Revisiting the safety of aspartame

Arbind Kumar Choudhary and Etheresia Pretorius

Aspartame is a synthetic dipeptide artificial sweetener, frequently used in foods, medications, and beverages, notably carbonated and powdered soft drinks. Since 1981, when aspartame was first approved by the US Food and Drug Administration, researchers have debated both its recommended safe dosage (40 mg/kg/d) and its general safety to organ systems. This review examines papers published between 2000 and 2016 on both the safe dosage and higher-than-recommended dosages and presents a concise synthesis of current trends. Data on the safe aspartame dosage are controversial, and the literature suggests there are potential side effects associated with aspartame consumption. Since aspartame consumption is on the rise, the safety of this sweetener should be revisited. Most of the literature available on the safety of aspartame is included in this review. Safety studies are based primarily on animal models, as data from human studies are limited. The existing animal studies and the limited human studies suggest that aspartame and its metabolites, whether consumed in quantities significantly higher than the recommended safe dosage or within recommended safe levels, may disrupt the oxidant/antioxidant balance, induce oxidative stress, and damage cell membrane integrity, potentially affecting a variety of cells and tissues and causing a deregulation of cellular function, ultimately leading to systemic inflammation.

INTRODUCTION

Non-nutritive sweeteners are high-intensity sweeteners that are used in small amounts to reduce the caloric and sugar content of food and beverages. The controversial increased use of non-nutritive sweeteners in so-called healthy food and beverages has recently come under the spotlight. In particular, aspartame, which was accidentally discovered in 1969, has received much attention because of its potent sweetness, which is 200 to 300 times greater than that of sucrose. Aspartame has a clean sugar-like taste, with no undesirable metallic or bitter taste. It is far cheaper than sugar and is an attractive option for manufacturers.4

Aspartame is a synthetic dipeptide formed by the reaction of L-aspartic acid with L-phenylalanine methyl ester.5 It was first marketed as NutraSweet and Equal and is now freely available in supermarkets. Aspartame is incorporated into more than 6000 products, including soft drinks, dessert mixes, frozen desserts and yogurt, chewable multivitamins, and breakfast cereals. It is also contained in about 600 pharmaceutical products and is, therefore, consumed by millions of people worldwide.10 Aspartame metabolites may reduce or potentiate drug action through various mechanisms.11 Metabolites amino acids, and proteins may (1) alter blood proteins to which drugs attach; (2) alter drug receptors on cell membranes; (3) change the sites at which impulses are transmitted along nerves to muscle; (4) cause metabolic abnormalities in elderly people that may enhance the vulnerability of this population to...
drug reactions; or (5) interfere with drug action. Safety issues associated with the use of aspartame include potential toxicity from aspartame metabolites, including methanol and/or its metabolite, formaldehyde.\textsuperscript{12,13}

This review examines the existing literature (published 2000–2016) describing the effects of aspartame on cells and organ systems when used within the safe dosage range. The interactions between aspartame and cells and organ systems are examined, and extensive references for current recommended safe dosages are provided. Finally, literature that considers the effects of aspartame on different cells in the body is reviewed.

**Biochemistry of aspartame**

Aspartame is an L-aspartyl-L-phenylalanine methyl ester\textsuperscript{14} (Figure 1) and is very stable under dry conditions when stored at temperatures ranging from 30\textdegree C to 80\textdegree C.\textsuperscript{15} It degrades at high temperatures and in aqueous solutions. The rate of degradation in aqueous solutions, however, depends on pH as well as temperature.\textsuperscript{16} At room temperature, aspartame is most stable at pH 4.3, with a half-life of 300 days. Degradation is minimal when pH ranges between 4.0 and 5.0 and reaches a maximum when heated under conditions of high humidity at a pH greater than 6.0.\textsuperscript{16} Under strongly acidic (pH < 4.0) or alkaline conditions (pH > 6.0), aspartame may generate methanol by hydrolysis. Under more severe conditions, such as elevated temperature or high pH, the peptide bonds are also hydrolyzed.\textsuperscript{17} This results in the release of free amino acids (particularly phenylalanine and aspartic acid). It should also be noted that the pH of diet sodas—a major vehicle for aspartame consumption—tends to be somewhere between 3.0 and 4.0. Interestingly, following breakdown in the gut or exposure to temperature changes, aspartame and its metabolites lose their sweetness.\textsuperscript{18,19} Conditions during storage vs during ingestion are thus different and may determine the formation of aspartame metabolites.\textsuperscript{20}

![Figure 1 Structure of aspartame (L-aspartyl-L-phenylalanine methyl ester).](image)

**Aspartame during storage.** Aspartame can be stored in a dry or an aqueous form. In the dry form, stability depends mainly on temperature, and stability decreases at temperatures < 30\textdegree C or > 80\textdegree C. In the aqueous form, stability is greatest at a pH of 4.3; beyond this pH, aspartame degrades into its 3 known metabolites (phenylalanine, aspartic acid, and methanol) and loses some of its sweetness. The aqueous form also becomes sweeter with increased temperature.\textsuperscript{21}

**Aspartame during ingestion.** Upon ingestion, aspartame is metabolized by gut enzymes (esterase and peptidase) into 3 amino acid isolates, phenylalanine (50\%), aspartic acid (40\%), and methanol (10\%).\textsuperscript{22} Phenylalanine is further metabolized in the liver into l-tyrosine by the enzyme phenylalanine hydroxylase. l-Tyrosine in turn, is converted into l-dopa (l-3,4-dihydroxyphenylalanine) by the enzyme tyrosine hydroxylase.\textsuperscript{23} l-Dopa is further converted into the catecholamines—dopamine, norepinephrine (noradrenaline), and epinephrine (adrenaline)—by the amino acid decarboxylase enzyme. Phenylalanine can cross the blood–brain barrier.\textsuperscript{24,25} Furthermore, elevations in plasma concentrations of phenylalanine and aspartic acid result in increased transport of these amino acids into the brain, modifying the brain’s neurochemical composition.\textsuperscript{26} Neuroendocrine changes, particularly increased concentrations of catecholamine resulting from phenylalanine and its hydroxylation product, tyrosine, have been observed in the brain.\textsuperscript{26}

Phenylalanine is a large neutral amino acid that competes with other important large neutral amino acids for binding on the large neutral amino acid transporter.\textsuperscript{26} However, excess phenylalanine concentrations are associated with decreased concentrations of catecholamine, serotonin, and dopamine.\textsuperscript{26} Aspartic acid is metabolized in the liver into l-lysine and l-methionine by the enzyme aspartate kinase. At high concentrations, aspartic acid may cross the blood–brain barrier and bind to the N-methyl-D-aspartate receptor (also known as the NMDA receptor or NMDAR)\textsuperscript{27} or to other glutamate binding sites, causing an influx of calcium ions into cells (Figure 2). Increased firing of action potentials and higher rates of neuron depolarization can potentiate neurodegeneration.\textsuperscript{26} The enzyme responsible for metabolism of methanol (CH\textsubscript{3}OH) is species dependant.\textsuperscript{28} In primates, methanol is metabolized into formaldehyde (HCHO) in the liver by alcohol dehydrogenase.\textsuperscript{29} In rodents, on the other hand, methanol is mainly metabolized by alcohol catalase and differences in the embryonic metabolism of CH\textsubscript{3}OH may determine species sensitivity, in which mouse embryos were more sensitive than the rat.\textsuperscript{30} Formaldehyde is oxidized into formic
acid (HCOOH) by formaldehyde dehydrogenase in both primates and rodents. Formic acid is metabolized more rapidly into carbon dioxide and water in rodents, as rodents produce more folic acid (tetrahydrofolate) than primates. Excess formic acid may lead to metabolic acidosis and tissue injury, with humans being uniquely sensitive because of their low hepatic folate concentrations.

Safety of aspartame consumption

The safety of aspartame and its metabolites (phenylalanine, aspartic acid, and methanol) has been discussed frequently, and blood concentrations of aspartame metabolites increase after consumption. An important consideration is that aspartame metabolites are also found naturally in foods (Table 1). Milk has approximately 6 times more phenylalanine and 13 times more aspartic acid than the same volume of an aspartame-sweetened beverage. Similarly, tomato juice has 6 times more methanol than an equivalent volume of an aspartame-sweetened beverage. Exposure to low levels of methanol, which occurs naturally in blood, urine, saliva, and expired air, is common. Methanol occurs in fresh citrus fruits and juices, vegetables, and fermented beverages. It is produced endogenously when compounds such as pectin are fermented. Naturally occurring phenylalanine, aspartic acid, and methanol are released at different rates to produce artificial or free forms of these compounds. Naturally occurring amino acids like aspartic acid and phenylalanine are bound to a protein, and thus they are released slowly into the body during digestion and metabolism. The so-called free forms of amino acids found in aspartame-sweetened beverages are released rapidly and in greater concentrations. For example, the methanol released during aspartame metabolism is a free form and is thus released directly into the bloodstream. It was also recently shown that, after an acceptable daily intake (ADI) of 40 mg per kilogram of body weight, blood methanol concentrations increased 3- to 6-fold over individual baseline values.

The daily consumption of chemically produced aspartame is increasing. Understanding the adverse effects of aspartame and its metabolites is thus essential. The safe levels of non-nutritive artificial sweeteners, especially aspartame in soft drinks, iced tea, concentrated fruit syrups, fruit and vegetable juices, flavored mineral water, energy drinks, milk-based desserts, candies, jelly, chewing gum, fruit yogurt, and ice cream are continuously monitored and scrutinized by governmental agencies. The European Food Safety Authority set the ADI of aspartame for humans at 40 mg/kg of body weight. The US Food and Drug Administration set an ADI of 50 mg/kg of body weight. The ADI of aspartame can be calculated as follows:

\[
\text{ADI (mg/kg)} = \frac{\text{aspartame (g/d)} \times \text{daily consumption (g/person/d)} \div \text{body weight (kg)}}{1000}
\]

Daily consumption of artificial sweeteners by women of childbearing age and by children has been estimated at 2.5 to 5.0 mg/kg. Typically, in adults, mean intake values of aspartame range from 5.6% of the ADI to, at most, 14.7% of the ADI. Mean intake values in children range from 21% of the ADI to, at most, 43.1% of the ADI.

The ADI of aspartame is also species dependent. Body surface area conversions should be used to convert between rat ADIs and human ADIs. The safe dose or ADI of aspartame for humans, ie, 40 mg/kg, is always corrected by a factor of 5 or 6 for rats, as rats metabolize aspartame faster than humans. From these calculations, it is accepted that rats may have an aspartame dosage of

<table>
<thead>
<tr>
<th>Food or beverage</th>
<th>Phenylalanine (g)</th>
<th>Aspartic acid (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartame-sweetened beverages (per 100 g)</td>
<td>1.186</td>
<td>0.983</td>
</tr>
<tr>
<td>Fat-free or skim milk (per 100 g)</td>
<td>0.175</td>
<td>0.288</td>
</tr>
<tr>
<td>Apple juice (per 100 g)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Tomato juice (per 100 g)</td>
<td>0.026</td>
<td>0.130</td>
</tr>
<tr>
<td>Orange juice (per 100 g)</td>
<td>0.043</td>
<td>0.437</td>
</tr>
<tr>
<td>Banana (raw)</td>
<td>0.049</td>
<td>0.124</td>
</tr>
<tr>
<td>Egg white (raw)</td>
<td>0.686</td>
<td>1.220</td>
</tr>
</tbody>
</table>
250 mg/kg/d (after species factor correction). It is therefore suggested that the oral lethal dose of aspartame in rats and mice is more than 10 g/kg/d.53

**DISCUSSION**

Absorption and toxicokinetic data that compare the effects of aspartame in humans and animals at the same dosages are not available. However, as noted previously, animals respond to aspartame and/or aspartame metabolites more rapidly than do humans.94 Extrapolating information from animal studies is one of the limitations of using data from animal studies. This review presents information from recent studies (2000–2016) that investigated excess dosages (> 40 mg/kg/d) (Table 3) and optimum safe dosages for humans (< 40 mg/kg/d) (Table 3). Table 4 summarizes the effects of aspartame administered at higher and at safe dosages. The effects of aspartame on different cells and organ systems are detailed below, in the remaining sections.

Effect of aspartame on blood cells and fibrin packaging

Injury to cell membranes by free radicals can lead to a change in cell membrane fluidity, impairing the vital functions of blood cells (erythrocytes, neutrophils, and lymphocytes).92 Most importantly, it can affect immunity by altering neutrophil and lymphocyte function.93 Oral administration of aspartame at both a higher dosage (> 40 mg/kg/d)55 and a safe dosage (< 40 mg/kg/d)55,70,71 can increase the production of free radicals and induce oxidative stress in blood cells (erythrocytes, neutrophils, and lymphocytes) by altering the oxidant/antioxidant balance. Oxidative stress in erythrocytes can lead to damage of the erythrocyte membrane108; impair the flow of erythrocytes through the microcirculation and the delivery of oxygen to the tissues91; induce erythrocyte aging91; and induce inflammation.109

Reactions between excessive amounts of reactive oxygen species (ROS) and superoxide radicals from activated neutrophils can exert cytotoxic effects and may induce overactivation of the nuclear repair enzyme poly-(adenosine diphosphate [ADP]-ribose) polymerase, which may cause adenosine triphosphate depletion and cellular injury.96 The activation of poly-(ADP-ribose) polymerase is also known to upregulate multiple pathways of proinflammatory signaling.96,91 Hence, oxidative stress in blood cells after aspartame consumption can modify the expression or activation of inflammatory mediators. The T lymphocytes (T cells) are rendered hyporesponsive to activating stimuli, but both exposure to ROS produced by activated neutrophils110 and prolonged exposure to high ROS concentrations94 induce selective loss of T-cell signaling molecules. This may lead to decreased T-cell proliferation94 and can ultimately result in apoptosis.111

Normally, in the coagulation process, thrombin, fibrin, and platelets play an important role in hemostasis. An increase in oxidant stress and a decrease in antioxidant levels are also associated with aberrant changes in platelet function.112 However, fibrin formation (and, therefore, fibrin packaging) and platelet activation were found to be changed during aspartame intake in an animal model.13 The hydrogen peroxide and peroxyl radicals that form when aspartame is ingested13,74 are likely involved in enhanced calcium mobilization, which may lead to platelet hyperactivity and hyperaggregability in patients with type 2 diabetes.95 Type 2 diabetes causes changes in the coagulation system, and hypercoagulability is a hallmark of the systemic inflammatory profile in type 2 diabetic patients. Hence, aspartame use may exacerbate the hypercoagulability already present in these patients.

Effect of aspartame on the brain

Once the cellular antioxidant capacity is overcome by the generation of ROS and reactive nitrogen species (RNS), cellular damage may occur.92 Neuronal cells are especially vulnerable to oxidative stress because of high concentrations of polyunsaturated fatty acids, which render them more susceptible to lipid peroxidation compared with other tissues.113 The excess free radicals can attach to fatty acids in the neuronal cell membrane, thereby interfering with neuronal cell function.97 The production of excess free radicals may also increase permeability of the blood–brain barrier in a time- and concentration-dependent manner.98

The consumption of higher dosages of aspartame (> 40 mg/kg/d) on the brain was also previously studied.56,60,62–64 Results suggest that higher aspartame dosages may result in changed enzyme activities.56–60,63–64 It was also found, in an in vivo voltammetry study that aspartame decreases evoked extracellular dopamine levels in the rat brain.62 Brain areas affected by aspartame may include the cerebral cortex, hypothalamus and hippocampus, as shown in a paper by Iyyaswamy and Rathinasamy in 2012.60 Areas like the hippocampus and medial prefrontal cortex play important roles in memory and decision making.114,115 Even the US Food and Drug Administration–approved ADI of aspartame (≤ 40 mg/kg) has been shown to be toxic to the brain.13,74,116 Inside neuronal cells, intense or prolonged oxidative stress causes overexpression of proinflammatory cytokines (interleukin 6 [IL-6], interleukin 1β [IL-1β], interleukin 8 [IL-8]), considered a hallmark of neuroinflammation.100,101 The proinflammatory cytokines
Table 2: Effects of high dosages of aspartame (> 40 mg/kg) on different cells and organ systems

<table>
<thead>
<tr>
<th>Reference</th>
<th>Cells/tissue</th>
<th>In vitro or animal model</th>
<th>Aspartame dosage</th>
<th>Route and duration of administration</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tsakiris et al. (2006)55</td>
<td>Blood</td>
<td>Human erythrocyte membrane</td>
<td>150 mg/kg or 200 mg/kg</td>
<td>Incubated for 1 h at 37°C</td>
<td>Significant decrease in AChE activity in human erythrocyte membranes when incubated with aspartame components</td>
</tr>
<tr>
<td>Simintzi et al. (2007)56,57</td>
<td>Brain</td>
<td>Suckling (21 days) Wistar rats</td>
<td>(150 or 200 mg/kg)</td>
<td>Incubated for 1 h at 37°C</td>
<td>Significant decrease in AChE activity in the frontal cortex and hippocampus with aspartame components</td>
</tr>
<tr>
<td>Ashok et al. (2013),58 Ashok et al. (2014),59 Iyyaswamy &amp; Rathinasamy (2012)60</td>
<td>Wistar albino male rats</td>
<td>75 mg/kg</td>
<td>Oral for 90 d</td>
<td>Imbalance of cell membrane homeostasis, leading to oxidative stress in discrete brain regions (cerebral cortex, cerebellum, midbrain, pons medulla, hippocampus, and hypothalamus) and changes in locomotor activity and anxiety levels. Histopathological alterations in brain regions were also observed</td>
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</tr>
<tr>
<td>Villareal et al. (2016)61</td>
<td>Male ICR mice (Mus musculus)</td>
<td>1000 mg/kg</td>
<td>Oral for 32 d</td>
<td>No significant difference on memory retention, but a neurotropic effect (apoptosis) was observed in the hippocampus (brain)</td>
<td></td>
</tr>
<tr>
<td>Abhilash et al. (2013)64</td>
<td>Wistar albino male rats</td>
<td>500 mg/kg and 1000 mg/kg</td>
<td>Oral for 90 d</td>
<td>Imbalance in antioxidant/prooxidant status, mainly through the glutathione-dependent system, which led to vascular congestion in the brain and a relatively potent effect of decreasing evoked extracellular dopamine (DA) levels when administered systemically under the conditions specified by the authors</td>
<td></td>
</tr>
<tr>
<td>Bergstrom et al. (2007)62</td>
<td>Sprague-Dawley rats</td>
<td>500 mg/kg</td>
<td>Single systemic dose</td>
<td>Increase in protein expression and activity of several cytochrome P450 enzymes (CYP1, 2, and 3) of phase I metabolizing enzymes in the brain (cerebrum and cerebellum)</td>
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<tr>
<td>Vences-Mejía et al. (2006)63</td>
<td>Wistar rats</td>
<td>75 and 125 mg/kg</td>
<td>Oral for 30 d</td>
<td>At highest dose of 5.625 mg/kg, repeated aspartame administration impaired memory and increased oxidative stress in brain. When a mild systemic inflammatory response was present, intraperitoneal administration of LPS (100 μg/kg) increased oxidative stress and inflammation in the brain, but not in the liver</td>
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<tr>
<td>Abdel-Salam et al. (2012),65 Abdel-Salam et al. (2012)66</td>
<td>Adult mice</td>
<td>0.625, 1.875, or 5.625 mg/kg</td>
<td>Subcutaneous for 2 wk</td>
<td>At highest dose of 5.625 mg/kg, repeated aspartame administration impaired memory and increased oxidative stress in brain. When a mild systemic inflammatory response was present, intraperitoneal administration of LPS (100 μg/kg) increased oxidative stress and inflammation in the brain, but not in the liver</td>
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<tr>
<td>Park et al. (2000)67</td>
<td>Adult mice</td>
<td>0.5 mg/g</td>
<td>Intraperitoneal</td>
<td>Impaired memory retention observed, as well as damaged neurons in the arcuate nucleus of the hypothalamus</td>
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<tr>
<td>Goerss et al. (2000)17</td>
<td>Male Long-Evans rats</td>
<td>200, 400, or 800 mg/kg</td>
<td>Intraperitoneal</td>
<td>Inverse relationship observed between striatum serotonin levels and aggression at high doses of aspartame</td>
<td></td>
</tr>
<tr>
<td>Ashok et al. (2014)68</td>
<td>Liver</td>
<td>Wistar albino male rats</td>
<td>75 mg/kg</td>
<td>Oral for 90 d</td>
<td>Altered levels of liver marker enzyme (Ygt) as well as histological changes in the liver observed</td>
</tr>
<tr>
<td>Abhilash et al. (2011)69</td>
<td>Wistar albino male rats</td>
<td>500 mg/kg and 1000 mg/kg</td>
<td>Oral for 90 d</td>
<td>Aspartame (1000 mg/kg) led to alterations in liver antioxidant status, mainly through the glutathione-dependent system and hepatocellular injury</td>
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</tr>
<tr>
<td>Ashok et al. (2014)68</td>
<td>Kidney</td>
<td>Wistar albino male rats</td>
<td>75 mg/kg</td>
<td>Oral for 90 d</td>
<td>Induced histological changes in the renal cortex (kidney)</td>
</tr>
</tbody>
</table>

Abbreviations: AChE, acetylcholinesterase; LPS, lipopolysaccharide.
### Table 3  Effects of safe dosages of aspartame (≤ 40 mg/kg) on different cells and organ systems

<table>
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<tbody>
<tr>
<td>Choudhary &amp; Devi (2014)[70]</td>
<td>Blood</td>
<td>Wistar albino male rats</td>
<td>40 mg/kg</td>
<td>Oral for 15, 30, and 90 d</td>
<td>Induced oxidative stress in serum, irrespective of duration of exposure</td>
</tr>
<tr>
<td>Arbind et al. (2014)[71]</td>
<td>Wistar albino male rats</td>
<td>Oral for 15, 30, and 90 d</td>
<td>Induced oxidative stress in blood cells (RBCs, neutrophils, and lymphocytes). Altered neutrophil function, irrespective of duration of exposure</td>
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<tr>
<td>Agamy (2009)[72]</td>
<td>Diabetic male Wistar rats</td>
<td>Intraperitoneal injection for 4 wk</td>
<td>Significant increase in AChE activity in serum</td>
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<tr>
<td>Pretorius &amp; Humphries (2007)[73]</td>
<td>Rabbit</td>
<td>Oral for 2 mo</td>
<td>Disruption in platelet activation and coagulation</td>
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</tr>
<tr>
<td>Tsakiris et al. (2006)[74]</td>
<td>Human erythrocyte membrane</td>
<td>Incubated for 1 h at 37°C</td>
<td>Significant decrease in AChE activity in the human erythrocyte membrane when incubated with aspartame components</td>
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<td>Ashok &amp; Sheeladevi (2015)[13], Ashok &amp; Sheeladevi (2014)[74]</td>
<td>Brain</td>
<td>Wistar albino male rats</td>
<td>40 mg/kg</td>
<td>Oral for 90 d</td>
<td>Alterations in neurobehaviors (emotional and anxiety behavior). Increased neuronal oxidative damage led to neuronal cell death (apoptosis) in discrete regions of brain (eg, cerebral cortex, cerebellum, midbrain, pons medulla, hippocampus, and hypothalamus)</td>
</tr>
<tr>
<td>Choudhary &amp; Sundareswaran (2016)[75]</td>
<td>Wistar albino male rats</td>
<td>Oral for 90 d</td>
<td>Alteration in EEG pattern (fronto-parietal and occipital regions)</td>
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<tr>
<td>Abu-Taweel et al. (2014)[76]</td>
<td>Mice</td>
<td>Oral for 30 d</td>
<td>Alterations in behavioral parameters (cognitive responses, memory retention, and learning capabilities), without significant changes in biochemical parameters</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kim et al. (2011)[77]</td>
<td>Zebra fish (10 wk old)</td>
<td>Oral for 12 d</td>
<td>Increased brain inflammation, impairment of learning and memory, and acute swimming defects were noted in hyperlipidemic rats</td>
<td></td>
<td></td>
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<td>Reference</td>
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<tr>
<td>Christian et al. (2004)</td>
<td>Male Sprague–Dawley rats</td>
<td>Rat dosage (250 mg/kg)</td>
<td>Oral, via the drinking water, for 3–4 mo</td>
<td>Altered T-maze performance and increased muscarinic cholinergic receptor densities or enzymes in certain brain regions</td>
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<tr>
<td>Abd El-Samad (2010)</td>
<td>Wistar albino male rats</td>
<td>Rat dosage (250 mg/kg)</td>
<td>Oral for 8 wk</td>
<td>Harmful effects (condensed nuclei and loss of characteristic pyriform shape of Purkinje cells) on cells in the cerebellar cortex</td>
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<tr>
<td>Ashok &amp; Sheeladevi (2015)</td>
<td>Liver</td>
<td>Wistar albino male rats</td>
<td>40 mg/kg</td>
<td>Oral for 90 d</td>
<td>Altered antioxidant status, expression of stress protein, and induced apoptotic changes in the liver</td>
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<tr>
<td>Choudhary &amp; Devi (2014), Kumar Choudhary et al. (2014)</td>
<td>Kidney</td>
<td>Wistar albino male rats</td>
<td>40 mg/kg</td>
<td>Oral for 15 d and 30 d</td>
<td>Induced oxidative stress in liver, regardless of duration of exposure. Levels of serum protein and bilirubin that reflect liver function were altered after 30 d of aspartame administration</td>
</tr>
<tr>
<td>Iman (2011)</td>
<td>Wistar albino male rats</td>
<td>40 mg/kg</td>
<td>Oral for 2, 4, and 6 wk</td>
<td>Induced oxidative stress in liver after 4 wk and 6 wk of treatment</td>
<td></td>
</tr>
<tr>
<td>Kim et al. (2011)</td>
<td>Zebrafish (10 wk old)</td>
<td>3 mM</td>
<td>Oral for 12 d</td>
<td>Inflammatory cells in the liver were infiltrated</td>
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<td>Choudhary &amp; Devi (2014), Kumar Choudhary et al. (2014)</td>
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<td>Wistar albino male rats</td>
<td>40 mg/kg</td>
<td>Oral for 15 d and 30 d</td>
<td>Induced oxidative stress in kidney and serum values that reflect kidney function (such as creatinine, urea, and uric acid) after 30 d of aspartame administration</td>
</tr>
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<td>Iman (2011)</td>
<td>Wistar albino male rats</td>
<td>40 mg/kg</td>
<td>Oral for 2, 4, and 6 wk</td>
<td>Induced oxidative stress in kidney after 6 wk</td>
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</tr>
<tr>
<td>Martins &amp; Azoubel (2007)</td>
<td>Wistar female rats</td>
<td>(14 mg/kg) heated to 40°C</td>
<td>Intragastric on days 9, 10, and 11 of pregnancy</td>
<td>Morphometric alterations in all renal structures (glomerulus, proximal and distal convoluted tubules, and collecting ducts) of the rat fetal kidney during organogenesis</td>
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</tr>
</tbody>
</table>
Table 3 Continued

<table>
<thead>
<tr>
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<tr>
<td>Choudhary &amp; Sundareswaran (2016),25 Choudhary &amp; Sundareswaran (2016)85</td>
<td>Heart</td>
<td>Wistar albino male rats</td>
<td>40 mg/kg</td>
<td>Oral for 90 d</td>
<td>Induced oxidative stress in the heart, impaired cardiac function, and reduced heart rate variability, as evidenced by sympathetic dominance and loss of vagal tone, but could not induce structural change</td>
</tr>
<tr>
<td>Gudadhe et al. (2013)85</td>
<td>Neonatal mice</td>
<td>100 μg/g</td>
<td>Intraperitoneal for 2 wk</td>
<td>Compensatory hypertrophy of myocytes as a toxic effect of aspartame</td>
<td></td>
</tr>
<tr>
<td>Choudhary &amp; Devi (2014),79 Kumar Choudhary et al. (2014),81 Choudhary &amp; Devi (2015),90 Choudhary &amp; Rathinasamy (2014)97</td>
<td>Immune system</td>
<td>Wistar albino male rats</td>
<td>40 mg/kg</td>
<td>Oral for 90 d</td>
<td>Altered the homeostasis of immune organs. Possible oxidative stress and imbalanced oxidant/antioxidant status, variations in serum cytokine levels, and alteration of cellular and humoral immunity</td>
</tr>
<tr>
<td>Okasha (2016)98</td>
<td>Sciatic nerve</td>
<td>Male albino rats</td>
<td>Rat dosage (250 mg/kg)</td>
<td>Oral for 3 mo</td>
<td>Degenerative changes observed, mainly in the myelin sheath in the form of focal and extensive demyelination</td>
</tr>
<tr>
<td>Palmnäs et al. (2014)99</td>
<td>Gut microbes</td>
<td>Male albino rats</td>
<td>5–7 mg/kg</td>
<td>Oral for 8 wk</td>
<td>Elevated fasting glucose levels and impaired insulin tolerance may both be mediated by alteration of gut microbiota</td>
</tr>
<tr>
<td>Suez et al. (2014)90</td>
<td>Mice</td>
<td>4% aspartame</td>
<td>Oral for 11 wk</td>
<td>Induced higher glucose excursions, mediated by alteration of gut microbiota</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: AChE, acetylcholinesterase; EEG, electroencephalogram; RBCs, red blood cells.
are important regulators of matrix metalloproteinases, which are zinc-containing enzymes produced by activated microglia. They are responsible for breaking down the extracellular matrix in cerebral blood vessels, which lead to damage of neurons and a disruption in the blood–brain barrier.118

Neurobehavioral parameters (like learning and memory) were impaired not only with higher dosages of aspartame but also with safe dosages. At high concentrations, ROS and RNS may lead to decreased synaptic plasticity by attenuating long-term potentiation and synaptic neurotransmission.99

**Effect of aspartame on the liver**

The liver is the principal detoxifying organ and maintains metabolic homeostasis. Free radical reactions are widely known to be involved in liver injury. Both higher dosages of aspartame and safe dosages have been shown to impair the antioxidant status of the liver, which may lead to hepatocellular injury. During oxidative stress, the balance between ROS and antioxidants shifts toward increased production of the former, which may affect the energetic and regenerative processes, resulting in hepatic damage. The cytotoxic effects of ROS and RNS in the liver may also lead to inactivation of the heme group and nitrosylation of iron–sulfur group. In addition, the inflammation caused by the presence of ROS results in the modulation of hepatocyte metabolism. Furthermore, Kupffer cells activated by free radicals are responsible for the release of proinflammatory cytokines. The toxicity of methanol released during the metabolism of aspartame was also shown to lead to apoptotic changes in the liver of Wistar albino rats.

**Effect of aspartame on the kidney**

The kidneys are vital organs, necessary for maintaining the composition and volume of body fluids, the acid–base balance, and the redox status. Oxidative stress may lead to kidney injury. Both higher dosages and safe dosages of aspartame may induce oxidative stress in the kidneys of rats. It was previously suggested that in the kidney, oxidative stress may contribute to the progression of kidney fibrosis and chronic kidney disease may be associated with inflammation and upregulated inflammatory cytokines.
Effect of aspartame on the heart (including cardiometabolic effects)

Over the past decade, a number of animal studies—as well as multiple large-scale, long-term, prospective observational studies in humans—have reported increased incidences of a range of cardiometabolic conditions among study participants with daily dietary exposure to aspartame. Fowler125 conducted an extensive review of results from animal research as well as from large-scale, long-term observational studies in humans on the cardiometabolic risks of aspartame use. Myocardial function is also modulated by the autonomic nervous system.126 Aspartame may lead to oxidative stress in cardiac tissue and has been shown to impair cardiac function, resulting in reduced heart rate variability, sympathetic dominance, and loss of vagal tone.75 The loss of protective vagal tone would explain the increased susceptibility to cardiovascular disease.126 An increasing number of physical illnesses appear to be associated with sympathetic dominance, reduced vagal tone, and reduced heart rate variability.127 The inflammatory markers fibrinogen and IL-6 are both moderately related to heart rate variability, which demonstrates a relationship between autonomic nervous system function and inflammatory and coagulant processes.128 This suggests that oxidative stress or inflammation caused by aspartame could affect heart rate variability. The toxic effect of aspartame may also induce structural changes in the myocardium that manifest as compensatory hypertrophy of myocytes.85

Effect of aspartame on the immune system

The oxidant/antioxidant balance is critical for immune cell function, since it maintains the integrity and functionality of cellular proteins, nucleic acids, and the cell membrane.93 Immune cells are particularly sensitive to oxidative stress because of the high percentage of polyunsaturated fatty acids in their plasma membranes.129 Aspartame (40 mg/kg/d) may act as a chemical stressor and induce oxidative stress by disturbing the oxidant/antioxidant balance, which may alter the ability of the immune system to maintain homeostasis, as observed in an animal model in which both nonimmunized and immunized rats were exposed to aspartame. This oxidant/antioxidant imbalance may lead to variations in serum cytokine levels and to alterations in both cellular and humoral immunity.86 The release of ROS has long been recognized as a typical consequence of immune cell stimulation, but excess production of ROS can promote inflammation by direct oxidative damage or by alteration of innate and adaptive mechanisms.130

Effect of aspartame on the gut microbiota

Non-nutritive sweeteners (including aspartame) may influence gut metabolism by changing the host metabolic phenotype, ultimately affecting the gut microbiota.131 Changes in the gut microbiota may interfere with the physiological responses that control homeostasis; alter the intestinal environment, thereby triggering inflammatory processes associated with metabolic disorders; or disrupt sweet-taste receptors in the gut that can affect glucose absorptive capacity and glucose homeostasis.

Aspartame, which has bacteriostatic properties, also has an anticyclic effect and is resistant to fermentation by oral bacteria.132 Aspartame’s ability to curb the growth of bacteria is not limited to oral bacteria but extends to the gut microbiota in animal models. Low-dose aspartame (5–7 mg/kg/d) consumed in drinking water over an 8-week period resulted in elevated fasting glucose levels and impaired insulin tolerance in diet-induced obese rats.89 Mice that drank water containing 4% aspartame and ate a high-fat diet for 11 weeks had higher glucose excursions after a glucose load; the elimination of this effect by antibiotic treatment suggested that changes in the metabolic phenotype of the mice was caused by alterations in the gut microbiota.90

CONCLUSION

Current scientific knowledge about the safety of aspartame, as reviewed here, is based mostly on animal studies.
studies. These studies suggest that aspartame, even at recommended safe dosages, might not be safe. Several of these studies (in vitro as well as in vivo) that investigated both higher and safe dosages indicate that aspartame or its metabolites cause an oxidant/antioxidant imbalance, induce oxidative stress, and damage membrane integrity (lipid, protein, and nucleic acid), possibly affecting most cells and tissues. Aspartame is directly involved in the development of oxidative stress, which is a hallmark of systemic inflammation (Figure 3). Several animal studies have also reported a deleterious effect of aspartame exposure on body weight, adiposity, and/or glucose tolerance and insulin levels. These are summarized in a 2016 review by Fowler. Thus, there is a need for additional detailed human studies and comprehensive characterizations of the physiological processes affected by aspartame. This is of particular importance, as diabetic and other individuals with gut dysbiosis may already be at increased risk of systemic inflammation because of the inflammatory nature of their conditions. Data reviewed in this paper suggest that aspartame use could not only exacerbate existing systemic inflammation but also cause inflammation if healthy individuals ingest it on a regular basis.

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Declaration of interest. The authors have no relevant interests to declare.

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