



Investigation of the toxic effects of aspartame as an artificial sweetener in food: effect on redox and inflammatory biomarkers in rat

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ARTICLE INFO	ABSTRACT
<p><i>Article history:</i> Received 08 Apr. 2023 Received in revised form 22 Jun. 2023 Accepted 28 Jun. 2023</p> <p><i>Keywords:</i> Aspartame; Toxicity; Oxidative stress; Antioxidant; Inflammation; Hematotoxicity</p>	<p>Aspartame (L-aspartyl-L-phenylalanine methyl ester) is a widely used synthetic sweetener. The safety of aspartame consumption remains controversial due to its widespread and sometimes indiscriminate use above the FDA-recommended level. The study focused on investigating the toxic effects of aspartame administration at high doses on redox and inflammatory biomarkers in male Wistar rats. Rats were divided into two groups: Group I was given normal saline (0.9%) orally and Group II was administered with aspartame (100 mg/kg body weight) for 30 days. Administration of aspartame significantly ($p < 0.05$) increased the levels of Malondialdehyde (MDA), protein carbonyl (PCO), and Advanced Oxidation Protein Products (AOPP) content which are prominent markers of oxidative stress. The assessment of antioxidant defenses in both the groups denoted a significant ($p < 0.05$) decrease in levels of Ferric Reducing Ability of Plasma (FRAP), Superoxide Dismutase (SOD), and Catalase in the aspartame-treated group in comparison to the control. The pro-inflammatory markers Tumour Necrosis Factor α (TNF-α), Interleukin-6 (IL-6) and C - reactive protein (CRP) were also significantly ($p < 0.05$) increased in the treated group. These results suggest that aspartame intake of 100 mg/kg body weight contributes to oxidative stress in erythrocytes which in turn may play a role in predisposing an individual to obesity, cardiovascular disease, and cancer. Aspartame should be strictly limited to the FDA-recommended levels since its indiscriminate use causes severe toxic effects.</p>

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1. Introduction

Aspartame (L-aspartyl L-phenylalanine methyl ester) is a non-nutritive sweetener that has been approved by

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the United States Food and Drug Administration (FDA) at the maximum dose of 50 mg/kg body weight (BW) for use in carbonated soft drinks and as an artificial sweetener (1).



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Upon ingestion, aspartame is metabolized into its constituent amino acids: phenylalanine & aspartic acid, and methanol (2). Among the metabolites of aspartame, phenylalanine is involved in the production of neurotoxins and hormones and its increased concentration produces neurotoxic effects in the brain (3), whereas methanol causes systemic toxicity in the body. Thus the consumption of aspartame must be regulated given the effect it produces on oxidative stress levels, oxidant/ antioxidant production, and brain health (4,5).

Considering its consumption as safe, as per FDA norms, aspartame is widely used by diabetic and obese (6) individuals as it does not negatively influence their blood glucose and insulin levels. However, its efficacy is questionable because of its by-product methanol which acts as a toxicant whose severe harmful effects on health include neurological diseases, multiple sclerosis, schizophrenia, and autism (7). The consumption of aspartame at high doses (beyond 50 mg/kg BW) may cause deteriorative effects in the brain leading to neurodegeneration, liver injury, inflammation in kidneys, and impaired cardiometabolic function (8). The side - effects of aspartame consumption may pertain to the generation of oxidized products of lipids (9).

Oxidative stress is a crucial factor influencing the health of any organism and this state is determined by the quality of food intake. A rise in oxidative stress occurs due to an increase in free radical generation leading to lipid and protein oxidation and inflammation (10). However, cells also have an antioxidant defense system that acts to minimize oxidative stress (11).

Although the FDA-approved level of aspartame consumption for humans is 50 mg/kg BW per day, it is frequently observed that aspartame is being indiscriminately used in local foods especially sweets without following the FDA-approved limit (12). This indiscriminate use of aspartame in high doses may lead to health issues which prompts us to make an effort to study its possible toxic effects.

The following study was undertaken to assess the toxicity of aspartame, at high doses, on lipid peroxidation, protein oxidation, antioxidant enzymes, and inflammatory biomarkers in male Wistar rats.

2. Materials and methods

2.1. Reagents and chemicals

Aspartame (N-L- α -aspartyl-L-phenylalanine-1-methyl ester, C₁₄H₁₈N₂O₅) was purchased from TCI Chemicals (India) Pvt. Ltd. All chemicals/biochemicals were of the highest purity available from Merck (India) and HIMEDIA labs (India).

2.2. Experimental study design and treatment

Rats (12-18 months age) corresponding to human middle age (13), were selected for the study. They were kept in standard living conditions and acclimatized in a circadian light/dark cycle at 23±2°C temperature with ad libitum drinking water and standard rat feed pellets. All protocols of animal care and laboratory methods were in accordance with the guidelines of the Committee of the Control and Supervision of Experiments on Animals (CPCSEA) and the Institutional Animal Ethics Committee (IAEC), University of Allahabad, India (IAEC/AU/2019(1)/09).

Rats (n = 6) were divided randomly into two groups:

Group I: Normal Control rats (C): Rats were administered with saline (0.9%) orally (30 days).

Group II: Aspartame (Asp): Rats were dosed with aspartame (100 mg/kg BW) dissolved in normal saline orally for 30 days.

The dose of aspartame was decided from a previously published report (14).

2.3. Collection of blood samples, separation of plasma and RBCs

On the completion of the experimental treatment of 30 days, the rats were sacrificed under mild anesthesia (pentobarbital 50 mg/kg body weight) and blood was collected by heparinized syringes through cardiac puncture. Plasma was isolated by centrifuging blood at 800 g for 10 min at 4°C, and packed red blood cells (PRBCs) were obtained. The PRBCs were washed thrice with cold-phosphate-buffered-saline (PBS) which was stored in glucose-phosphate-buffered-saline (GPBS) at 4°C. While experiments on PRBCs were done on the same day, the plasma was stored at -80°C. Serum was isolated by allowing blood to clot in non-heparinized syringes.

2.4. Determination of FRAP (Ferric reducing the ability of plasma)

FRAP assay was used to measure the total antioxidant capacity of the plasma as per the established protocol (15). This assay utilizes the ability of ferric ions to get reduced to ferrous ions. Absorbance values were measured at 593 nm. Readings were taken at 30 s intervals for 5 min, values are expressed as (μmol Fe (II)/L plasma).

2.5. Estimation of Reduced Glutathione (GSH)

Erythrocyte GSH was assayed by following the protocol (16). This method is based on the reducing ability of 5, 5'-dithiobis (2-nitrobenzoic acid) (DTNB) to reduce the -SH group to form an anionic product (yellow) the OD of which is measured at 412 nm. GSH concentration is expressed in terms of mg/mL PRBCs.

2.6. Determination of Superoxide Dismutase (SOD) and Catalase activity

Superoxide dismutase (SOD) was determined following the reported method (17). Catalase activity of all treated groups in plasma was measured by the H₂O₂ degradation assay following the protocol (18).

2.7. Estimation of Malondialdehyde and protein.

Determination of MDA content was done by the established protocol (19). Plasma protein was measured by the method of Lowry (20) using bovine serum albumin as standard.

2.8. Determination of Protein Carbonyl and advanced oxidation protein products (AOPP)

The procedure of Levine (21) was used to measure plasma protein carbonyl levels. An absorption coefficient of 22,000 M⁻¹ cm⁻¹ was used to calculate carbonyl content was calculated using. Data was expressed in units of nmol/mg protein. AOPP was determined by using the spectrometric method (22) The concentration of AOPP is reported as μmol of chloramine-T equivalent/liter of plasma (μmol/L).

2.9. Estimation of serum levels of the pro-inflammatory cytokines IL-6, TNF, and C-reactive protein

Serum cytokine levels were measured according to guidelines provided by Krishgen Biosystem, India, and previously described (23). The results are expressed in pg/mL and ng/mL.

2.10. Statistical analysis

Statistical analysis was performed using the software Graph Pad Prism version 5.01. The results are expressed as mean \pm standard deviation (SD) of six independent experiments. Statistical differences were analyzed by unpaired t-test, ($p < 0.05$) was considered statistically significant.

3. Results

3.1. Effect of aspartame supplementation on antioxidant biomarker levels in erythrocytes

Antioxidant parameters like FRAP shown in (Fig. 1A) show a significant decrease ($p < 0.05$) of (24.85%) during aspartame treatment concerning control group. The GSH level (Fig 1B) decreased significantly ($p < 0.05$) by (50%) in the aspartame-treated group concerning control group. Antioxidant enzyme SOD (Fig. 1C) was significantly decreased ($p < 0.05$) by (32.09%) during aspartame intake when compared to the control group. The catalase level shown in (Fig. 1D) also decreased significantly ($p < 0.05$) by (45.63%) in the aspartame group compared to the control.

The lipid peroxidation product determined by the MDA level (Fig. 2A) showed a significant increase ($p < 0.05$) by (20.02%) during aspartame intake in comparison to control rats.

The biomarkers of protein oxidation determined by the PCO level (Fig. 2B) increased significantly ($p < 0.05$) by (46.77%) in the treated group concerning control

whereas, the AOPP level (Fig. 2C) increased significantly ($p < 0.05$) by (30.49%) during aspartame intake when compared to control.

The TNF- α level reported in (Fig. 3A) showed a significant increase ($p < 0.05$) of (57.18%) in the aspartame group when compared to control. The IL-6 level reported in (Fig. 3B) also showed a significant increase ($p < 0.05$) of (73.26%) in the aspartame group in comparison to the control group. The CRP levels (Fig. 3C) showed a significant increase ($p < 0.05$) of (53.20%) in the treated group compared to control.

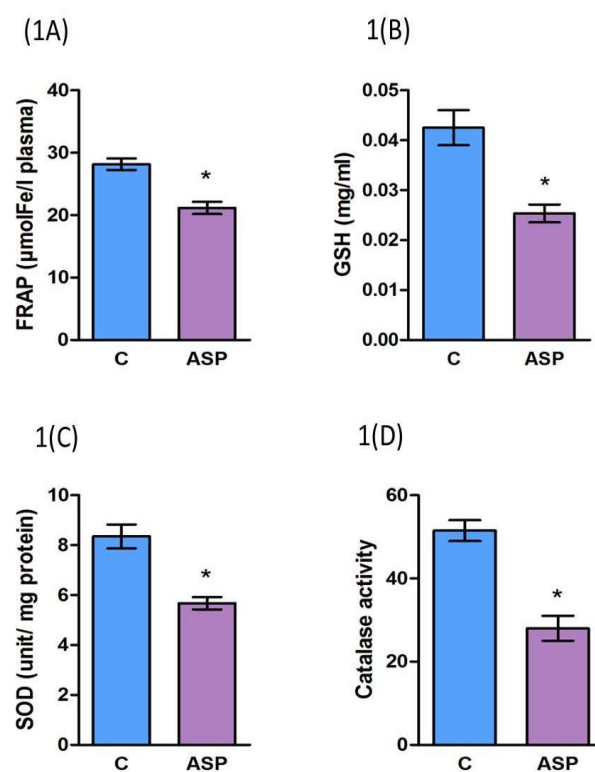


Figure 1. Effect of aspartame supplementation on pro-oxidant biomarker levels in erythrocytes

Effect of aspartame supplementation on the antioxidant level in rats. (1A) FRAP levels are reported in terms of ($\mu\text{mol Fe/l plasma}$). * denotes a significant decrease ($p < 0.05$) in comparison to the control group. (1B) Non-enzymatic antioxidant level determined by Glutathione (GSH) content is reported in terms of (mg/ml). * significantly lower ($p < 0.05$) when compared to control. (1C) Non-enzymatic antioxidant SOD is reported in terms of (unit/mg protein) * denotes a significant decrease ($p < 0.05$) in comparison to the control group. (1D) Catalase activity (KU/L). * denotes a significant decrease ($p < 0.05$) in comparison to the control group

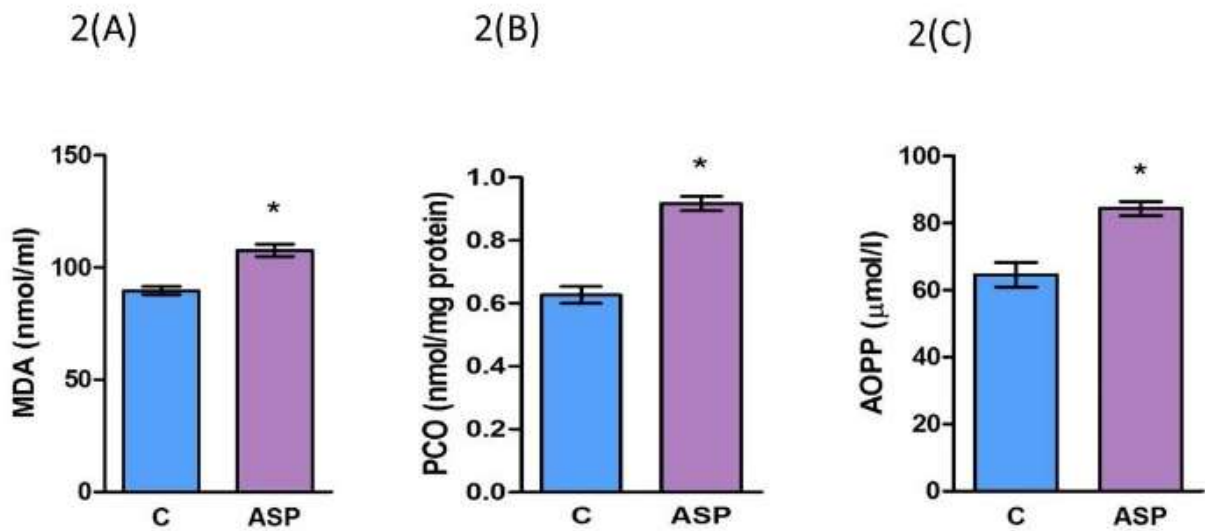


Figure 2. Effect of aspartame supplementation on pro-inflammatory biomarker levels

Effect of aspartame supplementation on the pro-oxidant level in rats. (2A) Malondialdehyde (MDA) content showing lipid peroxidation expressed as (nmol/mL). * denotes a significant increase ($p < 0.05$) in comparison to the control group. (2B) Protein carbonyl (PCO) content reported in terms of (nmol/mg protein). * Significantly higher ($p < 0.05$) when compared to control. (2C) AOPP content expressed as ($\mu\text{mol}/\text{mL}$). * Significantly higher ($p < 0.05$) when compared to control

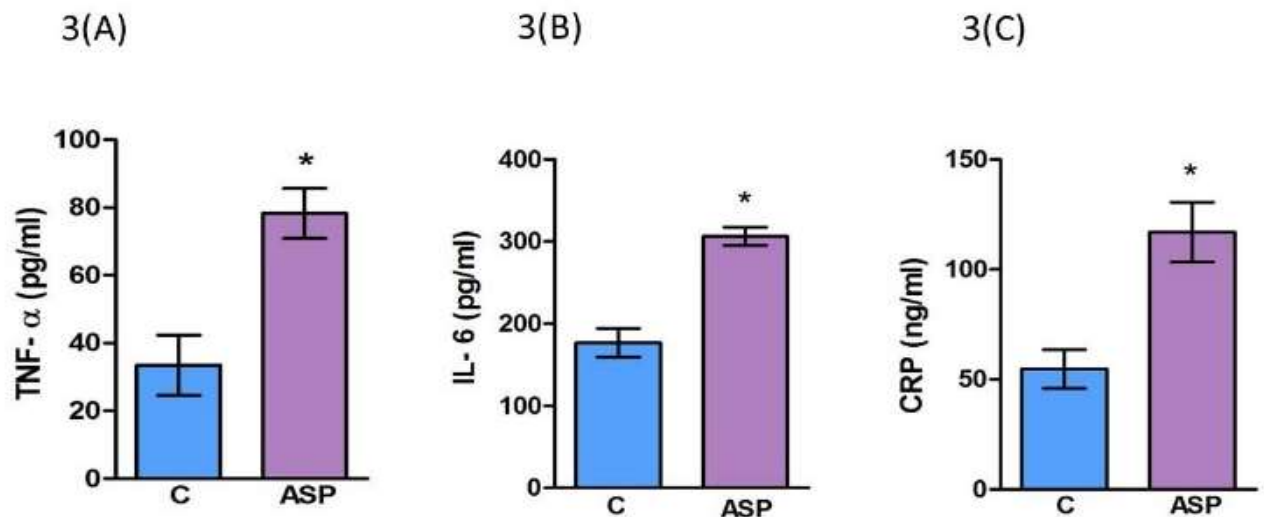


Figure 3. Effect of aspartame supplementation on pro-inflammatory biomarker levels. 3(A) TNF- α reported as (pg/mL). (3B) IL-6 reported as (pg/mL). 3(C) CRP level expressed as (ng/mL). * Significantly higher ($p < 0.05$) when compared to control

4. Discussion

Aspartame toxicity and oxidative stress are closely connected (24). Due to an imbalance in the antioxidants/oxidative system at tissue level, aspartame causes the generation of reactive oxygen species and cytotoxicity. A high dose of aspartame produces free radicals that can destroy membranes by oxidizing lipids, proteins, and DNA, in addition to a rise in inflammatory biomarkers (25). The body constantly produces ROS as a result of oxidative metabolism and mitochondrial bioenergetics (26). Aspartame metabolites cause oxidative stress due to various reasons rather than aspartame itself (27). Obesity, cardiovascular disease, and cancer are among the many disorders that are caused due to oxidative stress (28). A high dose of aspartame causes oxidative stress and a rise in inflammatory biomarkers due to a rise in methanol in circulation (29).

The FRAP level decreased significantly in aspartame-administered rats which could be because of methanol formation, one of the major metabolic products produced during aspartame metabolism. The microsomal oxidizing system that metabolizes methanol is known to produce free radicals (3).

GSH is a non-enzymatic antioxidant and a crucial component of the antioxidant defense system of the cell (30). GSH performs detoxification of Reactive Oxygen Species (ROS)- induced products of chemical origin and thereby maintains a balanced redox state in cells (31,32). The decrease in GSH level in erythrocytes can be attributed to methanol-induced oxidative stress. Formaldehyde is produced after aspartame ingestion whereas, GSH is responsible for formaldehyde metabolism. Therefore formation of formaldehyde is a

causative factor for the reduction observed in GSH content in the aspartame-treated rats (33).

Aspartame intake increased the levels of lipid peroxidation, as observed by the increase in MDA content in the present study. The rise in lipid oxidized products is a result of the depleted free radical scavenging activity and increased oxidative stress (34). Therefore, the administration of aspartame disrupts the unsaturated bonds in lipid molecules that subsequently lead to the generation of lipid-oxidized products (1).

Superoxide Dismutase (SOD) and Catalase provide essential antioxidant defense in cells. These enzymes suppress the damage caused by increased free radicals formation and therefore control the oxidative stress levels in cells (35). However, it has been observed that the rats administered with aspartame showed a decrease in SOD and catalase levels in comparison to the control rats. The lowered antioxidant enzymes SOD and catalase and increased oxidative molecules in aspartame-treated rats can damage erythrocyte membranes and produce lipid and protein oxidized products (29).

The formation of products of protein oxidation due to oxidative reactions is measured in terms of the Protein Carbonyl (PCO) content and Advanced Oxidation Protein Products (AOPP) formed (36). The PCO and AOPP content increased significantly during aspartame intake which suggests the rise in products of protein oxidation and therefore decline in protein thiol content (3). Moreover, a decrease in the GSH levels in aspartame-ingested rats is also indicative of an increase in PCO and AOPP content since GSH is a major thiol contributor.

Inflammatory cytokine (IL-6 and TNF- α) levels are higher in the aspartame group of rats, as reported

earlier (3). The possible hypothesis is that upon consumption, aspartame was metabolized in the gut and generated methanol, increased plasma methanol level which might have induced oxidative stress, and enhanced the expression of NF- κ B and NF- κ B dependent pro-inflammatory cytokines (TNF- α and IL-6) (14).

5. Conclusion

In conclusion, aspartame intake at doses of 100 mg/kg body weight causes an increase in the level of pro-oxidants in rats and damage to biomolecules due to a higher rate of free radical generation. Aspartame usage at doses much higher than FDA that is 50 mg/kg body weight may, in part, explain the higher incidence of cancer and other diseases linked to oxidative stress.

Conflict of Interests

No potential conflict of interest was reported by the authors.

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