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Research Article
Effects of Short-term Consumption of Aspartame on Some Biochemical and Hematological Parameters in Female Swiss Albino Mice

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Abstract

Background and Objectives: Aspartame is a popular artificial sweetener used by a wide range of consumers as an alternative sweetener to reduce the calorie intake. Uncontrolled used of aspartame may have many unknown deleterious effects on the population. The objectives of the present study were to investigate the adverse effects of aspartame (ASP) on biochemical and hematological parameters of female Swiss albino mice. **Materials and Methods:** Adult female mice (n = 5 each group) were divided into two groups viz. control and treatment. Treatment group received aspartame orally (40 mg kg^{-1} or 0.04 mg g^{-1} b.wt./day), while control group received double distilled water only. Animal care and handling was performed according to the standard guidelines. After 30 days mice were sacrificed for estimation of alkaline phosphates, glutamic oxaloacetic transaminase, glutamic pyruvate transaminase and hematological parameters. **Results:** The results indicate that ASP exposure significantly ($p \leq 0.05$) elevated alkaline phosphates (ALP), glutamic oxaloacetic transaminase (GOT) and glutamic pyruvate transaminase (GPT), while as a significant ($p \leq 0.05$) decrease was observed in hematological parameters (i.e.) white blood cells (WBCs), red blood cells (RBCs), hemoglobin (Hb) percentage as well as total protein content, in case of ASP treated mice compared to the control group. Creatinine content was increased in case of ASP treated mice as compared to control. **Conclusion:** The observed changes show that ASP consumption may have deleterious effects. Therefore, the current study indicates that consumption of ASP was harmful to mice in relation to hematological and biochemical analysis and needs more scientific research to investigate its effects on other parameters.

Key words: Aspartame, hematological parameters, biochemical parameters, body weight, organ weight

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Growing evidences against sugar-sweetened beverage (SSB) consumption which leads to increased weight gain and other negative health outcomes has prompted many individuals to resort to artificial, non-nutritive sweetener (NNS) substitutes as a means of reducing SSB intake[1]. These artificial, non-nutritive sweeteners include Neotame, Saccharin, Sucralose and the most used one i.e., Aspartame[2]. Aspartame is produced commercially from the methyl ester of two amino acids, L-aspartic and L-phenyl alanine[3]. It is an artificial sweetener possessing 180-200 times the sweetness potency of sucrose and has a calorie value of 4 Kcal g^{-1}. Aspartame is found in a number of foods, drinks, pharmaceuticals (vitamins and sugar-free cough drops), puddings, fillings and chewing gum, candies, tabletop sweeteners etc[4]. Many researchers have been already described side effects of aspartame consumption but still aspartame is available under many brand names for free and uncontrolled consumption. Aspartame is used in a variety of food products, however, aspartame-related neurological disturbances such as dizziness, headaches, gastrointestinal symptoms, mood alterations, allergic type reactions and alterations in menstrual patterns have also been reported by Abu-Taweel *et al.*[5]. It has been found that children and adults consume aspartame unintentionally to an excess amount than the Food and Drugs Administration (FDA) recommendation[6] which leads to serious health complications because of its metabolites, aspartic acid, phenylalanine and methanol. Low calorie artificial sweetener, aspartame (N-alpha-aspartyl-L-phenylalanine) is known to be toxic even at low doses (below than daily admissible intake 40 mg kg^{-1}/b.wt.)[7]. Aspartame consumption leads to health effects due to the formation of metabolites, especially methanol (10%), aspartic acid (40%) and phenylalanine (50%)[8]. The misuse and intake of aspartame that exceeds the recommended maximum daily intake of aspartame (40 mg kg^{-1} b.wt.) have not been described much[9].

The prolonged consumption of aspartame resulted in increased methanol concentration and its metabolites (aspartic acid, phenylalanine and methanol) responsible for the generation of oxidative stress[10]. Phenylalanine is an amino acid essential for the production of monoamines in the brain and found in nearly all foods. However, it has been found that increment in certain brain amino acid levels occurs after aspartame consumption[11]. Since its approval by the FDA for use as an artificial sweetener, aspartame has been the subject of much debate with respect to its numerous health effects[12,13].

The aim of the present study was to investigate the effects of aspartame (ASP) at a dose level admissible daily intake (ADI) generally recognized as safe for human consumption (i.e., 40 mg kg^{-1} b.wt.) biochemical and hematological parameters of female swiss albino mice.

MATERIALS AND METHODS

Ethics statement: All the research procedures and the protocol of the current study were approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The animals were handled according to the principles of laboratory care framed by CPCSEA. Before the experiment, proper approval (No. 1885/GO/S/16/CPCSEA/IAEC//B.U./08 Dt. 18/06/16) was obtained from the Institutional Animal Ethical Committee (IAEC). Barkatullah University, Bhopal (M.P.), India.

Animals and experimental design: Ten female Swiss albino mice (20-23 g b.wt.) used in this study were procured from Jawaharlal Nehru Cancer Hospital and Research Centre. Animals were kept at the animal house of Department of Biosciences, Barkatullah University, Bhopal, India. All the animals were maintained under standard laboratory conditions at 25°C temperature and 12 h light/dark cycle, with food (standard fed pellets supplied by M/S. Hindustan Lever Ltd. India) and water *ad libitum*. The study was performed in the Laboratory of Endocrinology, Department of Biosciences, Barkatullah University, Bhopal during the month of August. Aspartame was purchased in powdered form from CDH- Laboratory Chemicals India. The dose selected for the present study is 40 mg kg^{-1} b.wt., because acceptable daily intake (ADI) of aspartame for human use as recommended by The European Food Safety Authority (EFSA)[14] and Food and Drug Administration (FDA)[15] is 40 mg kg^{-1} b.wt./day. In order to confine within the human recommended admissible daily intake (ADI), this dose was chosen. Aspartame was dissolved in distilled water and administered orally (40 mg kg^{-1} b.wt./day).

Mice were divided into two groups, viz., control and aspartame treated group. The treatment group received aspartame (0.04 mg g^{-1} b.wt.) orally with the help of cannula for 30 days. On 31st day the mice were sacrificed by cervical dislocation. Blood was collected by cardiac puncture for hematological parameters. The blood was freshly used for blood count assay. Kidney, liver, spleen and brain were quickly removed and washed in ice-cold 0.9% sodium chloride (NaCl) chilled solution.

Body weight and organ weight: Body weight of the experimental animals (control as well as treatment group) were taken at 0, 15 and 30 days of the experiment. The organs (liver, kidney, brain and spleen) were weighed at the end of the experiment. The body weight gain was calculated as:

$$\text{Body weight gain} = \text{Final body weight-Initial body weight}$$

Whereas, relative organ weight was calculated as:

$$\text{Relative organ weight} = \frac{\text{Weight of organs (g)}}{\text{Final weight (g)}} \times 100$$

Biochemical study: Protein contents of the samples were assayed by the method of Lowry *et al.*[16]. Glutamic oxaloacetic transaminase (GOT) and glutamic pyruvate transaminase (GPT) were assayed by the methods of Reitman and Frankel[17]. Alkaline Phosphatase (ALP) was assayed by the method of Lowry *et al.*[16]. Creatine was assayed by Jaffe's method of Bergmeyer[18].

Hematological study: Haemoglobin concentration was determined according to the method described by Jaffe[19]. Red blood cell (RBC) count and white blood cell (WBC) count were determined by the method of Wintrobe[20].

Statistical analyses: Data are expressed as mean±standard deviation (SD). Experimental data obtained were statistically processed with the Student's "t" test to observe significant differences ($p \leq 0.05$).

RESULTS

The data from the groups for the individual parameters are presented as bar diagrams and tables with mean±SD.

Effect of aspartame on body weight and organ weight: At the 1st day of the experiment the mean values of the body weight ranged from 22.16-23.16 g. After 15 days and at the end of the experiment, there was a significant decrease in body weight of the aspartame treated mice compared to control group (Table 1). Likewise, significant decrease in organ weights (liver, kidney, brain and spleen) of aspartame treated mice compared to control was found (Table 2).

Effect of aspartame on GOT, GPT and ALP of kidney and liver: A significant increase in GOT, GPT and ALP were found in treated mice as compared to control group after 30 day of aspartame dosing. Similarly kidney GOT, GPT and ALP values were found elevated after 30 day of treatment of aspartame (Table 3, 4).

Effect of aspartame on protein and creatinine of liver and kidney: Amount of total protein in liver and kidney tissue were decreased significantly after the aspartame consumption. As is evident from figures, kidney creatinine value also changes in respect of aspartame treatment (Fig. 1).

Effect of aspartame on hematological parameters: Hematological parameters including RBC, WBC and Hb percentage were significantly reduced after the respective treatment of aspartame for 30 days (Fig. 2-4).

Table 1: Changes in body weights

Duration	Control average body weight±SD	Treatment average body weight±SD
Initial	23.16±1.379	22.16±1.379
Day 15	28.36±1.716	25.61±0.830*
Day 30	30.54±1.749	27.54±1.794**

*Significant difference at p<0.05, **Significant difference at p<0.01, ***Significant difference at p<0.001

Table 2: Changes in organ weights (g)

Organs	Control organ weight±SD	Treatment organ weight±SD
Kidney	0.173±0.023	0.145±0.014*
Brain	0.4293±0.018	0.4021±0.012*
Spleen	0.1125±0.021	0.0886±0.004*
Liver	1.068±0.428	0.602±0. 419*

*Significant difference at p<0.05, **Significant difference at p<0.01, ***Significant difference at p<0.001

Table 3: Effect of aspartame on enzymatic parameters of liver

Enzyme values tissue weight (mg g^{-1})	Control	ASP treated	SD control	SD ASP	p-value
ALP	55.08	56.73*	±0.13939	±0.22405	0.030941
GOT	10.09	11.58*	±0.47274	±0.24783	0.00015
GPT	14.82	15.08*	±0.37765	±0.43425	0 .015733

*Significant difference at p<0.05

Table 4: Effect of aspartame on enzymatic parameters of kidney

Enzyme values tissue weight (mg g^{-1})	Control	ASP treated	SD control	SD ASP	p-value
ALP	1.808	1.998*	±0.12418	±0.12661	0.018983
GOT	17.59	18.36*	±0.39981	±0.18876	0.000852
GPT	10.78	11.09*	±0.21721	±0.19677	0.025076

*Significant difference at p<0.05

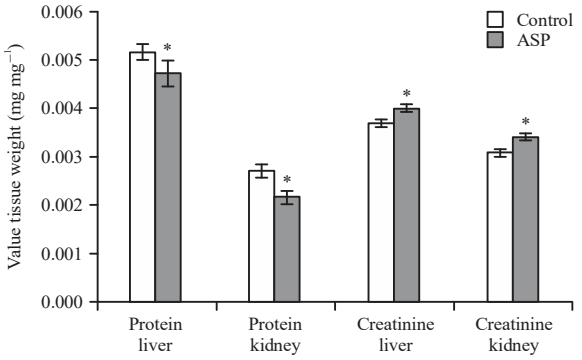

Fig. 1: Effect of aspartame on protein and creatinine level of liver and kidney

*Significant difference at p<0.05

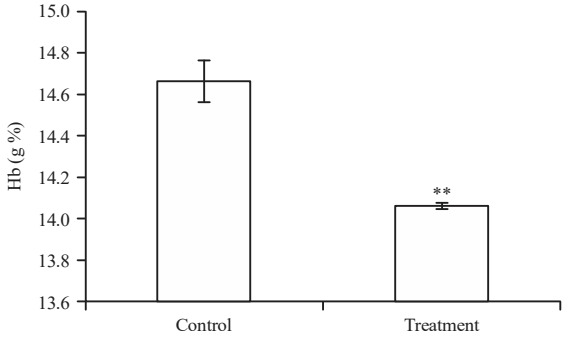

Fig. 2: Effect of aspartame on Hb percentage

**Significant difference at p<0.01

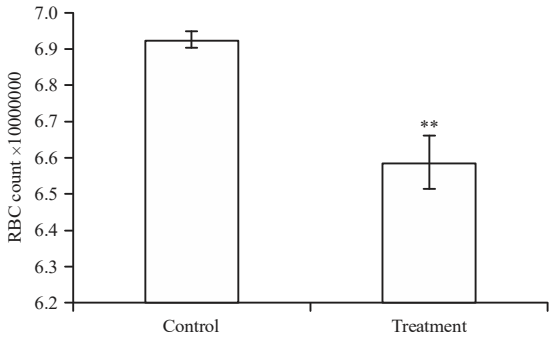

Fig. 3: Effect of aspartame on RBC count

**Significant difference at p<0.01

DISCUSSION

In the present study, decreased body weight and organ weight was observed in aspartame treated mice. This may be due to oxidative damage as reported by Schalm *et al.*[21] and Skrzydlewska and Szynaka[22]. They reported that oxidative damage caused marked decrease in organ weight upon

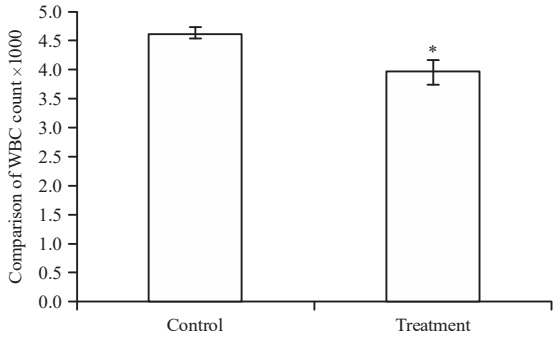

Fig. 4: Effect of aspartame on WBC

*Significant difference at p<0.05

methanol intoxication. Formaldehyde, the first metabolite of methanol is already known to increase the population of shrunken cells, dead cells and hydrolipid cells[23]. This result is supported by Nakao *et al.*[24], who reported that the body weight and fat mass decreased in overweight subjects supplemented with aspartame for 10 weeks. The mechanism by which aspartame could induce reduction in body weight has been described by many authors. Aspartame induced satiety in human beings and thereby leads to weight loss, which could have an association with post-absorptive effect of rising circulating levels of phenylalanine[25]. The phenylalanine constituent of aspartame had two effects, suppression of food intake in humans and animals[26] and increase in cholecystokinin secretion which delays the gastric emptying[27]. It was also observed that decrease in the body weight was associated with diminution of Neuropeptide Y (NPY) in its principal hypothalamic site of synthesis[28]. The NPY promotes weight gain and fat deposition as it both inhibits lipolysis and stimulates denovolipogenesis[29]. Thus, the beneficial effects of aspartame on the body weight could be related to the decreased effects of NPY on the lipid metabolism[28].

The measurement of glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), alkaline phosphatase (ALP) enzyme activities in tissues and body fluids can be used to estimate the degree of toxicity of a chemical compound on organs/tissues. The ALP is a membrane associated enzyme, which plays a role in the process of dephosphorylation. Increment in its activities can be used as an indicator of liver injury in dose and duration dependent studies[30]. Upon consumption of aspartame, its metabolites increase in the blood[31] and are mainly metabolized by the liver, since the liver is the chief organ in the breakdown, where metabolism of xenobiotics of drugs and chemical takes place to a large extent.

A significant increase was observed in the liver and kidney marker enzymes, GOT, GPT and ALP during the present study. These results were in agreement with Stegink[32] and Kamal *et al.*[33], who reported that the activities of GOT and ALP increased significantly after consumption of aspartame to healthy adult albino rats for 5 weeks. The elevation in liver enzyme activities could be due to drastic physiological effects caused by free radicals interaction with cellular membranes or may be related to breakdown of liver parenchyma[34]. The liver cells play an important role in both synthesis and secretion of ALP into the bile. Therefore, the alterations in ALP activity caused by aspartame may be attributed to early cholestatic liver damage which primarily affects the liver parenchyma, thus making ALP a sensitive index in the diagnosis of infiltrative diseases[35].

A decrease in the level of total protein was observed in aspartame treated mice. Protein depletion results in increased toxicity to ASP, which is associated with a significantly decreased rate of hepatic metabolism[36,30].

The results presented in this study suggested that the number of WBC, RBC and Hb decreased in ASP treated rats. The reduced WBC number is due to a redistribution of cells into damaged organs such as the liver, rather than loss of cells[37]. These findings agree with those of Dhabhar and McEwen[38] and Prasad and Rai[39] where general reductions in hemoglobin and hematocrit levels and erythrocyte counts were found in the case of male and female mice fed on diets containing saccharin. However, the changes due to ASP was not significant in their study. The findings also agreed with the results obtained by Abdallah[40], who reported that parental rats and their offspring fed on sodium saccharin for 30 days developed anemia as indicated by the decrease in red blood cells and hemoglobin. The observed decreased hemoglobin content could be attributed to depressed erythropoiesis caused by direct action of saccharin on hematopoietic tissue[41,42].

CONCLUSION

The present study reveals that aspartame consumption alters the enzyme activity in the liver and kidney by probably elevating the free radical levels. It is concluded that aspartame consumption is not safe and it is necessary to be careful when using it as a sweetener in beverages and food. It is essential to note that the dosage used in this study is food and drug administration (FDA) permitted human consumption level Therefore, this investigation emphasizes the need to caution people who are using aspartame routinely. This study will help the researchers to uncover the critical areas of aspartame related toxicity that many researchers were not able to explore completely. Thus a new theory on aspartame-induced alterations may be arrived after the new investigations.

SIGNIFICANCE STATEMENT

"This study discovered the deleterious after effects of aspartame consumption at a dose level which EFSA and FDA have declared as safe for human consumption. The changes observed during the study can be beneficial to explore other possible after effects of aspartame that may result from consumption of aspartame for longer durations. Also, it is the need of the hour to re-think about the controversies within the available literature related to beneficial or harmful effects of aspartame. This study will help the researchers to uncover the critical areas of human studies keeping in mind the large number of population who are consuming the aspartame routinely. The important thing about the present study is the admissible daily intake (ADI), generally recognized as safe with no adverse effects. However, it was found that even this dose is not safe for general population. The study recommends that the use of aspartame should either be limited or the dose should be reduced.

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