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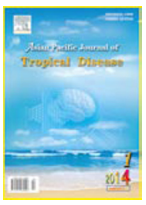

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Serum biochemical responses under oxidative stress of aspartame in wistar albino rats

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PEER REVIEW

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Comments

The study attempted by the authors is genuine and sincere by comprehensively carrying out related parameters to achieve their objective of confirming aspartame toxicity and its impact on the hepatic, renal and hematological parameters.
Details on Page S409

ABSTRACT

Objective: To study whether the oral administration of aspartame (40 mg/kg body weight) for 15 d, 30 d and 90 d have any effect on marker enzymes, some selective liver and kidney function parameter, lipid peroxidation and antioxidant status in serum. To mimic human methanol metabolism, folate deficient animals were used.

Method: Animal weight, complete hemogram, marker enzyme in serum, some selected serum profile reflect liver and kidney function, plasma corticosterone level, and in serum, lipid peroxidation, nitric oxide, enzymatic and non-enzymatic antioxidant level was measured .

Result: After 15 d of aspartame administration animals showed a significant change in marker enzymes, and antioxidant level. However, after repeated long term administration (30 d and 90 d) showed a significant change in some selected serum profile reflects liver and kidney function, along with marker enzymes, and antioxidant level.

Conclusions: This study concludes that oral administration of aspartame (40 mg/kg body weight) causes oxidative stress in Wistar albino rats by altering their oxidant/antioxidant balance.

KEYWORDS

Aspartame, Serum, Marker enzymes, Oxidative stress, Antioxidant

1. Introduction

Aspartame is widely consumed by humans who are diabetic and who are under weight loss regime. Aspartame (L-aspartyl-L-phenylalanine methyl ester) also known as NutraSweet, after oral administration to humans and experimental animals, aspartame is rapidly and completely metabolized to 50% phenylalanine, 40% aspartic acid and 10% methanol[1]. Methanol is being increasingly recognized as a substance that damages the liver cells where it is oxidized to formaldehyde and later to formate[1]. These processes are accompanied by elevation of NADH level and the formation of superoxide anion, which may be involved in lipid peroxidation[2]. Also, methanol intoxication is associated with mitochondrial damage and increased microsomal proliferation resulting in increased production of oxygen radicals[3]. And these factors together with the excess of formaldehyde formed during acute methanol intoxication cause significant increase in lipid peroxidation[2]. After aspartame consumption, the concentration of its metabolites is increased in blood[4]. There are many enzymes found in the serum that did not originate from the extracellular fluid. During tissue damage, some of these enzymes find their way into the serum through leakage arising from altered membrane permeability. Serum enzymes [alkaline phosphatase (ALP), acid phosphatase (ACP), aspartate

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aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH) and γ-glutamyl transpeptidase (γGT)] measurements are therefore a valuable tool in clinical diagnosis, providing information on the effect and nature of pathological damage to any tissue. The measurement of the activities of these marker enzymes in tissues and body fluids can be used in assessing the degree of assault and the toxicity of a chemical compound on organ/tissues[5,6]. The metabolism of xenobiotics to a large extent takes place in the liver. The byproduct of such metabolism sometimes is more toxic than the initial substance. This can cause hepatic damage and the emergency of hepatic disorders. The kidney is one of the organs responsible for the maintenance of constant extracellular environment through its in environment in the excretion of such purine catabolite as urea, creatinine, blood urea nitrogen (BUN) and uric acid as well as electrolyte balance. Abnormal concentration of this catabolite and some electrolyte in plasma or serum is a clear indication of renal function impairment[7]. The kidney function may be assessed from the level of some electrolyte (such as Na^+, and K^+,) and metabolite (such as creatinine, urea, uric acid and BUN) in the serum[8,9].

Oxidative stress was originally defined as the disequilibrium between prooxidants and antioxidants in biological systems[10]. Oxidative damage in a cell or tissue occurs when the concentration of reactive oxygen species (O^{2-}, H_2O_2, and OH-) generated exceeds the antioxidant capability of the cell[10]. The status of lipid peroxidation as well as altered levels of certain endogenous radical scavengers is taken as direct evidence for oxidative stress[14]. Therefore, serum of Wistar albino male rats was monitored for the above described parameters on exposure of aspartame (40 mg/kg body weight).

2. Materials and methods

2.1. Chemicals

Pure aspartame powder and methotrexate was purchased from Sigma Aldrich chemical, St. Louis, USA, and all other chemical used were of analytical grade obtained from Sisco Research Laboratory, Mumbai, India.

2.2. Animal model

Animal experiments were carried out after obtaining clearance from the Institutional Animal Ethical Committee (IAEC No: 02/03/11) and the Committee for the Purpose of Control and Supervision of Experiments on Animals. The experimental animals were healthy, inbreed adult male Wistar albino rats, weighing approximately 200-220 g (12 weeks of age). The animals were maintained under standard laboratory conditions and were allowed to have food and water *ad libitum* (standard rat feed pellets supplied by M/s. Hindustan Lever Ltd., India) for control animals and for making rat folate deficient were given special folate deficient diet for 37 d[11] and methotrexate(0.1 mg/100 g body weight) *i.p.* every other day for two weeks[12]. Methotrexate folate deficiency was confirmed by estimating the urinary excretion of formamino-glutamic acid (FIGLU)[13] prior to the experiment. Rats on a folate deficient diet excreted an average of 70 mg FIGLU/kg body weight per day (Range 25-125 mg) while animals on the control diet excreted an average of 0.29 mg/kg body weight per day (Range 0.15-0.55 mg). These folate deficient animals showed a significant increase in FIGLU excretion when compared to the control animals ($P<0.05$). After that animals were daily administered aspartame (40 mg/kg body weight)[14] dissolved in normal saline orally (by means of needle lavage) for 15, 30 and 90 d. All the rats were housed under condition of controlled temperature (26±2) °C with 12 h light and 12 h dark exposure.

2.3. Experimental design

Every groups consisting of 6 animals each. Group I were the control animals which were administered normal saline orally (by means of needle lavage) thought out the experimental protocol. Since human beings have very low hepatic folate content compare to rats[15]. In methanol metabolism conversion of formate to carbon dioxide is folate dependent. Hence in the deficiency of folic acid, methanol metablism could take the alternate pathway (microsomal pathway) in human[16]. Hence to mimic human methanol metabolism, folate deficient rats were used. The folate deficient animals were further divided into 4 groups. Group II was folate deficient diet fed control, Group III was folate deficient animals treated with aspartame for 15 d (40 mg/kg body weight), Group IV was folate deficient animals treated with aspartame (40 mg/kg body weight) for 30 d. Group V was folate deficient animals treated with aspartame (40 mg/kg body weight) for 90 d.

2.4. Sample collection

Blood samples were collected between 8 and 10 a.m. to avoid circadian rhythm induced changes. Stress-free blood samples were collected as per the technique described by Feldman S, *et al*[17]. At the end of experimental period all the animals were exposed to mild anesthesia and blood was collected from internal jugular vein, and used for doing complete hemograms, plasma and serum was separated respectively from anti-coagulated and coagulated blood by centrifugation at 3000 r/min at 4 °C for 15 min and kept at -80 °C until biochemical estimations.

2.5. Biochemical determinations

All assays were performed in serum. Nitric oxide levels were measured as total nitrite + nitrate levels with the use