treated group when compared to control*, the MTX treated groups#. Asp: aspartame; MTX: methotrexate.

Table 2 **Effect of aspartame (75 mg/kg b.wt) on reduced GSH, GPx activity, and thiols in different rat brain regions**

Brain regions	Control	MTX	Asp+MTX
GSH (μg/mg protein)			
Cerebral cortex	0.042±0.004	0.038±0.004	0.023±0.004 * #
Cerebellum	0.046±0.002	0.044±0.003	0.036±0.002 * #
Midbrain	0.052±0.002	0.046±0.002	0.037±0.002 * #
Pons medulla	0.048±0.003	0.042±0.001	0.025±0.003 * #
Hippocampus	0.026±0.002	0.025±0.003	0.021±0.002 * #
Hypothalamus	0.030±0.003	0.024±0.005	0.016±0.002 * #
GPx (units/minute·mg protein)			
Cerebral cortex	5.36±0.21	6.13±0.87	8.15±0.27 * #
Cerebellum	4.41±0.35	5.46±0.67	7.78±0.35 * #
Midbrain	5.31±0.34	5.25±0.60	8.11±0.44 * #
Pons medulla	5.56±0.22	6.15±0.83	9.28±0.21 * #
Hippocampus	5.18±0.72	6.16±0.57	7.70±0.31 * #
Hypothalamus	4.65±0.50	5.45±0.56	8.65±0.48 * #
Thiols (μmol/L·mg protein)			
Cerebral cortex	6.2±0.57	5.16±0.53	2.58±0.51 * #
Cerebellum	6.46±0.48	5.43±0.33	2.31±0.56 * #
Midbrain	6.57±0.44	4.87±0.56	2.82±0.72 * #
Pons medulla	5.91±0.85	5.13±0.42	2.45±0.50 * #
Hippocampus	6.20±0.42	5.34±0.39	2.94±0.27 * #
Hypothalamus	5.46±0.52	4.18±0.38	1.7±0.38 * #

The data are presented as mean±SD (n=6). Significance fixed at $P<0.05$. The aspartame treated group compared to control*, and the MTX treated groups#. Three replicate experiments were done by the one-way analysis of variance (ANOVA) (n=6). The control group was compared with the MTX group and the aspartame MTX group. The MTX group was compared with the aspartame+ MTX group. Asp: aspartame; MTX: methotrexate.

hypothalamus (2.01±0.147), the SOD activity was not different from controls as well as from methotrexate treated rats ($P<0.05$). The results are shown in ***Table 3***. The CAT activity in the methotrexate treated rats did not differ from the controls. However, the aspartame treated methotrexate rats showed a significant increase in the CAT activity from the control and methotrexate treated rats, with the cerebral cortex (6.82±0.40), cerebellum (6.91±0.51), pons-medulla (7.11±0.41), hippocampus (7.13±0.37) and hypothalamus (7.23±0.35). Only in the midbrain (6.53±0.54), there was a significant increase in the CAT activity from control and remained similar to the methotrexate treated rats ($P<0.05$). The results are shown in ***Table 3***. The lipid peroxidation levels in the methotrexate treated rats did not differ from the controls. However, the aspartame and methotrexate treated rats showed a significant increase in the lipid peroxidation level in the entire brain regions including the cerebral cortex (2.50±0.17), cerebellum (2.43±0.21), midbrain (2.26±0.17), pons-medulla (2.23±0.17), hippocampus (2.37±0.15) and hypothalamus (2.21±0.09) from the control as well as from the methotrexate treated rats ($P<0.05$).

Discussion

Aspartame is an artificial sweetener used in day to day life and is consumed in many ways such as beverages, chewing gum, food products and other edible products. This study strengthened the study design that upon consumption, aspartame was metabolized in the gut and generated methanol which is freely available in the blood after 24 hours in the system. Our findings corroborate our previous findings that long term exposure of aspartame showed detectable methanol in the plasma which might have induced oxidative stress[30]. Kruse[31] has reported that methanol is the toxicant among the aspartame metabolites that causes systemic toxicity. According to Jeganathan and Namasivayam[32], methanol is toxic to the brain as the increased blood methanol level can lead to severe shifts in brain monoamine levels. It is well known that the nervous system is highly susceptible to methanol intoxication.

Oxidative stress is the general phenomenon of oxidant exposure and antioxidant depletion, or oxidant-antioxidant balance[33]. The central nervous system (CNS) is vulnerable to free radical damage because of brain′s high oxygen consumption, its abundant lipid content, and the relative

Table 3 **Effect of aspartame (75 mg/kg b.wt) on SOD, CAT and LPO in rat brain discrete regions**

Brain regions	Control	MTX	Asp +MTX
SOD (Units/minute/mg protein)			
Cerebral cortex	0.62±0.01	0.66±0.08	1.01±0.09 * #
Cerebellum	0.77±0.04	0.79±0.08	1.03±0.09 * #
Midbrain	0.64±0.03	0.65±0.10	1.25±0.18 * #
Pons medulla	0.75±0.03	0.72±0.10	1.38±0.12 * #
Hippocampus	1.40±0.11	1.38±0.19	1.52±0.14 * #
Hypothalamus	1.84±0.17	1.90±0.31	2.01±0.14 * #
CAT (Amount of H_2O_2 utilized/minute·mg protein)			
Cerebral cortex	4.85±0.30	5.13±0.70	6.82±0.40 * #
Cerebellum	4.83±0.34	5.58±0.61	6.91±0.51 * #
Midbrain	5.13±0.25	5.95±0.63	6.53±0.54 * #
Pons medulla	5.98±0.34	6.30±0.19	7.11±0.41 * #
Hippocampus	6.12±0.33	6.35±0.27	7.13±0.37 * #
Hypothalamus	6.16±0.33	6.48±0.31	7.23±0.35 * #
LPO (nmol/L of malondialdehyde/mg protein)			
Cerebral cortex	1.70±0.18	1.61±0.24	2.50±0.17 * #
Cerebellum	1.63±0.12	1.57±0.23	2.43±0.21 * #
Midbrain	1.60±0.13	1.58±0.26	2.26±0.17 * #
Pons medulla	1.65±0.13	1.55±0.19	2.23±0.17 * #
Hippocampus	1.63±0.15	1.56±0.21	2.37±0.15 * #
Hypothalamus	1.62±0.28	1.53±0.28	2.21±0.09 * #

The data are presented mean±SD (n=6). Significance fixed at $P<0.05$. The aspartame treated group compared to control*, and the MTX treated groups#. Three replicate experiments were done by the one-way analysis of variance (ANOVA) (n=6). The control group was compared with the MTX group and the aspartame MTX group. The MTX group was compared with the aspartame+ MTX group. Asp: aspartame; MTX: methotrexate.

paucity of antioxidant enzymes as compared with other tissues. In this study, marked elevation in free radicals was indicated by increase in SOD, catalase and GPx in the entire brain regions studied. Elevated SOD indicates excess superoxide radical production. According to McCord[34], prolonged exposure to free radicals, even at a low concentration, may damage biologically important molecules. It has been reported that when the amount of super oxides produced overwhelms the SOD/catalase system, can damage cell membranes, DNA and cell proteins[35]. However, in spite of increase in SOD and GPx activity in the brain, elevated LPO level indicates that the activity increase may not be sufficient to cope up with free radical generation. Mourad[36] report supports our present observation that oral administration of aspartame (40 mg/ kg) led to a significant elevation in LPO level in the liver tissue after four and six weeks of treatment. Furthermore, they also observed a significant increase in LPO level in the renal tissue at the end of six weeks of treatment. Vidyasagar et al.[37] reported that increased SOD levels were only partially effective in combating the oxidative damage. This may be because, in spite of increase in SOD, GPx and catalase enzyme activity, there was an increase in the LPO level in the entire brain regions studied. Moreover, the limitation of GPx and catalase may be based on their location within the cells.

In the present study, increase in GPx activity was associated with decrease in the GSH. The decrease in GSH levels has obvious implications, as it is a substrate for GPx[38]. The elevated LPO observed after aspartame treatment regardless of the duration of treatment may be due to decrease in GSH as GSH itself can remove free radicals. GSH itself could protect against oxidative insults[39]. The decrease in GSH observed throughout the brain may be due to methanol, a metabolite of aspartame. The decrease in GSH concentration was well correlated with the methanol metabolism that depends upon GSH[40]. Abilash et al.[41-42] concluded that long term consumption of aspartame leads to hepatocellular injury and alterations in liver antioxidant status mainly through glutathione dependent pathway, which is also parallel to the present findings.

Oxidative stress is based on changes in post translational protein thiol modifications. Changes of the primary structure of proteins also cause modification in their secondary and tertiary structures. There is also a decrease in protein thiol in this study, which indicates protein thiol oxidation. Oxygen radicals can also induce formation of peroxides[43]. Patsoukis et al.[44] and Nikolaos et al.[45]

observed decreased protein thiol in the brain due to oxidative damage. As free radicals induced denaturation, aggregation and fragmentation of protein molecules have been reported, alteration in protein thiol observed in this study supports the toxicity of aspartame at protein level modifications. The decrease in glutathione may cause this decrease in protein thiol. The present study demonstrates increased free radicals resulting in an imbalanced anti-oxidant status in the brain after aspartame consumption. After an acute exposure to aspartame, detectable methanol continues to circulate in the blood even after 24 hours; methanol and its metabolites may be responsible for the generation of oxidative stress in the brain regions.

Conclusion

The observed alteration in aspartame fed animals may be due to its metabolite methanol and elevated formate. Elevated free radicals due to methanol may have induced oxidative stress. This study confirms the presence of toxic metabolites after aspartame administration. However, further studies are required to understand aspartame action in depth.

Acknowledgement

The author is grateful for the valuable suggestion offered by Dr. NJ Parthasarathy and Co-authors. The financial assistance was provided by the University of Madras.

References

[1] Garriga MM, Metcalfe DD. Aspartame intolerance[J]. *Ann Allergy,* 1988,61(Pt 2):63-69.

[2] Gombos K, Varjas T, Orsós Z, et al. The effect of aspartame administration on oncogene and suppressor gene expressions[J]. *In Vivo*, 2007,21(1):89-92.

[3] Newsome RL. Sweeteners: Nutritive and non-nutritive. In: *The Scientific Status Summaries of the Institute of Food Technologies Expert Panel on Food Safety and Nutrition, Chicago: Institutive of Food Technologies*, 1986.

[4] Maher TJ, Wurtman RJ. Possible neurologic effects of aspartame, a widely used food additive[J]. *Environ Health Persp*, 1987,75:53-57.

[5] Fernstrom JD, Fernstrom MH, Gillis MA. Acute effects of aspartame on large neutral amino acids and monoamines in rat brain[J]. *Life Sci*, 1983,32(14):1651-1658.

[6] Haschemeyer RH, Haschemeyer AEV. Proteins. In: *A Guide to Study by Physical and Chemical Methods, New York: John Wiley & Sons*, 1973:1-30.

[7] Dailey JW, Lasley SM, Burger RL, et al. Amino acids, monoamines and audiogenic seizures in genetically epilepsy-prone rats: effects of aspartame[J]. *Epilepsy Res*, 1991,8(2):122-133.

[8] Diomede L, Romano M, Guiso G, et al. Interspecies and interstrain studies on the increased susceptibility to metrazol-induced convulsions in animals given aspartame[J]. *Food Chem Toxicol*, 1991,29(2):101-106.

[9] Yokogoshi H, Roberts CH, Caballero B, et al. Effects of aspartame and glucose administration on brain and plasma levels of large neutral amino acids and brain 5-hydroxyindoles[J]. *Am J Clin Nutr*, 1984,40(1):1-7.

[10] Kruse JA. Methanol poisoning[J]. *Intensive Care Med*, 1992,18(7):391-397.

[11] Jeganathan PS, Namasivayam A. Methanol induced biogenic amine changes in discrete areas of rat brain: Role of simultaneous ethanol administration[J]. *Ind J Physiol Pharmacol*, 1998,32(1):1-10.

[12] Tephly TR, McMartin KE. Methanol metabolism and toxicity. In: *Aspartame-Physiology and Biochemistry, New York: Marcel Dekker*, 1984:111-140.

[13] Tephly TR. The toxicity of methanol[J]. *Life Sci*, 1991, 48(11):1031-1041.

[14] Osterloh JD, Pond SM, Grady S, et al. Serum formate concentrations in methanol intoxication as a criterion for hemodialysis[J]. *Ann Intern Med*, 1986,104(2):2000-2003.

[15] McMartin KE, Martin-Amat G, Noker PE, et al. Lack of a Role for Formaldehyde in Methanol Poisoning in the Monkey[J]. *Biochem Pharm*, 1979,28(5):645-649.

[16] Lee EW, Garner CD, Terzo TS. Animal model for the study of methanol toxicity: comparison of folate-reduced rat responses with published monkey data[J]. *J Toxicol Environ Health*, 1994,41(1):71-82.

[17] Eells JT, Henry MM, Lewandowski MF, et al. Development and characterization of a rodent model of methanol-induced retinal and optic nerve toxicity[J]. *Neurotoxico*, 2000,21(3):321-330.

[18] Arthur SL, Donald BH, Catherine B, et al. Safety of Long-term Large Doses of Aspartame[J]. *Arch Intern Med*, 1989,149(10):2318-2324.

[19] Gonzalez-QA, ObregonF, Urbina M, et al. Effects of chronic methanol administration on amino acid and monoamines in retina, optic nerve, and brain of the rat[J]. *Toxicol Appl Pharmacol*, 2002,185(2):77-84.

[20] Tabor H, Wyngarden.A method for determination of formiminoglutamic acid in urine[J]. *J Clin Inrest*, 1962,37(6):824-828.

[21] Feldman S, Conforti N. Participation of dorsal hippocampus in the glucocorticoids feedback effect on adrenocortical activity[J]. *Neuroendocrino*, 1980,30(1):52-55.

[22] Glowinski J, Iverson LL. Regional studies of catecholamines in the rat brain[J]. *I J Neurochem*, 1996,13(8):655-669.

[23] Dorman DC, Moss OR, Farris GM, et al. Pharmacokinetics of inhaled methanol and methanol derived formate in normal and folate deficiency cynomolgus monkeys[J]. *Toxicol Appl Pharmacol*, 1994,128(2):229-238

[24] Pecina R, Bonn G, Burtscher E et al. High performance liquid chromatographic elution behavior of alcohols, aldehydes, ketones, organic acids and Carbohydrates on a strong catino-exchange stationary phase[J]. *J Chromatogr*, 1984, 287:245-258

[25] Ohkawa H, Ohishi M, Yagi. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction[J]. *Anal biochem*, 1970,95(2):351-358.

[26] Marklund S, Marklund G. Involvement of the Superoxide anion radical in the auto oxidation of Pyrogallol and a convenient assay for superoxide dismntase[J]. *J Biochem*, 1974,47(3):469-474.

[27] Asru K, Sinha. Calorimetric assay of Catalase[J]. *Anal Biochem*, 1972,47(2):389-394.

[28] Rotruck JT, Pope AC, Ganther HE, et al. Selenium biochemical role as a component of glutathione peroxidase[J]. *Sci*, 1973,179(4073):588-590.
[29] Sedlack J, Lindsay RH. Estimation of total, protein bound and non-protein sulfhydryl groups in the tissue with Elman's reagent[J]. *Anal Biochem*, 1968,25(1):192-205.
[30] Ashok I, Sheeladevi R. Effect of chronic exposure to aspartame on oxidative stress in brain discrete regions of albino rats[J]. *J Biosci*, 2012,37(4):1-10.
[31] Kruse JA. Methanol poisoning. Review article[J]. *Intensive Care Med*, 1992,18(7):391-397.
[32] Jeganathan PS, Namasivayam A. Methanol induced biogenic amine changes in discrete areas of rat brain: Role of simultaneous ethanol administration[J]. *Ind J Physiol Pharmacol*, 1998,32:1-10.
[33] Bidlack R Wayne, Lancaster, Pa. TECHNOMIC. Phytochemicals a new paradigm[M]. *Food Toxicology*, 1998,179.
[34] McCord JM. The evolution of free radicals and oxidative stress[J]. *Am J Med*, 2000,108(8):65218-65222.
[35] Blum J, Fridovich I. Inactivation of glutathione peroxidase by superoxide dismutase radical[J]. *Arch Biochem Biophys*, 1985,240(2):500-508.
[36] Mourad IM, Noor NA. Aspartame (a widely used artificial sweetener) and oxidative stress in the rat cerebral cortex[J]. *Int J Pharm Biomed Sci*, 2011,2(1):4-10.
[37] Vidyasagar J, Karunakar N, Reddy MS, et al. Oxidative stress and antioxidant status in acute organophosphorous insecticide poisoning[J]. *Indian J Pharmacol*, 2004,36(2):76-79.
[38] Sáez JC, Bennett MVL, Spray DC. Hepatocyte gap junctions, metabolic regulation and possible role in liver metabolism[M]. In: *Transduction in Biological Systems, New York: Plenum Publishing Corp,* 1990:231-243.
[39] Winterbourn CC, Metodiewa D. The reaction of superoxide with reduced glutathione[J]. *Arch Biochem Biophys*, 1994,314(2):284-290.
[40] Pankow D, Jagielki S. Effect of methanol on modifications of hepatic glutathione concentration on the metabolism of dichloromethane to carbon monoxide in rats[J]. *Hum ExpToxicol*, 1993,12(3):227-231.
[41] Abhilash M, Paul MV, Varghese MV, et al. Effect of long term intake of aspartame on antioxidant defense status in liver[J]. *Food Chem Toxicol*, 2011,49(6):1203-1207.
[42] Abhilash M, Sauganth Paul MV, Mathews V, et al. Long-term consumption of aspartame and brain antioxidant defense status[J]. *Drug Chem Toxicol*, 2012,36(2):1-6.
[43] Gebicki S, Gebicki JM. Formation of peroxides in amino acids proteins exposed to oxygen free radicals[J]. *Biochem J,* 1993,289(Pt 3):743.
[44] Patsoukis N, Zervoudakis G, Panagopoulos NT, et al. Thiol redox state (TRS) and oxidative stress in the mouse hippocampus after pentylenetetrazol-induced epileptic seizure[J]. *Neurosci Lett*, 2004,357(2):83-86.
[45] Nikolaos P, George Z, Nikolaos TP, et al. Thiol redox state (TRS) and oxidative stress in the mouse hippocampus after pentylenetetrazol-induced epileptic seizure[J]. *Neurosci Lett*, 2004,357:83-86.