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Studies on the effects of aspartame on memory and oxidative stress in brain of mice

O.M.E. ABDEL-SALAM[1], N.A. SALEM[1], M.E.S. EL-SHAMARKA[1], J.S. HUSSEIN[2], N.A.S. AHMED[1], M.E.S. EL-NAGAR[1]

Departments of Toxicology and Narcotics[1], and Medical Biochemistry[2], National Research Centre, Cairo, Egypt

Abstract. – OBJECTIVE: The dipeptide aspartame (N-L-alpha-aspartyl-Lphenylalanine, 1-methyl ester; alpha-APM) is one of the most widely used artificial sweeteners. The present study aimed to investigate the effect of repeated administration of aspartame in the working memory version of Morris water maze test, on oxidative stress and brain monoamines in brain of mice.

MATERIALS AND METHODS: Aspartame (0.625, 1.875 or 5.625 mg/kg) was administered once daily subcutaneously for 2 weeks and mice were examined four times a week for their ability to locate a submerged plate. Malondialdehyde (MDA), reduced glutathione (GSH), nitric oxide levels (the concentrations of nitrite/nitrate) and glucose were determined in brain.

RESULTS: Only at the highest dose of 5.625 mg/kg, did aspartame significantly impaired water maze performance. The mean time taken to find the escape platform (latency) over 2 weeks was significantly delayed by aspartame 5.625 mg/kg, compared with the saline-treated control group. Significant differences occurred only on the first trial to find the escape platform. Significant increase in brain MDA by 16.5% and nitric oxide by 16.2% and a decrease in GSH by 25.1% and glucose by 22.5% occurred after treatment with aspartame at 1.875 mg/kg. Aspartame administered at 5.625 mg/kg significantly increased brain MDA by 43.8%, nitric oxide by 18.6% and decreased GSH by 32.7% and glucose by 25.8%. Aspartame caused dose-dependent inhibition of brain serotonin, noradrenaline and dopamine.

CONCLUSIONS: These findings suggest impaired memory performance and increased brain oxidative stress by repeated aspartame administration. The impaired memory performance is likely to involve increased oxidative stress as well as decreased brain glucose availability.

Key Words:
partame, Water maze, Oxidative stress, Brain, Mice.

Introduction

The dipeptide aspartame (N-L-alpha-aspartyl-Lphenylalanine, 1-methyl ester; alpha-APM) is one of the most widely used artificial sweeteners in the world, with a sweetness potency of about 160-200 times that of sucrose on a weight basis. Aspartame was approved by Food Drug Administration (FDA) for use in dry applications (e.g., tabletop, gelatins) in 1981 followed by approval for use in carbonated soft drinks in 1983 then as a general sweetener in 1996[1]. Aspartame is being used in over 6.000 consumer packaged goods and in nearly 500 pharmaceutical products, including children's medicines. In the United States, more than 70% of aspartame sales are attributed to soft drinks[2]. Ever since its approval by the FDA for use as an artificial sweetener, aspartame has been the subject of much debate as concerns its health effects such as increasing brain cancer rates[3]. High doses of aspartame can also generate major neurochemical changes in rats[4,5]. Aspartame is metabolized by digestive esterases and peptidases in the intestinal lumen to methanol and to its constituent amino acids phenylalanine and aspartic acid or absorbed by intestinal mucosal cells where it is hydrolyzed to its components, followed by absorption of these components into the systemic circulation. Phenylalanine enters the plasma free amino acid pool from the portal blood after partial conversion to tyrosine by hepatic phenylalanine hydroxylase.

Memory or the retention of learned information is fundamental to human beings; memory impairment such as that occurring in normal aging or in pathological conditions, e.g., Alzheimer's, disease is a serious medical and social problem. It has been proposed that excessive aspartame ingestion might be involved in the pathogenesis of certain mental disorders and also in compromised learning and emotional functioning[6]. Oxidative stress, defined as a breach in the balance between free radical production and antioxidant defense mechanisms, has been implicated in the pathogenesis of several brain disorders such as Alzheimer's disease, Parkinson's

Corresponding Author: Omar M.E. Abdel Salam, MD; e-mail: omasalam@hotmail.com

disease, multiple sclerosis, schizophrenia and autism[7-11]. Oxidative stress also plays an important role in the decline in cognitive functions associated with aging or caused by different pathological states, where a reduction of oxidative and nitrosative stress was associated with improvement of the age-related memory deficits[12,13], the stress-induced decline in learning and memory[14,15], and the memory impairment induced by acute ethanol[16]. Indeed, the high rate of oxidative metabolic activity, high polyunsaturated fatty acid (PUFA) content, the monoamine neurotransmitter and iron content and the relatively low antioxidant capacity are among several factors which account for the increased susceptibility of the brain to oxidative stress[17].

In the present study, the effect of aspartame in the working memory version of Morris water maze test and on oxidative stress in brain of mice was investigated. The doses of aspartame used correspond to 10-60 mg after being converted to that of mice[18].

Materials and Methods

Animals

Swiss male albino mice 20-22 g of body weight were used. Standard laboratory food and water were provided *ad libitum*. Animal procedures were performed in accordance with the Ethics Committee of the National Research Centre and followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985). Equal groups of 6 mice each were used in all experiments. The doses of aspartame used correspond to 10-60 mg (1/2-3 tablets) after being converted to that of mice according to Paget and Barnes conversion tables[18].

Cognitive Testing

The Morris water maze (MWM) was performed to test spatial learning and memory. The MWM is a paradigm that requires the mice to use spatial memory to find a hidden platform just below the surface of a pool of water, and to remember its location from the previous trial[19]. Therefore, the mice must use distal cues to effectively locate it. Accurate navigation is rewarded by escape from the pool. The maze consisted of a glass tank, narrowed to 20 cm wide, 40 cm in height, 70 cm in length, filled to a depth of 21 cm with water maintained at 25°C. The escape glass platform was hidden from sight, submerged 1 cm below the surface of the water at the end of the tank[20]. Mice rapidly learn to swim directly to the escape platform and climb out. Once the mice reached the platform, it remained there for 15 sec (trial 1; reference memory or acquisition trial). At the end of each trial, the mice was towel dried, returned to its home cage (where a heat lamp was available), and 3 min elapsed before the next trial (trials 2 and 3; working memory or retrieval trial), which used the same platform location and start position as trial 1. The latency to find the platform (sec) was assessed with a stopwatch. Aspartame (0.625, 1.875 or 5.625 mg/kg) was administered daily by subcutaneous (s.c.) injection and mice were tested four times per week for 2 weeks. The control group received saline instead (0.2 ml, s.c.).

Biochemical Studies

At the end of the study, mice were euthanized by decapitation, brains were then removed, washed with ice-cold saline solution (0.9% NaCl), weighed and stored at -80ºC for the biochemical analyses. The brain was homogenised with 0.1 M phosphate buffer saline (PBS) at pH 7.4, to give a final concentration of 10% w/v for the biochemical assays. For the determination of monoamine neurotransmitters, frozen samples were homogenized in cold 0.1 N-perchloric acid.

Determination of Brain Lipid Peroxidation

Lipid peroxidation was assayed by measuring the level of malondialdehyde (MDA) in the brain tissues. Malondialdehyde was determined by measuring thiobarbituric reactive species using the method of Ruiz-Larrea et al[21], in which the thiobarbituric acid reactive substances (TBARS) react with thiobarbituric acid to produce a red colored complex having peak absorbance at 532 nm.

Determination of Brain Reduced Glutathione Content

Reduced glutathione (GSH) was determined in brain tissue by Ellman's method[22]. The procedure is based on the reduction of Ellman´s reagent by –SH groups of GSH to form 2-nitro-s-mercaptobenzoic acid, the nitromercaptobenzoic acid anion has an intense yellow color which can be determined spectrophotometrically (UV-VI8 Recording Spectrophotometer, Shimadzu Corporation, Australia). A mixture was directly prepared in a cuvette: 2.25 ml of 0.1 M K-phosphate buffer, pH 8.0; 0.2 ml of the sample; 25 µl of Ellman's reagent (10 mM 5,5′-dithio-bis-2-nitroben-

zoic acid in methanol). After 1 min the absorbance was measured at 412 nm and the GSH concentration was calculated by comparison with a standard curve.

Determination of Brain Nitric Oxide Level

Nitric oxide (NO) measured as nitrite was determined by using Griess reagent, according to the method of Moshage et al[23], where nitrite, stable end product of nitric oxide radical, is mostly used as indicator for the production of NO.

Determination of Brain Glucose

Brain tissue glucose content was determined according to the method of Trinder[24]. Glucose in the presence of glucose oxidase is converted to peroxide and gluconic acid. The produced hydrogen peroxide reacts with phenol and 4-amino-antipyrine in the presence of peroxidase to yield a colored quinonemine, which is measured spectrophotometrically.

Determination of Brain Monoamines

Determination of brain serotonin, noradrenaline and dopamine was carried out using high performance liquid chromatography (HPLC) system (Agilent Technologies, Boeblingen, Germany), Agilent technologies 1100 series, equipped with a quaternary pump (Quat pump, G131A model). Separation was achieved on ODS reversed phase column (Agilent Technologies, Wilmington, DE, USA) (C18, 25 × 0.46 cm i.d. 5 µm). The mobile phase consisted of potassium phosphate buffer/methanol 97/3 (v/v) and was delivered at a flow rate of 1 ml/min. UV detection was performed at 270 nm and the injection volume was 20 µl. The concentration of both catecholamines and serotonin were determined by external standard method using peak areas. Serial dilutions of standards were injected and their peak areas were determined. A linear standard curve was constructed by plotting peak areas versus the corresponding concentrations. The concentration in samples was obtained from the curve.

Drugs

Aspartame (Amrya Pharm. Ind., Cairo, Egypt) was used and dissolved in isotonic (0.9% NaCl) saline solution immediately before use. The doses of aspartame used in the study were based upon the human dose after conversion to that of rat according to Paget and Barnes conversion tables[18].

Statistical Analysis

Data are expressed as mean ± SEM. The data were analyzed by one way ANOVA and by repeated measures (session × treatment) ANOVA, followed by Duncan's multiple range test, using SPSS software (SAS Institute Inc., Cary, NC, USA). A probability value of less than 0.05 was considered statistically significant.

Results

Effect of Aspartame on Spatial Memory

Spatial memory was tested in the Morris water maze test. Aspartame substantially impaired water maze performance. The average mean latency and standard error of the mean over 2 weeks for the saline (group 1), aspartame 0.625 mg/kg (group 2), aspartame 1.875 mg/kg (group 3) and aspartame 5.625 mg/kg (group 4) was 4.0 ± 0.16, 4.18 ± 0.21, 4.73 ± 0.16, 4.84 ± 0.28 sec, respectively. The mean time taken to find the escape platform (latency) was significantly delayed by aspartame 5.625 mg/kg compared with the saline-treated control group (Figures 1, 2). There was a significant main drug effect (F = 4.82, p = 0.003), a significant main effect of days (F = 11.62, p = 0.0001) but no significant main effect of trials (F = 0.35, p = 0.70). There was a significant trial × time interaction (F = 2.89, p = 0.0001) and drug × trial interaction (F = 2.3, p = 0.03).

A repeated measure ANOVA was done to compare latency to find the hidden plate in the first, second, and third trial over 2 weeks. In the first trial, the average mean for the saline (group 1), aspartame 0.625 mg/kg (group 2), aspartame 1.875 mg/kg (group 3) and aspartame 5.625 mg/kg (group 4) was 3.58 ± 0.27, 4.32 ± 0.38, 4.65 ± 0.32, 5.65 ± 0.66 sec, respectively (Figure 3). On the first trial there was a significant main effect of drug (F = 5.3, p = 0.002), and a significant main effect of time (F = 6.9, p = 0.0001). There was a significant drug × time interaction (F = 2.23, p = 0.003). Aspartame at the dose of 0.625 or 1.875 mg/kg did not significantly alter latency to find the hidden plate. Only at the dose of 5.625 mg/kg, did aspartame significantly delayed mean time taken to find the escape platform.

In the second trial the average mean for the saline (group 1), aspartame 0.625 mg/kg (group 2), aspartame 1.875 mg/kg (group 3) and aspartame 5.625 mg/kg (group 4) was 4.18 ± 0.28,

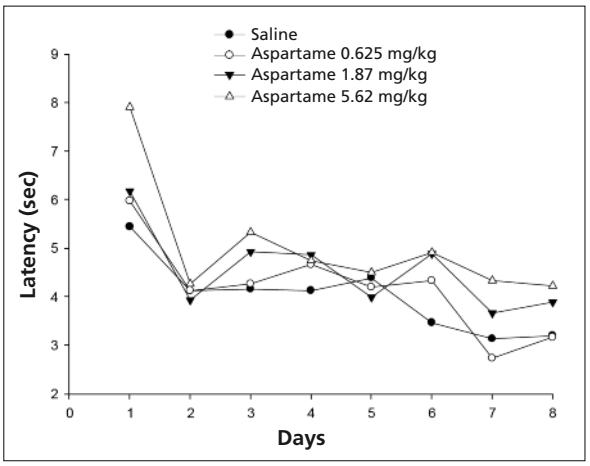

Figure 1. The mean latency (*in seconds*) to locate a submerged plate in the Morris water maze test over two weeks. Mice received daily injections of saline or aspartame (0.625, 1.875 or 5.625 mg/kg) and were tested four times weekly for two weeks. Error bars are omitted for the purpose of clarity.

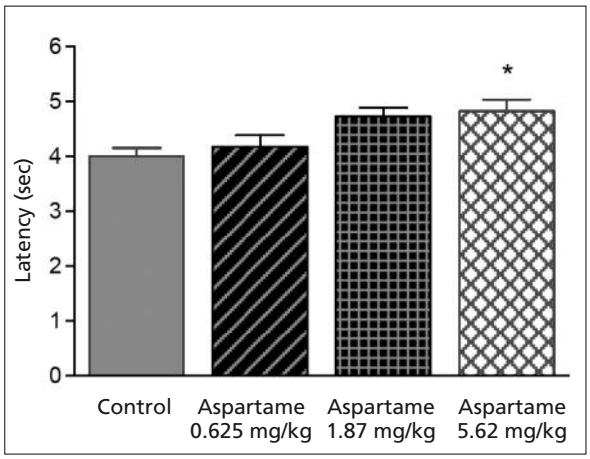

Figure 2. The average mean latency (*in seconds*) and standard error of the mean to locate a submerged plate in the Morris water maze test over two weeks. Mice received daily injections of saline or aspartame (0.625, 1.875 or 5.625 mg/kg) and were tested four times weekly.

4.22 ± 0.41, 4.18 ± 0.23, 4.9 ± 0.28 sec, respectively (Figure 4). There was no significant main effect of drug ($F = 1.32$, $p = 0.29$), but a significant main effect of time ($F = 6.2$, $p = 0.0001$). Aspartame did not significantly alter latency to find the hidden plate.

In the third trial the average mean for the saline (group 1), aspartame 0.625 mg/kg (group 2), aspartame 1.875 mg/kg (group 3) and aspartame 5.625 mg/kg (group 4) was 4.26 ± 0.24, 4.07 ± 0.30, 4.66 ± 0.24, 4.68 ± 0.34 sec, respectively (Figure 5). There was no significant main effect for drugs ($F = 1.48$, $p = 0.22$) but a significant main effect of time ($F = 3.19$, $p = 0.003$) and a significant time × drug interaction ($F = 1.65$, $p = 0.04$). Aspartame did not significantly alter latency to find the hidden plate.

Effect of Aspartame on Brain Malondialdehyde

The administration of aspartame at the dose of 0.625 mg/kg did not change brain malondialdehyde level. However, the level of MDA was significantly increased by 16.5 ($p < 0.05$) and

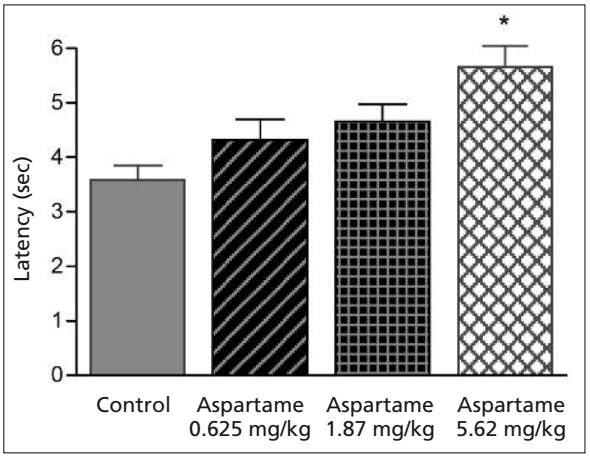

Figure 3. The average mean latency (*in seconds*) ± SEM of first trial to locate a submerged plate in the Morris water maze test over two weeks. Mice received daily injections of saline or aspartame (0.625, 1.875 or 5.625 mg/kg) and were tested four times weekly.

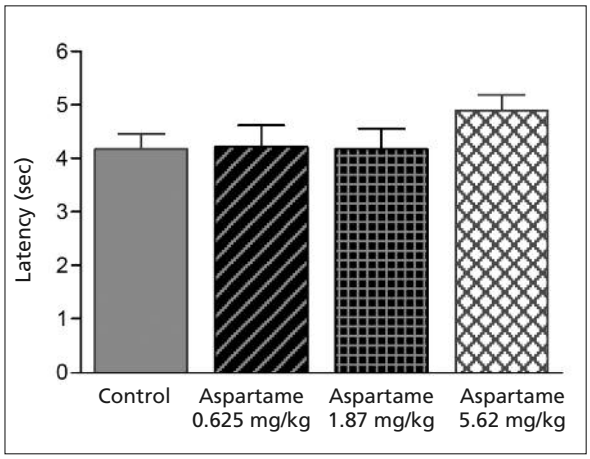

Figure 4. The average mean latency (*in seconds*) ± SEM of the second trial to locate a submerged plate in the Morris water maze test over two weeks. Mice received daily injections of saline or aspartame (0.625, 1.875 or 5.625 mg/kg) and were tested four times weekly.

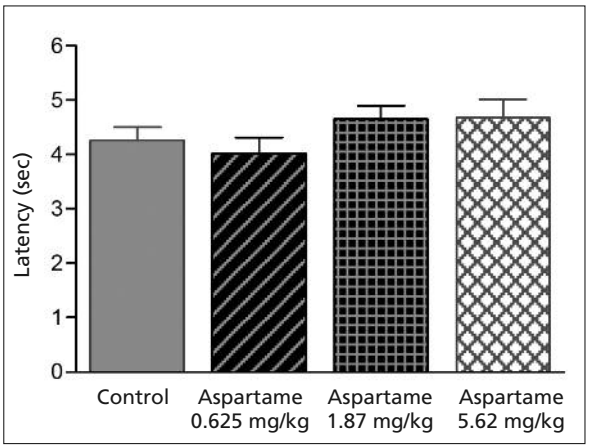

Figure 5. The average mean latency (in seconds) ± SEM of the third trial to locate a submerged plate in the Morris water maze test over two weeks. Mice received daily injections of saline or aspartame (0.625, 1.875 or 5.625 mg/kg) and were tested four times weekly.

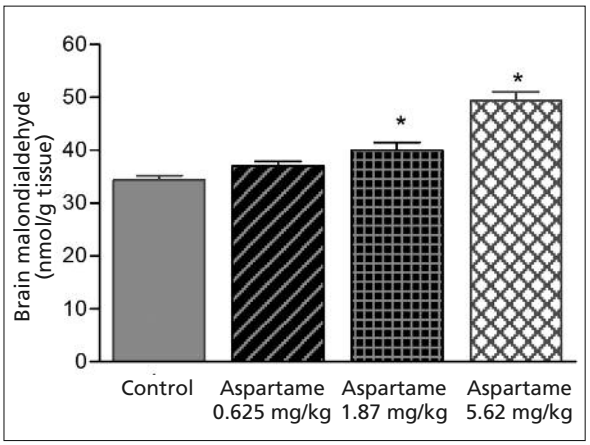

Figure 6. The effect of aspartame administration (0.625, 1.875 or 5.625 mg/kg, s.c.) on the brain tissue levels of malondialdehyde (MDA: nmol/g. tissue) in mice. Data are expressed as the mean ± SE. n = 6. Data were analyzed by one-way analysis of variance, followed by Duncan multiple range test for post hoc comparison of group means. Effects with a probability of $p < 0.05$ were considered to be significant. Asterisks indicate significant change from saline control group.

43.8% ($p < 0.05$) by aspartame at 1.875 and 5.625 mg/kg, respectively *vs* saline control value (40.0 ± 1.54 and 49.38 ± 1.62 *vs* 34.35 ± 0.8 nmol/g tissue) (Figure 6).

Effect of Aspartame on Brain Nitric Oxide

Significant increase in NO was observed after the administration of aspartame at 1.875 and 5.625 mg/kg, respectively (16.1 and 18.6% increase *vs* saline group, $p < 0.05$).Values of nitric oxide were: 44.5 ± 1.3, 46.8 ± 1.4 and 47.8 ± 1.8 µmol/g for aspartame doses of 0.625, 1.857 and 5.625 mg/kg, respectively *vs* saline control value of 40.3 ± 1.5 µmol/g (Figure 7).

Effect of Aspartame on Brain Reduced Glutathione

Reduced glutathione decreased by 25.1 ($p < 0.05$) and 32.7% ($p < 0.05$) after the administration of aspartame at the doses of 1.875 and 5.625 mg/kg, respectively compared with the saline-treated group. Values of GSH were: 6.9 ± 0.8, 5.43 ± 0.5 and 4.88 ± 0.41 µmol/g for aspartame doses of 0.625, 1.875 and 5.625 mg/kg, respectively *vs* saline control value of 7.25 ± 0.5 µmol/g. Aspartame at the dose of 0.625 mg/kg did not significantly alter GSH concentrations (Figure 8).

Effect of Aspartame on Brain Glucose

Concentrations of glucose were decreased by 22.5 ($p < 0.05$) and 25.8% ($p < 0.05$), following aspartame treatment at the doses of 1.875 and 5.625 mg/kg, respectively compared with the saline-treated group (27.6 ± 2.7 and 26.4 ± 3.1 *vs* 35.6 ± 2.3 µg/g tissue). Aspartame at the dose of 0.625 mg/kg did not significantly alter glucose concentrations (Figure 9).

Brain Monoamines

A dose-dependent inhibition of serotonin, noradrenaline and dopamine was produced by aspartame (Table I).

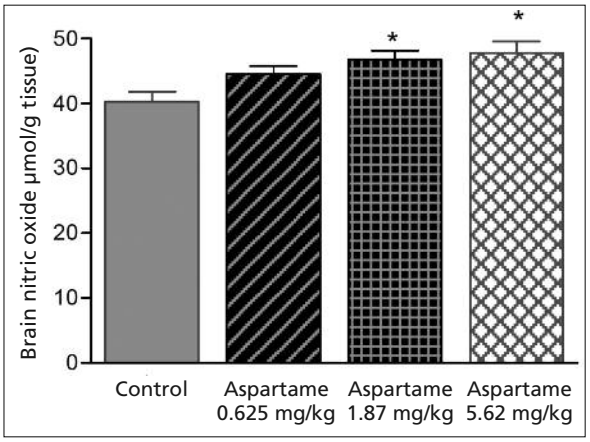

Figure 7. The effect of aspartame administration (0.625, 1.875 or 5.625 mg/kg, s.c.) on the brain tissue levels of nitric oxide (µmol/g tissue) in mice. Data are expressed as the mean ± SE. n = 6. Data were analyzed by one-way analysis of variance, followed by Duncan multiple range test for post hoc comparison of group means. Effects with a probability of $p < 0.05$ were considered to be significant. Asterisks indicate significant change from saline control group.

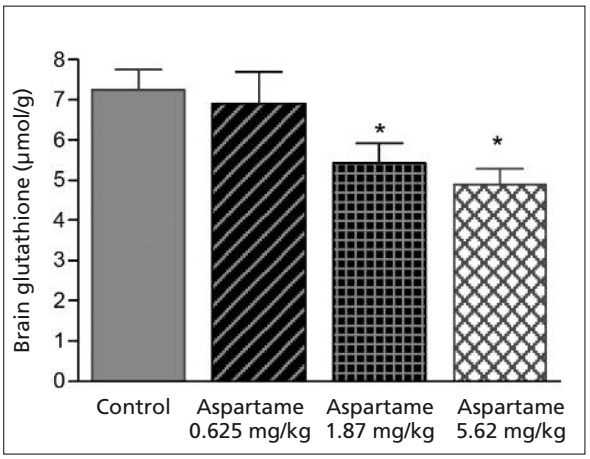

Figure 8. The effect of aspartame administration (0.625, 1.875 or 5.625 mg/kg, s.c.) on the brain tissue reduced glutathione (GSH: µmol/g tissue) in mice. Data are expressed as the mean ± SE. n = 6. Data were analysed by one-way analysis of variance, followed by Duncan multiple range test for post hoc comparison of group means. Effects with a probability of $p < 0.05$ were considered to be significant. Asterisks indicate significant change from saline control group.

Discussion

The present study provides evidence that aspartame, a widely used sweetening agent in human diet, impairs water maze performance and increases oxidative stress in mice brain. Aspartame substantially impaired water maze performance. The mean time taken to find the escape platform (latency) was significantly delayed by repeated administration of aspartame at 5.625 mg/kg compared with the saline-treated control group. Significant differences occurred only on the first trial to find the escape platform. The effect of aspartame on memory has been examined in humans and in experimental animals, with inconclusive results. In healthy volunteers, a single dose of aspartame (15 mg/kg) had no effect on reaction-time, cognition, or memory tested at 2 and 24 hours after dosage[25]. In pilots, no detectable performance decrements were associated with aspartame dose of 50 mg/kg[26]. However, in healthy smokers nicotine increased hits and decreased misses on a continuous performance task when participants were given the sucrose-containing beverage, but not when they were given the aspartame-containing beverage. Participants who drank the sucrose-containing beverage performed significantly better on the spatial memory task than those who drank the aspartame-containing beverage[27]. In rats, experiments suggested that acute or repeated (14 days) aspartame (500 or 1.000 mg/kg) had no significant effect on a spatial, reference memory task in the Morris water maze[28]. In rats also, aspartame (250 mg/kg/day in the drinking water for 3 or 4 months) caused a significant increase in time to reach the reward in the T-maze, suggesting a possible effect on memory due to the artificial sweetener. Aspartame has also been reported to increase muscarinic cholinergic receptor densities in brain[29].

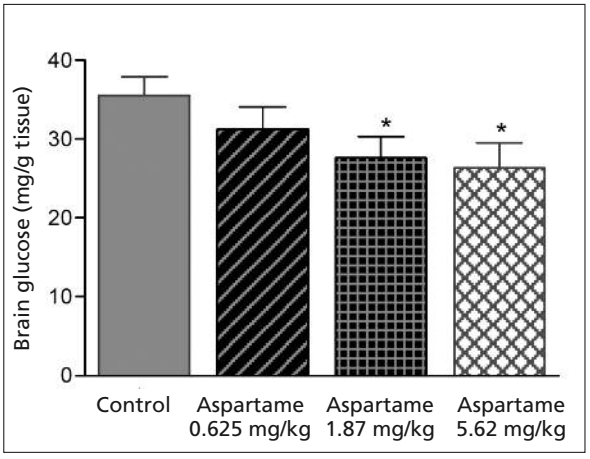

Figure 9. The effect of aspartame administration (0.625, 1.875 or 5.625 mg/kg, s.c.) on the brain tissue glucose (µg/g tissue) in mice. Data are expressed as the mean ± SE. n = 6. Data were analyzed by one-way analysis of variance, followed by Duncan multiple range test for *post hoc* comparison of group means. Effects with a probability of $p < 0.05$ were considered to be significant. Asterisks indicate significant change from saline control group.

Findings in the present study also suggested increased oxidative stress after repeated aspartame administration. This finding derives its importance from the fact that increased oxidative stress has been linked to neurodegenerative diseases as well as to age-related cognitive deficits[9,30]. Because of its high ATP demand, the brain consumes O_2 rapidly, and is thus susceptible to interference with mitochondrial function, which can in turn lead to increased $O_2\bullet^-$ formation[31]. Prime targets for free radical reactions are the unsaturated bonds in membrane lipids. Consequent peroxidation results in a loss in membrane fluidity and receptor alignment[32]. Lipid peroxidation was assayed by measuring the level of malondialdehyde, arising from the free radical degradation of PUFA in the brain tissues[33]. In the present study, the administration of aspartame increased brain malondiadehyde in a dose-dependent manner suggesting oxidative damage to

Table I. Effect of repeated aspartame administration on serotonin, dopamine and noradrenaline in mice brain.

	Saline	Aspartame 0.625 mg/kg	Aspartame 1.875 mg/kg	Aspartame 5.625 mg/kg
Serotonin (μg/g tissue)	3.03 ± 0.16	3.31 ± 0.18	2.17 ± 0.21*	1.56 ± 0.18*
Dopamine (μg/g tissue)	2.78 ± 0.21	2.34 ± 0.22	1.78 ± 0.18*	1.73 ± 0.21*
Noradrenaline (μg/g tissue)	2.33 ± 0.18	2.61 ± 0.13	1.67 ± 0.12*	1.20 ± 0.16*

Results are mean ± S.E. Six mice were used per each group. Data were analyzed by one way ANOVA and means of different groups were compared by Duncan's multiple range test. $p < 0.05$ was considered statistically significant. *$p < 0.05$ vs saline control group.

macromolecules such as lipids. Oxidative stress can be the result of increased free radicals production or alternatively decreased endogenous antioxidants. In this context, brain nitric oxide is increased following aspartame. Nitric oxide (nitrogen monoxide, NO) is an important intercellular messenger in the brain. Its synthesis from L-arginine is catalysed by the enzyme nitric oxide synthase (NOS) which exists in neuronal (nNOS), inducible (iNOS), endothelial (eNOS) as well as a mitrochondrial (mNOS) isosorms[34]. NO is produced in the brain by neurons, astroglia and endothelial cells. NO is also generated in response to inflammatory agents and cytokines by the action of iNOS[35]. Once i-NOS has begun to produce nitric oxide it will continue to do so for several hours and at concentrations of the order of μM that are high enough to be toxic to the target cell[36]. High levels of NO have been linked with neurotoxicity[37]. Nitric oxide is a stable free radical (NO•), but can react rapidly with molecular oxygen (O_2), superoxide anion ($O_2^{\bullet -}$) and transition metals. The reaction of NO• with O_2 results in the generation of NOx compounds (including NO_2•, N_2O_3 and N_2O_4), which can either react with cellular amines and thiols, or simply hydrolyze to form the end metabolites nitrite (NO_2^-) and nitrate (NO_3^-). The reaction of NO• with $O_2^{\bullet -}$ yields peroxynitrite ($ONOO^-$), a powerful oxidant that mediates cellular injury[34,38].

In face of free radicals production, the thiol glutathione (glycyl-glutamic acid-cysteine) is the most important cellular free radical scavenging system in the brain[39]. Glutathione depletion in the brain has been shown to disrupts short-term spatial memory[40]. Glutathione is decreased after repeated aspartame administration suggesting consumption of this important antioxidant defense mechanism by increased free radicals production due to aspartame administration which theoretically can further increase the vulnerability of the brain tissue to other oxidative insults.

In this investigation, the administration of aspartame was found to decrease brain monoamines. Other researchers suggested alterations in neurotransmitters due to aspartame intake. Thus, Coulombe and Sharma[5] observed increased norepinephrine and dopamine in various regions 3h after aspartame (13, 130 and 650 mg/kg) intake in mice. Goerss et al[41] found a significant increase in striatal serotonin concentrations in rats after i.p. administration of 200, 400, and 800 mg/kg of aspartame. Yokogoshi et al[4] reported no effect on serotonin and 5-hydroxyindoleacetic acid whole brain concentrations after oral aspartame (200 mg/kg) administration to rats. Aspartame, however, completely blocked the increase in 5-hydroxyindoles caused by glucose consumption. Troii et al[42] found no effect for aspartame on brain dopamine or norepinephrine levels. Serotonin levels were slightly increased when a protein-free diet was consumed, but aspartame added minimized this increase of brain serotonin levels. Perego et al[43] observed no change in monoamines or their metabolites in striatum, hippocampus and nucleus accumbens after 1h of oral aspartame administration at doses of 1000 and 500 mg/kg in rats. In rats also, acute doses of up to 2000 mg/kg, failed to induce significant changes in brain serotonin or dopamine levels[44]. More recently, Bergstrom et al[45] reported a significant decline in evoked extracellular dopamine levels in the striatum of rats within 1 h of a single systemic dose (500 mg/kg i.p.).

The present study employed aspartame doses relevant to those used in humans. The acceptable daily intake levels of aspartame established by U.S. Food and Drug Administration and European Food Safety Authority is 50 and 40 mg/kg/day, respectively[46]. The doses of aspartame used correspond to 10-60 mg (i.e., ½ to 3 tablets) after being converted to that of mice[18]. However, rodents metabolize aspartame at a much greater rate than humans and a conversion

factor around 5[47-49] and up to 60[50] has been used to estimate equivalent dosages. Thus the doses used in our study are notably low and below the human daily intake levels of aspartame.

It might be argued that the route of administration do not mimic the human intake of the agent i.e. the oral route. Aspartame was given *via* subcutaneous route so as to ensure rapid and efficient absorption before trials. Rapid and reliable responses are usually obtained by parenteral routes and smaller doses are required because less drug is lost in reaching the bloodstream during its absorption and distribution. It was also aimed to avoid possible gustatory effects that may alter or mask the effect of the hydrolytic products of aspartame. Several studies have employed the intraperitoneal or parenteral route[41,45,47,49,51-53] for the administration of aspartame. Authors also found that the effect of aspartame is similar despite the route of administration. Oral aspartame was found to be at least as effective as the parenterally administered sweetener in raising regional brain levels of tyrosine (using aspartame doses of 200 or 300 mg/kg)[47]. Quantitative differences, however, have been reported; aspartate, phenylalanine, tyrosine and glutamate levels increased more after the injection, than the intubation, of aspartame (176 mg/kg)[51]. Aspartame's methyl ester group is susceptible to hydrolysis and it is most likely that aspartame undergoes rapid hydrolysis in the subcutaneous tissue or peritoneum to its principal constituents, aspartic acid and phenylalanine, and methanol followed by absorption into the general circulation.

The exact mechanism and the degradation constituent (s) by which aspartame can affect memory and increase oxidative stress in the brain is not clear. Aspartame consists of 39.5% aspartic acid, 50% phenylalanine and 10.5% methyl ester[55]. Aspartame is rapidly broken down in the body to aspartate, phenylalanine and methanol. When absorbed, aspartic acid is transformed into alanine plus oxaloacetate[56]. Aspartate may also be incorporated into body constituents such as other amino acids, proteins, pyrimidines, asparagine, and N-acetylaspartic acid[57]; phenylalanine is transformed mainly into tyrosine and, to a lesser extent, phenylethylamine and phenylpyruvate. Once in the systemic circulation, phenylalanine is taken up across the blood-brain barrier into the central nervous system. Phenylalanine is a precursor for tyrosine, 3,4 dihydroxyphenylalanine (DOPA), dopamine, norepinephrine, and epinephrine. Aspartame yields approximately 10% methanol by weight[1]; Methanol rapidly enters the portal circulation and is oxidised in the liver to formaldehyde, which is further oxidised to formic acid and then to CO_2. Formaldehyde has a half-life of about 1.5 min, so there is no accumulation in the tissue[46]. It has been suggested, however, that aspartame ingestion can lead to the formation of formaldehyde adducts in the organs and tissues[58]. Formaldehyde can enhance cellular oxidative stress via reacting with free radicals[59]. Plasma aspartate was significantly increased at 0.25 hr after the aspartame intake[60]. Aspartic acid is an excitatory neurotransmitter in brain, a direct N-methyl-D-aspartate (NMDA) agonist. Studies have shown increased phenylalanine in blood and brain after aspartame administration in humans and in experimental animals[47,49]. The increase in aspartic acid and phenylalanine levels in brain could be a likely explanation for the increased oxidative stress observed after aspartame.

In summary, these findings suggest that the mice's brain is susceptible to the components arising from aspartame degradation. Aspartame significantly impaired water maze performance, increased malondialdehyde, nitric oxide and decreased glutathione and glucose in brain. Such findings are likely to have important implications because of the wide spread use of aspartame in many food and beverage preparations.

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