Toxopathological and Cytogenetic Effects of Commercial Sweetener Aspartame after Chronic Oral Administration in Rat Pups

Ali, I. Al-ameedi[1], Zahraa M. Ayad, Falah Mousa Kadhim AL-Rekabi[3]

[1]Department of Physiology and Pharmacology-College of Veterinary Medicine – Al-Qasim Green University-Babylon-Iraq, [2]Department of Nursing, Altoosi University College-Najaf-Iraq, [3]Department of Physiology,Biochemistry&pharmacology, College of Veterinary Medicine-University of Baghdad-Iraq

Abstract

Objective: This study was done to evaluate the potential toxic effect of aspartame (APS) artificial sweetener after chronic oral administration in rat pups as a model for children that consumption high quantities of different types of sweets and juices from the peddler, regularly and daily.

Methods: Twenty-four rat pups were divided equally into three groups. Therapeutic dose group (T1) and double dose group(T2) received 0.08, 0.16 g/kg. body weight(BW), respectively, while control group (C) received distilled water orally for 90 (d)days.

Results: The result of present study revealed that there was an increased consumption of food, water and arising nervous signs(aggressive) in T1 and T2 groups comparing with control. The cytogenetic study includes the mitotic index and blast index showing a substantial decline relative to the control one in both treated groups. While the blast index showed a significant reduction($P<0.05$) in T2 relative to T1 and the control group. No chromosomal aberration observed in all groups exposed to aspartame. Different histopathological lesions were recorded in the liver of T1 group represented by inflammatory cell (neutrophil & mononuclear cell) aggregation around the blood vessels. While noted in a double dose group (0.16g/kg.bw), a granulomatous lesion with mild change in fat is shown.

Conclusion: Used daily for lengthy periods ASP has cytogenetic and pathological risks.

Keywords: *Aspartame, chronic toxicity, pups, cytogenetics.*

Introduction

The passion of human beings of sweet foods is inborn, the studies proved a preference for sweet-tasting nutrition in newborns, human beings draw near to caves, even ancient cave paintings show a Neolithic man taking honey from a bee's nest(1). It's a dipeptide artificial sweetener that is widely used in all ages as a non-nutritive sweetener in foods and drinks, a high intensity sweetener most commonly found in low calorie beverages, chewable multi-vitamin, breakfast cereals, dessert mixes, diet Soda, tabletop sweeteners added to tea or coffee and food products, and pharmaceuticals which has been approved as a sweetener for liquid carbonated beverages(2). ASP has fair acid stability but poor heat stability(3). After ingestion, aspartame is immediately absorbed from the intestinal lumen and metabolized to phenylalanine, like aspartic acid, and methanol(4). ASP is metabolized by digestive esterase and peptidases in the intestinal lumen to methanol and its constituent amino acids phenylalanine and aspartic acid or absorbed by intestinal mucosal cells were hydrolyzed to its components(5).Followed by absorption into the systemic circulation, phenylalanine enters the plasma free amino acid pool from the portal blood after

Corresponding Author
lec.dr.zahraa m Ayad
Altoosi university college, nursing department
zahraa_m_ayad@altoosi.edu.iq

partial conversion to tyrosine by hepatic phenylalanine hydroxylase(6).Methanol has previously been shown to result in the generation of ROS(7).

Materials and methods: Twenty-four (24) pups of the albino rat, aged 30-40 d with weight range (100-150g), supplied from the animal house of the College of veterinary medicine of Al-qasim green university, accordance to the ethical standard of working on laboratory animals. They were housed and maintained in a conventional animal facility, with controlled conditions of temperature (20 ± 5ºC). The animals were fed on special formula feed pellets and given water *ad libitum*, throughout the experiment, each group of rats was housed in a plastic cage containing hard-wood chip as bedding.

Chemicals: Aspartame obtained from NutraSweet–Monsanto company_ America.

Preparation of concentration for chronic toxicity study: Dose of Aspartame measured according to(8). When approved it as a sweetener.

Therapeutic dose (T1): (0.08g/ kg.BW): to obtain 80mg/kg.bw of Aspartame dissolve 8g in 100ml distilled water, and dosing 1 ml for each 100g (rat) daily for 90 d.

Double dose (T2): In the same way in therapeutic dose but in double dose give 2 ml for each 100g (rat) daily for 90 d to obtain 0.16g/kg.bw of ASP.

The control group(C): Was given 1ml of distilled water for each 100g (rat) orally daily.

Histopathological examination: The samples prepared according to(9).

Cytogenetic analysis: According to(10).

$$\text{Mitotic index(MI)} = \frac{\text{Cells in metaphase}}{1000} \times 100$$

$$\text{Blast index (BI} \frac{\text{Number of lymphoblast}}{1000} \times 100$$

Statistical analysis: - Statistical analysis was applied by one-way ANOVA and the mean difference is significant at the 0.05 level in using statistical package for social sciences (SPSS), Version 10.

Results

Chronic toxicity: After 90 days of oral administration the animals of T1 and T2 groups showed some physiological and neurological signs listed in table (1).

Table (1): Clinical signs of rat pups exposed to 0.08, 0.16g/kg.BW of the ASP.

Groups	Signs	Time of appearance
T1 Therapeutic dose 0.08g/kg.BW	1-increase in consumption of feed and water. 2-increase weight gain. 3-Nervous signs (aggressiveness).	14 d 32 d 81 d
T2 Double dose 0.16g/ kg.BW	1-increase in consumption of feed and water. 2-increase weight gain. 3-Nervous signs (aggressiveness).	12 d 23 d 72 d

Table (2): Body weight gain in rat pups exposed chronically to ASP.

Periods Groups	Zero time Mean± SEM	After chronic exposure Mean± SEM	Weight gain(g)
C	130.3±2.1 Ab	159.6±2.3 Ca	29.3
T1	122.4±1.6 Bb	223.1±3.7 Ba	100.7
T2	127.6±1.9 Bb	254.7±4.0 Aa	127.1

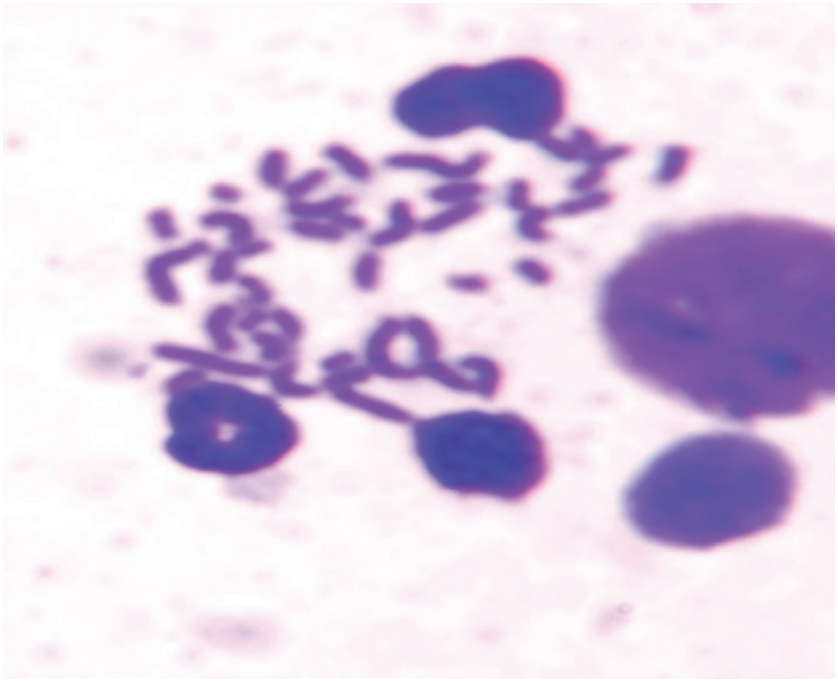
-Different capital letters denote significant differences ($p<0.05$) between groups.

- Different small letters denote significant differences ($p<0.05$) within groups. -N=8

-L.S.D=6.4

Cytogenetic analysis:

Chromosomal Aberration: The results of the chromosomal analysis revealed there was no chromosomal aberration in stem cells of bone marrow in all groups (Fig.1).



Fig(1): stem cells of the bone marrow of male albino rats received (0.08 and 0.16g/kg.BW)of ASP for 90 d (Giemsa stain× 100, × 40).

Table (3): The effect of chronic exposure of deferent doses of ASP on the blast index and mitotic index in rat pups.

parameter **groups**	**M. I** **Mean± SEM**	**B. I** **Mean± SEM**
C	6.28±3.6 A	4.49±20.9 A
TI	3.21±1.8 B	4.34±21.1 A
T2	2.39±0.8 C	3.20±12.2 B
	L.S.D =0.4	L.S.D=0.5

-Different capital letters denote significant differences ($p<0.05$) between groups. -N=8

Blast index and Mitotic index: The results revealed a significant decrease($P<0.05$) of mitotic index (number of cells in mitosis/1000 in cells of the bone marrow) of exposed groups (T1 and T2) comparing with the control group and in a dose-dependent manner. While the blast index (number of lymphoblasts/1000 in cells of the bone marrow) showed a significant decrease in T2 group comparing with T1 and C groups.

Histopathological finding:

The histological examining of the liver section in the control group of rat pups showed normal tissue. Furthermore, in sections of the liver of animal received 0.08g / kg.bw of ASP for 90 d there was inflamatory cell (neutrophile & mononuclear cell) aggregation around the blood vessels. While in double dose group (0.16g/ kg.bw) observed shows granuolomatous lesion with moderate fatty change.

Fig(2): Histopathological finding of the liver of rat pups after chronic exposure to ASP.

Discussion

The results showed that ASP increases the consumption of food and water, the appetite increase through ASP metabolites by different mechanisms one of these mechanisms is that an increased phenylalanine concentration releases cholecystokinin(12).Which is an endogenous anorectic agent(11). As a precursor of catecholamine neurotransmitters(13). Phenylalanine may facilitate intake via the hypothalamic adreno-receptors implicated in the central appetite control mechanisms, stimulating appetite(14). Increased fluid intake can be associated with the intensive sweet taste of ASP and its hedonic impact. ASP is about 200 times sweeter than sucrose(6). On the other hand, the result showed there was an increase in weight gain of animals exposed to as this is the natural result when you have increase in food consumption and that's who recorded by(16).When reported increased body weight and fluid intake in a group treated with ASP. There are opposing opinions about the effect of ASP on body weight changes. Some studies reported increased body weight caused by ASP(15,2). While others showed that ASP is efficient in body weight loss(16,17).We observed predicted increased appetite and high weight gain in ASP-treated rats. From the foregoing it is clear that the methanol, which is a by-product of aspartame, may be responsible for the alteration observed in the free-radical-scavenging system. Since methanol is freely permeable through membranes and lipids, it also gets distributed in the brain tissues and may cause damage. Increased production of free radicals and increased oxidative damage to proteins in distinct brain regions, retina and optic nerve after methanol administration(18,19). Also, we agree with(20),who was reported that the aspartame intake has been reported to be responsible for neurological and behavioral disturbances in sensitive individuals. Oxidative stress is an imbalance between the elevated level of ROS and the impaired function of the antioxidant. Overproduction of ROS can induce the death of immature cultured cortical neurons(21). And DNA damage(22). Marko D, *et al* have reported that increased ROS can trigger cell damage. The increase in ROS level may be due to methanol that is released during aspartame metabolism in the GI tract, as the pathway leads to formaldehyde and format production by the catalase enzyme. Cell death could occur in response to high oxidative stress(23,24). The result of the cytogenetic study revealed there are significant decreases ($P<0.05$) in MI of animals exposed to a therapeutic and double dose of ASP comparing with control which received DW. In contrast, the result of BI showed significant decrease ($P<0.05$) in the T2 group comparing with T1 and C groups. Aspartame is hydrolyzed to several products including methanol, which can be further metabolized to formaldehyde, which is a DNA cross-linking agent, known to induce chromosome damage in mammalian cells(25).We thought that metabolites (methanol, formaldehyde, formic acid, and acetic acid) that respectively released when ingestion of Aspartame play a vital role in inhibition of MI and BI, and that agrees with *Rencuzogullari, et al 2004*(2).*when they* reported that aspartame showed cytotoxic effect by decreasing the mitotic index at all concentration and treatment periods. Furthermore, the present study showed no chromosomal aberration in stem cells of exposed pups, on the opposite to AlSuhaibani who observed that aspartame induced a significant increase of chromosome aberration frequencies in mice compared to control(26). *Rencuzogullari et al*(2), studied the genotoxic effects of aspartame on human lymphocytes *in vitro* using chromosomal aberration test, SCE test, and micronucleus test. They found that aspartame induced a significant increase in chromosomal aberrations(27). Other authors evaluated the effect of blends of aspartame and acesulfame-k on induction of chromosomal aberration in bone marrow cells of male mice. The authors observed an increase in the percentage of cells with chromosomal

aberrations with increasing doses of the two sweeteners(28).Also, our results are supported by(29), who tested the possibility of micromolar formaldehyde, a metabolite of methanol derived from aspartame exerts cytotoxicity. *Strachan and Read* explained the occurrence of numerical aberrations through two main mechanisms after aspartame exposure(30). The result of histological sections of the liver of rat pups received 0.08 g/kg.bw of ASP for 90 d showed there was inflamatory cell (neutrophile & mononuclear cell) aggregation around the blood vessels. While in double dose group (0.16g/kg.bw) observed shows granuolomatous lesion with moderate fatty change . that's maybe due to increase the ROS in the tissue of liver and other vital organs due to long term exposure to aspartame. Hydroxyl radicals oxidize polyunsaturated fatty acids in an increased in biological membranes to induce the formation of lipid peroxides(31). The results indicated that exposure to ASP increased lipid peroxidation in the liver and other organs, this idea supported by Mourad IM and Noor NA 2011 When recorded that ROS increase in long-term ASP treatment in brain, liver and kidney tissues. The high level of ROS may be due to methanol formation during ASP metabolism and formaldehyde release as part of methanol metabolism(32). This has been well confirmed by Parthasarathy, *et al* who observed an increase in the LPO level in the lymphoid organs after methanol administration(33).The present study with an agreement with Abdel-Salam, *et al* who reported that increase aspartame concentrations than Acceptable daily intake (0.04g) the effects begin to appear on many vital organs like liver, brain and kidney(34).

Conclusion

According to our results, we can conclude that aspartame has a genotoxic risk. Therefore, it is necessary to be careful when using it in food and beverages as a sweetener**.**

Conflict of Interest: The authors declare no conflicts of interest.

Ethical Clearance: Permissions for carrying out the study were obtained from the Research Ethics Committee at Al-Qasim green university Babylon province-Iraq.

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