Original article

Long term effect of aspartame (Artificial sweetener) on membrane homeostatic imbalance and histopathology in the rat brain

Iyaswamy Ashok*, Rathinasamy Sheeladevi, Dapkupar Wankhar

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

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Background: The study focused to long-term effect of aspartame on membrane bound enzymes, oxidative stress markers and histopathology in brain regions of Wistar albino rats. Hence it is essential to observe whether the chronic aspartame administration (75 mg/kg b. wt) could release methanol and induce oxidative stress in the rat brain. Many reports are available on the use of aspartame as it releases methanol during metabolism.

Methods: To mimic the human methanol metabolism the methotrexate treated rats were included to study the aspartame effects and the gamma glutamyl transpeptidase, NO, H2O2 and the membrane bound enzymes were observed in brain discrete regions.

Results: There was a significant increase in all the parameters except with a significant decrease in membrane bound ATPases and creatine kinase. Luxol fast blue (LFB) staining were performed on brain cerebellum region which showed histopathological changes in aspartame treated MTX animals which showed a marked decrease in the density of white matter, intensity of staining and no. of stained neuron cells when compared to control and MTX control animals.

Conclusion: Moreover, the increases in some of these enzymes were due to methanol per se and its metabolite may be responsible for the generation of oxidative stress and histopathology in brain regions.

Some information is available on the aspartame induced toxicity at various levels. Moreover, most of the recent studies on aspartame, have been carried out to understand the mechanisms of neurotoxicity and cancer. Na+- K+-ATPase is responsible for the generation of the membrane potential through the active transport of sodium and potassium ions in the CNS necessary to maintain neuronal excitability. Ca2+-ATPase is responsible for fine-tuning of intracellular calcium levels. Moreover, the role of Mg2+-ATPase is to maintain high brain intracellular Mg2+ which can control rates of protein synthesis and cell growth. ATPase's are sensitive to peroxidation reaction and lipid peroxides. ATPase intimately associated with the plasma membrane and participates in the energy requiring translocation of sodium, potassium, calcium and magnesium ions. In the earlier report on aspartame for (75 mg/kg body weight), there was a marked increase in free radical generation in the entire brain regions. Free radicals can cause membrane damage through peroxidation of unsaturated fatty acids in the phospholipids making up the cell membrane. Peroxidation of membrane is accompanied by alteration of the structural and functional characteristics of membranes. They can also cause damage to fundamental cellular components such as nucleic acid
lesions, gene damage, and gene repair activity, leading to subsequent cell death by necrotic or apoptotic mode. Lipid peroxidation changes the activities of various lipid dependent membrane-bound enzymes such as ATPases, generation of free radicals such as peroxy, alkoxyl and aldehyde. Free radicals can cause severe damage to the membrane bound enzymes such as Ca2+ ATPase, Mg2+ ATPase and Na+K+ ATPase. Hence the activity of these membrane bound enzyme during aspartame ingestion forms the focus of this study.

Oxidative stress is considered to be crucial in the neural degeneration. The toxicity of ROS can be further increased by forming peroxynitrite (ONOO−), which is a molecule that causes oxidation and nitration of tyrosine residues on proteins. Other important oxidant species are hydrogen peroxide (H2O2) and the hydroxyl free radical (OH−). An elevation of H2O2, OH− and NO can result in the oxidative stress, which are considered as the biomarkers of oxidative stress. Catalase is the key enzyme of methanol oxidation in the brain of rodents, gamma-glutamyl transferase (GGT) is the most sensitive and widely employed marker enzyme to assist in diagnosis of alcohol systemic toxicity, elevated levels of these enzymes may indicate alcoholic hepatitis or cirrhosis. Rat brain creatine kinase expression is highest in the brain but is also detectable at lower levels in some other tissues. In the brain, the creatine kinase is thought to be involved in the regeneration of ATP necessary for transport of ions and neurotransmitters.

Large doses of both aspartame as well as these individual metabolites have been tested in humans and other animals producing a controversial report. It has been reported that not only the metabolites of methanol but methanol per se as well is toxic to the brain. The severity of clinical findings in methanol intoxication correlated better with formate levels. Formate is metabolized twice as fast in the rat as in the monkey. The rodents do not develop metabolic acidosis during methanol poisoning, owing to their high liver folate content and in order to create similar results in human beings only folate deficient rodents are required to accumulate formate in order to develop acidosis. Hence, in this study in order to mimic the human situation, a folate deficiency status is induced by administering MTX. The focus of this study is to observe what happens to membrane bound enzymes, oxidative stress markers and histopathology in the rat brain regions during the long-term oral administration of aspartame (75 mg/kg b.wt.)

2. Materials and methods

2.1. Animals

Wistar strain male 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 prior to the experiment and MTX was administered for a week before the experiment. The animals were handled according to the principles of laboratory care framed by the committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. Prior to the experimentation, proper approval was obtained from the Institutional Animal Ethical Committee (No: 01/032/2010).

2.2. Chemicals

Aspartame and methotrexate were purchased from Sigma–Aldrich Co., St. Louis, USA. Nitric oxide assay kit colorimetric Cat#482650 was purchased from Calbiochem, USA. All the other chemicals were of analytical grade obtained from Sisco research laboratory, Mumbai, India.

2.3. Experimental design

2.3.1. Aspartame dose

In order to confine within the human exposure limit, this dose was selected. A 1 L (approx. 1 quart) aspartame-sweetened beverage contains about 56 mg of methanol was used. Heavy users of aspartame-containing products consume as much as 250 mg of methanol daily, or 32 times above the EPA limit. However early reports on aspartame for the dose (75 mg/kg body weight) was controversial. This provided additional interest to use this dosage in our study. Aspartame mixed in sterile saline was administered orally (75 mg/kg body weight) and this dosage based on the earlier report.

2.3.2. Groups

The rats were divided in to three groups, namely, saline control, MTX-treated control, and MTX-treated aspartame administered groups. Each group consisted of six animals. MTX in sterile saline was administered (0.2 mg/kg/day) subcutaneously for 7 days to folate-deficient treated as well as to folate-treated aspartame groups. One week after treatment with MTX, folate deficiency was confirmed by estimating the urinary excretion of formiminoglutamic acid (FIGLU). From the eighth day, only the MTX-treated aspartame group received the aspartame, whereas the other two groups received equivalent volumes of saline as an oral dose and all animals were handled similarly. The chronic dose of aspartame was given for 90 days and all the animals were fed folate-deficient diet except the control animals till 90 days.

2.4. Sample collections

The blood samples and isolation of brain was performed between 8 and 10 a.m. to avoid circadian rhythm induced changes. The animals were sacrificed using higher dose of long acting pentathol sodium (100 mg/kg b.wt).

2.5. Brain dissection

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 cranium was peeled off, and using a blunt forceps, the brain was dropped onto the ice-cold glass plate, leaving the olfactory bulbs behind. The whole process of removing brain took less than 2 min. After removing the brain, it was blotted and chilled. Further dissection was made on ice-cold glass plate. The discrete regions of brain (cerebral cortex, cerebellum, midbrain, pons medulla, hippocampus and hypothalamus) were dissected according the method given by Glowinski and Iverson. The homogenate (10% w/v) of the individual regions were prepared in a Teflon-glass tissue homogenizer, using ice-cold Tris HCl (100 mm, pH 7.4) buffer and centrifuged separately in refrigerated centrifuge at 3000 rpm for 15 min. The supernatant was used for analyzing the parameters in this study.

2.6. Estimation of Na+ K+ ATPase

The activity of Na+ K+ ATPase (ATP: Phosphohydrolase – EC. 3.6.1.3) in the tissue was estimated by the method of Bonting et al. The liberation of inorganic phosphorous by the enzyme action (incubation of the tissue extract) could be measured and development of blue color, which was read at 620 nm against the reagent
2.7. Estimation of Ca^{2+} ATPase

The activity of Ca^{2+}-ATPase (ATP: Phosphohydrolase — EC. 3.6.1.3.) in the tissues was estimated as described by Hjerten and Pan.\(^{30}\) The liberation of inorganic phosphorous from the tissue upon its incubation in a medium could be measured, and the development of blue color, which was read at 620 nm against the reagent blank using spectrophotometer. The activity of Ca^{2+}-ATPase in the tissue is expressed as \(\mu\) mol of phosphorous liberated/min/mg protein.

2.8. Estimation of Mg^{2+} ATPase

The activity of Mg^{2+}-ATPase (ATP: Phosphohydrolase — EC. 3.6.1.3.) in the tissues was estimated by the methods of Ohnishi et al.\(^{31}\) The inorganic phosphorous liberated from the tissue upon its incubation in a medium could be measured, and the development of blue color, which was read at 620 nm against the reagent blank using spectrophotometer. The activity of Mg^{2+}-ATPase in the tissue was expressed as \(\mu\) mol of phosphorous liberated/min/mg protein.

2.9. Assay of \(\gamma\)-glutamyl transpeptidase (EC 2.3.2.2)

The activity of \(\gamma\)-glutamyl transpeptidase was estimated according to the method of Orlowski and Meister.\(^{32}\) The amount of \(p\)-nitroaniline in the supernatant was measured at 410 nm. The activity of \(\gamma\)-glutamyl transferase was expressed as \(\mu\)mol of \(p\)-nitroaniline formed/min/mg protein.

2.10. Hydrogen peroxide

The hydrogen peroxide generation was assayed by the method of Pick and Keisari.\(^{33}\) Horse radish peroxidase converts hydrogen peroxide into water and oxygen. This causes oxidation of phenol red, which forms adduct with dextrose which has maximum absorbance at 610 nm. The hydrogen peroxide generated was expressed as mM of H\(_2\)O\(_2\) generated/mg protein.

2.11. Determination of nitrite using nitric oxide assay kit

To investigate nitric oxide formation it is essential to measure nitrite (NO\(_2\)), which is one of two primary, stable and nonvolatile breakdown products of NO. This assay relies on a diazotization reaction that was originally described by Griess.\(^{34}\) The original reaction has been described as Griess reagent system is based on the chemical reaction, which uses sulfanilamide and N-1-naphthylethylenediamine dihydrochloride (NED) under acidic (phosphoric acid) conditions.

2.12. Creatine kinase Activity (EC 2.7.3.2.)

It was estimated by the method of Okinaka et al.\(^{35}\) In this method, creatine kinase catalyses the conversion of creatine to creatine phosphate. Phosphate reacts with ammonium molybdate to form phospho molybdate. The hexavalent molybdenum of phospho molybdate is reduced by ANSA to give blue color complex, which is measured at 640 nm. The colour thus developed was read spectrophotometrically at 640 nm after 20 min. The enzyme activity was expressed \(\mu\) mol of phosphorous liberated/min/mg protein.

2.13. Histopathology

Animals were deeply anesthetized with ketamine hydrochloride. Rats were then perfused transcardially with phosphate-buffered saline, followed by buffered 10% formalin. The brain, was removed, and preserved in formalin until processed for histology. Then kept on running water to remove formalin pigments and dehydrated with ascending grades of alcohol. After impregnation with paraffin wax, the paraffin blocks were made. They were processed and sections were cut with 10 \(\mu\)m in thickness using “Spencer Lens, rotatory microtome (no 820, New York, USA) and then stained with Luxol fast blue and eosin stain as follows for brain.

2.14. Statistical analysis

Statistical analysis was carried out using the SPSS statistical package version 17.0. The results are expressed as mean ± STD and the data were analyzed by the one-way analysis of variance (ANOVA) followed by Turkey’s multiple comparison tests when there is a significant ‘F’ test ratio. The level of significance was fixed at \(p \leq 0.05\).

3. Results

The data from various groups for the individual parameters are presented as bar diagram with mean ± STD.

3.1. Na^+- K^+-, Ca^{2+}- and Mg^{2+}-ATPase

The results are given in Fig. 2. The Na^+- K^+-ATPase levels in the MTX treated animals did not differ from the controls. However, the aspartame treated MTX animals showed a marked decrease in the Na^+- K^+-ATPase level in the entire brain regions studied such as cerebral cortex (df 2, \(F = 80\)), cerebellum (df 2, \(F = 33\)), midbrain (df 2, \(F = 88\)), pons-medulla (df 2, \(F = 68\)), hippocampus (df 2, \(F = 18\)), hypothalamus (df 2, \(F = 158\)), from the control as well as from the MTX treated animals.

The results are given in Fig. 3. The Ca2+ ATPase activity in the MTX treated animals did not differ from the controls. However, the aspartame treated MTX animals showed a marked decrease in the Ca2+ ATPase activity in cerebral cortex (df 2, \(F = 117\)), cerebellum (df 2, \(F = 247\)), midbrain (df 2, \(F = 203\)), pons-medulla (df 2, \(F = 56\)), hippocampus (df 2, \(F = 71\)) and hypothalamus (df 2, \(F = 24\)) from the controls as well as from the MTX treated animals.

The results are given in Fig. 4. The Mg^{2+}-ATPase activity in the MTX treated animals did not differ from the controls. However, the aspartame treated MTX animals showed a marked decrease in the Mg^{2+}-ATPase activity in cerebral cortex (df 2, \(F = 56\)), cerebellum (df 2, \(F = 14\)), midbrain (df 2, \(F = 92\)), pons-medulla (df 2, \(F = 22\)), hippocampus (df 2, \(F = 45\)) and hypothalamus (df 2, \(F = 86\)) from the control as well as from the MTX treated animals.

3.2. \(\gamma\)-Glutamyl transpeptidase (EC 2.3.2.2)

The results are given in Fig. 6. The \(\gamma\)-glutamyl transpeptidase activity in the MTX treated animals did not differ from the controls. However, the aspartame treated MTX animals showed a uniform marked increase in the \(\gamma\)-glutamyl transpeptidase activity in the entire brain regions such as cerebral cortex (df 2, \(F = 32\)), cerebellum (df 2, \(F = 175\)), midbrain (df 2, \(F = 37\)), pons-medulla (df 2, \(F = 162\)), hippocampus (df 2, \(F = 99\)), hypothalamus (df 2, \(F = 47\)) from control and MTX treated animals.
3.3. Hydrogen peroxide

The results are given in Fig. 5. The hydrogen peroxide activity in the MTX treated animals did not differ from the controls. However, the aspartame treated MTX animals showed a marked increase in

![Fig. 5. Effect of aspartame on hydrogen peroxide in the brain discrete regions of rats (μ moles of H₂O₂ generated/mg protein). Data are expressed as mean ± SD, n = 6. *P < 0.05 when compared with control group and folate deficient group.](image-url)
the hydrogen peroxide activity from control and MTX treated animals, in cerebral cortex (df 2, F = 25), cerebellum (df 2, F = 34), midbrain (df 2, F = 44), pons-medulla (df 2, F = 62), hippocampus (df 2, F = 12) and hypothalamus (df 2, F = 25).

3.4. Nitric oxide

The results are given in Fig. 8. The nitric oxide activity in the MTX treated animals did not differ from the controls. However, the aspartame treated MTX animals showed a marked increase in the nitric oxide activity from control and MTX treated animals, in cerebral cortex (df 2, F = 25), cerebellum (df 2, F = 34), midbrain (df 2, F = 44), pons-medulla (df 2, F = 62), hippocampus (df 2, F = 12) and hypothalamus (df 2, F = 25).

3.5. Creatine kinase Activity

The results are given in Fig. 7. The creatine kinase activity in the MTX treated animals did not differ from the controls. However, the aspartame treated MTX animals showed a marked decrease in the creatine kinase activity in cerebral cortex (df 2, F = 9), cerebellum (df 2, F = 25), midbrain (df 2, F = 59), pons-medulla (df 2, F = 242), hippocampus (df 2, F = 76) and hypothalamus (df 2, F = 329).

3.6. Histopathology of brain

Luxol fast blue (LFB) staining was performed on brain cerebellum region. The results are given in (Fig. 1). Upon gross examination, the white matter tracts, as visualized by LFB, appeared thinner in Asp + MTX treated animals when compared to control and MTX controls. At higher magnification, LFB staining revealed an array of densely stained myelinated fibers traversing through the white matter in the aspartame treated MTX animals. In contrast, the aspartame treated MTX animals cerebellar white matter appeared disorganized, less intensely stained and littered with ‘holes’. Furthermore, the number of densely stained cell bodies in the white matter was decreased in aspartame treated MTX animals and the morphology matter was first observed, suggesting the major afferent and efferent projections were undergoing widespread degeneration when compared to the control animals and MTX treated control animals. The quantification of density of white matter (Fig. 1a), intensity of staining (Fig. 1b) and number of stained cells (Fig. 1c) were done in three groups, which statistically showed a marked decrease in aspartame treated animals when compared to control and MTX control.

4. Discussion

The observed results support the toxic nature of aspartame when consumed repeatedly for a prolonged period. Upon ingestion, aspartame is immediately absorbed from the intestinal lumen and metabolized to phenyalanine, aspartic acid and methanol. Following aspartame consumption, the concentrations of its metabolites are increased in the blood. In our earlier report for the same dose of aspartame the increase in blood methanol levels was observed. A small amount of aspartame significantly increases the plasma methanol level. Moreover this increase in blood methanol level was associated with a marked increase in the free radical generation in brain regions of aspartame treated MTX animals. This increase in free radical may be the cause behind the decrease in the activity of membrane bound ATPases as well as decrease in creatine kinase activity observed in this study. As lipid per oxidation alters the membrane integrity, Na⁺-K⁺-ATPase, the enzyme that maintains Na⁺ and K⁺ gradients across the plasma membrane, was reported to be inhibited by ROS in the brain. Strength the argument. According to Zhang et al and Polizzi et al, the Na⁺-K⁺-ATPase is very sensitive to the plasma membrane structure changes and therefore measuring its activity represents a valuable indicator of the early and late stages of tissue injury. Further, the decrease in the activity of these membrane bound enzymes could not be ignored as Na⁺-K⁺-ATPase is responsible for the generation of the membrane potential through the active transport of sodium and potassium ions in the neurons in CNS necessary and to maintain neuronal excitability. Ca²⁺-ATPase is responsible for fine-tuning of intracellular calcium levels. Moreover, the role of Mg²⁺-ATPase is to maintain high brain intracellular Mg²⁺ which can control rates of protein synthesis and cell growth. Na⁺-K⁺-ATPase, Mg²⁺-ATPase and Ca²⁺-ATPase in the plasma membrane also keeps the intracellular sodium low but intracellular magnesium and potassium high when compared with the levels in extracellular fluids. Thus these enzymes are vital for neuronal functions and to maintain the resting membrane potential and nerve conduction. The report of methanol induced increased production of free radicals and increased oxidative damage to proteins in distinct brain regions, retina and optic nerve has been reported. It has been demonstrated that striatal neurons are more vulnerable to glutamate neurotoxicity when Na⁺-K⁺-ATPase activity is reduced.

It is relevant to point out that in the earlier report on aspartame, there was marked increase in the corticosteroid level in the plasma for the same dose which indicate that the dietary sweetener aspartame could act as a chemical stressor. However, this elevation in the corticosteroid may be contributing factor for the free radical generation and there by altering the membrane bound enzymes. McIntosh et al reported a decreased activity of the antioxidant enzymes in the brain of rats treated with gluco corticoids. Along with this Manolli et al reported that steroid hormones released by
Moreover, this increase in H$_2$O$_2$ and nitric oxide after aspartame for the alteration observed in the membrane bound enzymes. This study after aspartame ingestion may be a contributing factor could be the targets for oxidative modiﬁcation of different cell types with a variety of biological functions. Serious issue. Metabolism could not be overlooked and must be considered as an important role in the anti-oxidative defense system of the cell. In this study are due to methanol, the byproduct of aspartame intake and thus justify the decrease in the adrenals in response to physical and psychological stressors and exposure to physiological levels of these hormones exacerbates reactive oxygen species (ROS) generation.

The nitric oxide (NO), a nitrogen free radical is produced by a number of different cell types with a variety of biological functions. Nitric oxide is a product of the oxidation of L-arginine to L-citrulline in a two-step process catalyzed by the enzyme nitric oxide synthase (NOS). Hydrogen peroxide (H$_2$O$_2$) is formed primarily through the action of superoxide dismutase, although peroxisomal oxidases are responsible for producing hydrogen peroxide in peroxisomes. H$_2$O$_2$ is a strong oxidant and diffuses easily across membranes, although the diffusion rate is dependent on the concentration gradient across the membrane. Lipid per oxidation results in the degradation of lipids via a free radical chain reaction and the reaction may be initiated by H$_2$O$_2$. Hence, the increase in H$_2$O$_2$ and nitric oxide in this study after aspartame ingestion may be a contributing factor for the alteration observed in the membrane bound enzymes. Moreover, this increase in H$_2$O$_2$ and nitric oxide after aspartame consumption could not be overlooked as McCord stated that prolonged exposure to free radicals, even at a low concentration, may result in the damage of biologically important molecules and the cells can be injured or killed when the ROS generation overwhelms the cellular antioxidant capacity.

The increased levels of serum enzymes such as aspartate aminotransferase, alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT) and lactate dehydrogenase (LDH) were observed in alcoholic patients. Hence the increase may be due to the methanol, which is a member in alcohol family. However Abhilash et al used 1000 mg/kg b. wt aspartame, they hypothesized that long term consumption of aspartame may cause liver injury which was marked by the increase in AST, ALT, ATP and GGT activities in serum. It is certainly possible that the enhanced activities of these enzymes observed in this study are due to methanol, the byproduct of aspartame metabolism, which is previously reported to produce altered oxidant/antioxidant balance.

$\gamma$-glutamyltransferase (GGT) is an enzyme involved in the transfer of the $\gamma$-glutamyl residue from $\gamma$-glutamyl peptides to amino acids, H$_2$O, and other small peptides. On the other hand, GGT is also involved in the synthesis of glutathione. The biosynthesis of cellular glutathione, the most important cell antioxidant, depends upon GGT activity; hence this enzyme may play an important role in the anti-oxidative defense system of the cell. In our earlier report there was a marked decrease in the GSH level in entire brain regions studied after aspartame consumption. Considering the decrease in GSH level after aspartame intake, one can conclude that probably to replenish the deficient GSH, the GGT activity might be increased in this study.

Creatine kinase (CK), also known as creatine phosphokinase (CPK) or phospho-creatine kinase is an enzyme expressed by various tissues and cell types. CK catalyses the conversion of creatine and consumes adenosine triphosphate (ATP) to create phosphocreatine and adenosine diphosphate (ADP). This CK enzyme reaction is reversible, such that also can generate ATP. Creatine kinase isoenzymes play an important role in the maintenance of ATP level in the CNS tissue. Active site of CK isoenzymes contains an essential cysteine residue and tyrosine residues, which could be the targets for oxidative modifications. Therefore, CK is likely to be one of the primary targets for ROS, which is overproduced during aspartame intake and thus justify the decrease in its activity. Lipid peroxidation and protein oxidation can impair the function of numerous cellular components including creatine kinase. However, the decrease in CK might alter the brain energy metabolism could not be over looked and must considered as serious issue.

Numerous reports and various issues, concerning the toxic effects of aspartame have continued to be raised. It has been implicated revealing high incidence of brain tumors in aspartame-fed rats compared to no brain tumors in concurrent control. Meanwhile, several experimental studies suggested that, astrocytomomas were the exact kind of brain tumor found in aspartame dosed rats. These free radicals had been shown to damage cellular proteins and DNA. The most immediate DNA damage was to the mitochondrial DNA. Free radicals had been shown to prevent uptake of excitotoxins by astrocytes as well, which would signiﬁcantly increase extra cellular aspartame metabolites levels. This created a vicious cycle that would multiply any resulting damage and malfunctioning of neurophysiologic system. It was also added that, aspartame metabolites induced amino acids imbalance within neuron micro environment, thus producing ultimate damage. In the present study, Luxol fast blue (LFB) staining was performed on brain cerebellum region. Upon gross examination, the white matter tracts, as visualized by LFB, appeared thinner in Asp + MTX treated animals when compared to control and MTX controls. At higher magnification, LFB staining revealed an array of densely stained myelinated fibers traversing through the white matter in the aspartame treated MTX animals. In contrast, the aspartame treated MTX animals cerebellar white matter appeared disorganized, less intensely stained and littered with ‘holes’. Furthermore, the number of densely stained cell bodies in the white matter was decreased in aspartame treated MTX animals and the morphology matter was ﬁrst observed, suggesting the major afferent and efferent projections were undergoing widespread degeneration when compared to the control animals and MTX treated control animals. On hydrolysis of aspartame, methanol was formed and it was converted in the liver to formaldehyde, which is known to be neurotoxin and carcinogen. This could lead to increase in the metabolizing enzymes of the cerebellum as well as other sites in rat brain. Formaldehyde attached to the DNA, RNA and proteins of the cells become difﬁcult to be removed, which might cause breaks in the DNA.

Previous investigators reported that, excessive aspartame stimulation could trigger the generation of large numbers of free radical species, both as nitrogen and oxygen species. These free radicals had been shown to damage cellular proteins and DNA. The most immediate DNA damage was to the mitochondrial DNA. The free radical generation leads to the alteration in antioxidant system, which induces oxidative stress leading to the cellular level damage in the brain cells. Our ﬁnding also explains same stated by some authors who reported that excitotoxins acting at different sites within the central nervous system could strip myelin from fibers and destroying neurons. The present study reveals that aspartame administration in the body system alters the enzyme activity in brain by possible raise in free radicals. The observed changes may be due to the methanol or its metabolite. Since it is consumed more by common people, it is essential to do more work on aspartame and create awareness regarding the usage of this artiﬁcial sweetener.

5. Conclusion

The observed results support the toxic nature of aspartame when consumed repeatedly for a prolonged period. The present study reveals that aspartame administration in the body system by possible raise in free radicals alters the enzyme activity in brain. Moreover there was an effect in long term aspartame administration on membrane bound enzymes, oxidative stress markers and histopathology in brain regions. Since it is consumed more by common people, it is essential to do more work on aspartame and create awareness regarding the usage of this artificial sweetener.
Further studies are required to evaluate the effect of aspartame in mere future.

Conflicts of interest

All authors have none to declare.

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