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Low Intake of Aspartame Induced Weight Gain and Damage of Brain & Liver Cells in Weanling Syrian Hamsters

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Abstract This study aims to investigate the health effects of aspartame on weanling male hamsters. 20 Golden Syrian hamsters drank only water (control) or water with 6, 11, and 18 mg aspartame/kg of body weight per day for 42 days. Food intake, weight gain, glucose blood level, and lipid profile were determined at the end of the experiment. The animals were sacrificed and histopathological examination of organs (liver, brain and heart) was done. Results revealed that animals in Aspartame groups (Asp.groups) consumed significantly larger amount of food than the control (13.4±5.9, 8.6±2.5 and 8.8±3.0 vs 4.2±2.5 g/day, in succession). Hamsters in the control group showed higher total cholesterol and HDL levels than hamsters in aspartame 6, 11, 18 groups (160±19 vs 101±13, 130±22, 141±15 mg/dl & 144±9 vs 120±12, 118±13, 99±17 respectively (P<0·05)). The control group showed a glucose concentration below those of aspartame groups, indicating no effect of aspartame on glucose blood level. While, there were no significant differences in the triglycerides and LDL levels between control group and Asp.groups. Histopathological changes were observed, especially in brain and liver cells. Aspartame increases appetite and weight gain of young hamsters. Therefore, authorities (the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the U.S. Food and Drug Administration (FDA), the European Food Safety Authority (EFSA) and the Agence Française de Sécurité Sanitaire des Aliments (French Food Safety Agency – AFSSA)) should reconsider the acceptable daily intake (ADI) of aspartame especially for children, they are more vulnerable than adults.

Keywords: *non-nutritive sweeteners, organs, hunger, children, Appetite*

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1. Introduction

Aspartame is everywhere, it has become one of the most controversial and consumed food additives after tarnishing by concerns over the safety of saccharin [1]. It can be found in over 6.000 products, including carbonated soft drinks, powdered soft drinks, chewing gum, confections, gelatins, dessert mixes, puddings and fillings, frozen desserts, yogurt, tabletop sweeteners, and some pharmaceuticals such as vitamins and sugar-free cough drops [2]. The consumption of non-nutritive sweeteners is increasing at an alarming rate because of the ever evolving pandemic of obesity and Type 2 diabetes mellitus [3]. However, the consumption per unit body weight is highest in children. A recent systematic review estimated that 4–18% of the soft drinks consumed by the children are artificially sweetened [4,5].

A number of studies have been carried out to confirm the safety of artificial sweeteners [6]. As [7] who reported that "aspartame consumed without or with carbohydrate, did not affect either hunger or food intake of children when compared with the sweeteners sodium cyclamate and sucrose, respectively". Furthermore, aspartame didn't produce discernible cognitive or behavioral effects in normal preschool children or in school-age children believed to be sensitive to sugar [8], and not related to pediatric brain tumor occurrence [9] or hematopoietic risk [10]. Even in doses up to 1,000 mg/kg had no significant neurobiological activity in male Fischer-344 adult rats as neurotoxicants, including convulsants, organochlorine insecticides and heavy metals [11].

On the other hand, there are opponent studies such [12,15] who reported a controversial correlative analysis suggesting that an increase in the incidence of brain tumours in industrialized countries may be linked to aspartame consumption. It caused a statistically significant, dose-related increase in lymphomas and leukaemias in female rats [13]. Aspartame's multipotential carcinogenicity at a dose level close to the acceptable daily intake for humans. Furthermore, life-span exposure to aspartame begins during fetal life, its carcinogenic effects are increased [14]. Another study, [15] reported that it could cause headaches, dizziness, anxiety, depression. While, chronic use of ASP can lead to the development of hyperglycemia, hypercholesterolemia and associated diseases [16], alteration the brain antioxidant status and

can induce apoptotic changes in brain [17], oxidative stress in brain regions [18]. However, excessive aspartame ingestion might be involved in the pathogenesis of certain mental disorders and also in compromised learning and emotional functioning [19].

American academy of pediatrics, 2010, reported that the health benefits of nonnutritive sweeteners were inadequately assessed in children and adolescent and as such they should not form a significant part of a child's diet [4]. So, this study investigates the effect of aspartame in low doses (< 40 mg/kg body weight) on food intake, weight gain and vital organs of young hamsters.

2. Materials and Methods

2.1. Materials

Pure aspartame (ASP) powder was purchased from ADWIA Co., Cairo, Egypt. Reagent kits were purchased from Bio-diagnostic Company, Giza, Egypt.

2.2. Animals and Experimental Design

20 male, weanling Syrian hamsters (*Mesocricetus auratus*) of 23 days old, were obtained from an inbred strain in the college of Veterinary Medicine, Cairo University. Hamsters maintained in a temperature-controlled room (25ºC) with a fixed 12 h light: 12 h darkness cycle per 42 days. They were individually housed in stainless steel cages containing hardwood chips. Animals in all groups were given a commercial diet. After one week, the hamsters were weighed (27.63±7.15 g), and divided into equal 4 groups, each 5 hamsters: control (Group I) ingested only water, Group II (Asp. 6) drank water + 6 mg aspartame/ kg, Group III (Asp.11) ingested water + 11 mg aspartame/ kg, and Group IV (Asp.18) drank water + 18 mg aspartame/ kg. All the animals were weighed two times weekly to determine the gain in body mass. Food and water were given *ad libitum*.

2.3. Hematological Analysis

After the experimental period, animals were fasted 14 hours and blood samples were withdrawn from each animal (retro-orbital plexus under mild sedation into serum separator tubes. The blood was allowed to clot at 23°C for 30 min., and subsequently placed at 4ºC until centrifugation. Serum was separated by low-speed centrifugation at 2000rpm for 20 minutes at room temperature. Serum was frozen until analysis. Serum total cholesterol, HDL cholesterol, triglycerides, and glucose were determined according to [20,21,22] respectively on the Hitachi 911 automated analyzer using reagent kits. LDL cholesterol was calculated by the Friedewald equation [23].

2.4. Histopathology Changes

A liver, brain and heart sections soaked in 10% buffered formalin solution were processed for normal histological section. The formalin-fixed, paraffin-embedded tissue samples were ultrasectioned (4µm thickness), and stained. Histopathological examination was done at the lab of Department of Histopathology, Faculty of Veterinary Medicine, Cairo university, Giza.

2.5. Statistical Analysis

Results were expressed as mean ± standard deviation. The significance of the difference between the means of treated and control groups was established by repeated-measures analysis of variance ($P<0.05$)

3. Results and Discussion

3.1. Body Weight, Feed and Water Intake

Different gain body weights were recorded in groups I, II, III and IV (22.5, 21.2, and 23.4% vs10.2%) at the end of the experiment. Animals in Asp. groups consumed a significantly larger amounts of food than those in the control group (13.4±5.9, 8.6±2.5 and 8.8±3.0 vs 4.2±2.5 g/day respectively at $P<0{\cdot}05$). While, the different concentration of aspartame showed no evidence of influencing liquid intake ($p<0.05$) (Table 1). The encountered results are similar to the results found by other authors as [24] who disclosed that aspartame had an effect on appetite, followed by a sustained increase in hunger ratings. Thus, the concentration of the sweetener, the sex of the subject and the time after chewing, were all important determinants of whether "sweetness" increased hunger. Also, [25,26] found that the increase in the consumption of the foods sweetened by nonnutritive sweeteners was not parallel with a decrease in the consumption of the foods sweetened by caloric sweeteners. In rodent studies repeatedly exposed to foods containing artificial sweeteners and fat replacers in the place of calories were less able to adjust their intake in response to similarly tasting, and often gained more weight than the rodents that experienced consistent sensory-nutrient pairings in their diet [27].

Furthermore, [28] suggested the mechanism of how the artificial sweeteners might lead to increase body weight & obesity by interfering with the fundamental equilibrium of physiological processes mediated by taste receptors. Other mechanisms were revealed by [29] which include:

I. Disruption of sensory signal by sweet taste to brain might lead to altered energy balance and thereby promoting overcompensation.
II. Increase palatability of the food items using non-nutritive sweeteners (NNSs) could cause overstimulation of reward center, which could lead to overcompensation.
III. Repeated exposure to NNSs could stimulate liking for sweet foods, including those containing simple sugars.

On the contrary, [30] indicated that the use of low-energy sweeteners (LES) showed no consistent association with a heightened appetite for sugar or sweet products. He added, in many instances, the use of LES is associated with a lower intake of sweet tasting substances, prevention of weight gain, weight loss, and/or maintenance of weight loss.

3.2. Blood Glucose and Lipid Profile

The data in table II show that the control had a lower glucose level than those in the three aspartame groups. This may be ascribed that aspartame had a negative effect on the blood glucose level in the treated groups. While,

there were no significant differences in the triglycerides, and LDL levels measured at the end of the experiment. Regarding the cholesterol level, the treated groups (aspartame 6, 11, 18 groups) showed decreasing by 36.87%, 18.75% and 11.87% in succession, as well as the high density lipoprotein were also decreased by 16.7%, 18.1% and 31.25%, consecutively in comparison with the control. Results showed significant differences among the treated groups and control in TC and HDL. But, the mechanism of hypolipidemic effect remains to be established [31]. It is clear that aspartame had a great effect on the blood parameters of the treated hamster groups. In this respect [32] indicated that the animals which exposed to aspartame during the prenatal period presented a higher consumption of sweet foods during adulthood and a greater susceptibility to alterations in metabolic parameters, such as increased glucose, LDL and triglycerides.

Table 1. Initial Body Weight, Final Body Weight, Gain Weight, Daily Feed and Liquid Intake (Mean±SD) of Hamsters

Variable	Treatment			
	Control	6mg/kg aspartame	11mg/kg aspartame	18mg/kg aspartame
Initial body weight (g)	24.3±0.7^{a}	32.5±4.5^{a}	27.8±0.95^{a}	25.6±4.8^{a}
Final weight (g)	27.5±1.3^{a}	42.3±6.3^{c}	32.4±4.8^{b}	29.6±4.3^{a}
Weight gain (%)	10.2±2.9^{a}	22.5±4.5^{b}	21.2±2.0^{b}	23.4±2.4^{b}
Daily feed intake (g)	4.2±2.5^{a}	13.4±5.9^{b}	8.6±2.5^{c}	8.8±3.0^{c}
Liquid intake (ml/day)	16.7±6.8^{a}	15.08±4.7^{a}	21.5±2.8^{a}	19.95±1.9^{a}

Letters abc indicate significant differences between treatments. Means in the same line with different letters differ significantly ($p<0.05$).

Table 2. Serum Glucose and Lipid Profile (Mean±SD) of Young Hamster

Variable	Treatment			
	Control	6mg/kg aspartame	11mg/kg aspartame	18mg/kg aspartame
Glucose (mg/dL)	118±14^{a}	132±9^{b}	138±10^{b}	134±13^{b}
Triglycerides (mg/dL)	220±21	188±55	180±15	191±57
TC(mg/dL)	160±19^{a}	101±13^{b}	130±22^{b}	141±15^{b}
HDL(mg/dL)	144±9^{a}	120±12^{b}	118±13^{b}	99±17^{b}
LDL(mg/dL)	7.5±4	10±9	8.5±9	9.5±7

Letters abc indicate significant differences between treatments. Means in the same line with different letters differ significantly ($p<0.05$).

3.3. Histopathology

Histological examinations of liver, brain and heart organs of hamsters were conducted at the end of the experiment. Representative images can be seen in Figure 1-Figure 3 for liver, brain and heart tissue sections. It could be observed that the impact of aspartame on hamster liver cells, which ingested a concentration of 6, 11 and 18 mg aspartame/kg body weight were largely hydropic degeneration of hepatocytes as shown in Figure 1.

In this respect [33] revealed that the aspartame-treated groups displayed elevated enzyme activities, lowered antioxidant values, and histological changes reflecting the hepatotoxic effect of aspartame. Also, long term consumption of aspartame could cause hepatocellular injury, altered the hepatic antioxidant balance and behavior in rats [34].

Pathologic changes were mainly observed in hamster brain cells. Brain sections showed numerous large necrotic areas, cellular odema, and local gliosis in Asp. 6, 11, 18 groups. However, animals in the control group showed nothing remarkable or minimal pathologic damage. These results are in harmony with [35] who reported that some people suffer neurological or behavioural reactions in association with aspartame consumption. Aspartame disturbs amino acid metabolism, protein structure and metabolism, integrity of nucleic acids, neuronal function, endocrine balances and changes in the brain concentrations of catecholamines. It and its breakdown products cause nerves to fire excessively, which indirectly causes a very high rate of neuron depolarization. The energy systems for certain required enzyme reactions become compromised, thus indirectly leading to the inability of enzymes to function optimally. The ATP stores in the cells are depleted, indicating that low concentrations of glucose are present in the cells, and this in turn will indirectly decrease the synthesis of acetylcholine, glutamate and gamma-aminobutyric acid(GABA) [36]. It also increases the levels of lipid peroxidation and nitrite in the brain. In addition, aspartame itself impairs cellular antioxidant status because of the decreased brain levels of glutathione (GSH), and glucose [37].

Moreover, [38] revealed that aspartame administration altered the functional activity in the brain by probably elevating the free radical levels. Moreover the long term FDA approved daily acceptable intake (40 mg/kg bwt) aspartame administration distorted the brain function and generated apoptosis in brain regions, or cytotoxicity and neural cell apoptosis as a result of Tau aggregation [39]. In addition, another aspartame metabolite, deketopiperazine, could be a central nervous system carcinogen [40].

Aspartame is completely hydrolyzed in the gastrointestinal tract to aspartic acid, phenylalanine and methanol, each being toxic at high levels. The ADI dose of aspartame led to a 3–6 fold increase of blood methanol concentration above the individual baseline values [41]. In this respect [42] explained mechanism of methanol effects, it caused decrease in GSH levels (GSH is a cofactor of formaldehyde dehydrogenase and is responsible for formaldehyde metabolism). Also, a significant decrease in protein thiols was noted after aspartame administration. Moreover, a decrease in glutathione reductase activity might also contribute to the decrease in GSH levels observed in the aspartame treated animals. In addition, methanol is oxidized to formaldehyde and formic acid, these metabolites are toxic. Formaldehyde is a known carcinogen that causes retinal damage, prevents DNA replication and causes birth defects [38,43].

Second metabolite, Aspartate, is a neurotransmitter in the brain by facilitating the transmission of information from neuron to neuron. The large amount of it in the brain kill certain neurons by allowing the influx of too much calcium into the cells. This influx triggers excessive amount of free radicals, which kill the cells, thus, giving this amino acid the name “excitotoxin” because they excite or stimulate the neural cells to death.

Phenylalanine, third metabolite of aspartame, has been associated with neurotoxicity and also affects the synthesis of inhibitory monotransmitters, and has been

shown to mediate neurological effects [35]. Excessive level of it in the brain could cause the decreased levels of serotonin in the brain, which could lead to emotional disorders [43].

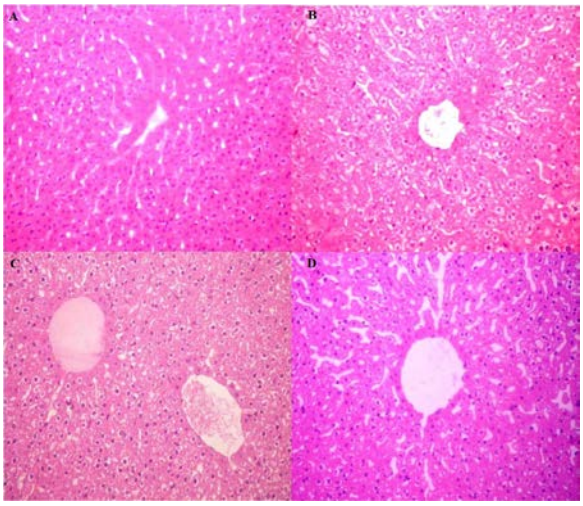

Figure 1. Comparison of the histopathology of liver in control (A), Asp. 6 (B), Asp.11 (C), and Asp.18 (D) groups (H and E X200)

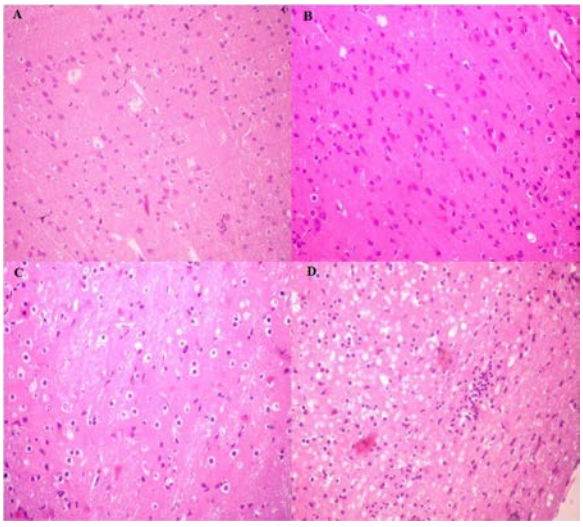

Figure 2. Comparison of the histopathology of brain in control (A), Asp.6(B), Asp.11(C), and Asp.18(D) groups (H and E X200)

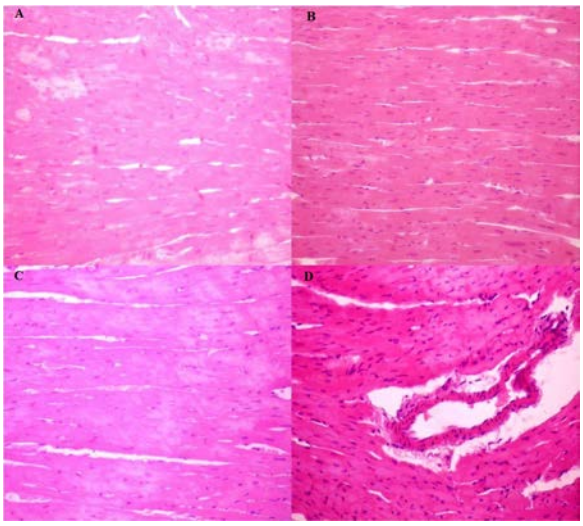

Figure 3. Comparison of the histopathology of heart in control (A), Asp.6 (B), Asp.11 (C), and Asp.18 (D) groups (H and E X200)

Figure 3 demonstrates that heart cells are generally less affected by aspartame cells where it is no change in heart cells in the treated groups and control group. Only aspartame18 group is showing perivascular edema.

4. Conclusion

This investigation clearly showed that aspartame concentrations less than 40 mg/kg body weight /day (acceptable daily intake of the US Food and Drug Organization) increased food intake and body weight gain in young hamsters. It caused histopathological changes in vital organs (liver and brain), especially damage in the brain even at low concentrations. Therefore, FDA & concerned organizations should be revised ADI of aspartame for children after its prevalence in food products. Exposure and susceptibility to chemical substances are more between children to smaller body weight, long-term effects from early exposure and immaturity of body systems.

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