Studying some Cytotoxic Parameters of Aspartame (Diet sweet) on Mature Albino Male Mice

دراسه بعض مؤشرات السميه الخلويه للمحلى الصناعي الاسباراتام على ذكور الفار الابيض

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Abstract

The present study was planned to evaluate some cytotoxic effect of aspartame intake trough determining the apoptosis cells from peripheral blood and sperm head abnormality from cauda epididymis. After one week of administration of various doses of asparatame (3.5,35,350 mg/kg) to mature albino male mice and distilled water treated group was served as control. The results were statistically analyzed to compare the data of individual dose with that of the control, apoptotic and necrotic cells as well as sperm head are determined. The results showed that induce apoptosis cells in all concentrations (3.5,35,350 mg/kg) and necrotic cells in doses (35, 350 mg/kg) while late apoptosis in doses (35, 3.5 mg/kg) on the other hand, asparatame is increased in sperm head abnormality in (350, 35 mg/kg) dose. According to these results, it was possible to conclude that aspartame have a cytotoxic risk. Therefore, it is necessary to be careful when using such materials in food and beverages as a sweetener.

Key words: Apoptosis, Aspartame, cytotoxic, food additives.

الملخص

وضعت الدراسه الحاليه لتقييم بعض التاثيرات السميه الخلويه للمحلى الصناعي الاسباراتام عن طريق تحديد موت الخلايا المبرمج من الدم المحيطي وتشوهات رؤوس النطف من البربخ. بعد مرور اسبوع من اعطاء جرع مختلفه للاسباراتام الى ذكور الفار الابيض البالغه واعطاء الماء المقطر لمجموعة السيطره، حللت النتائج احصائيا لمقارنة البيانات بين الجرع المختلفه ومجموعه السيطرة، التي من خلالها حددت خلايا الموت المبرمج وكذلك رؤوس النطف. أظهرت النتائج زياده في حث الخلايا التي يسببها موت الخلايا المبرمج في التراكيز (350،35،3.5 ملغم / كلغم) والخلايا الميتة في التركيزين (350,35 ملغم / كلغم) بينما تأخر موت الخلايا المبرمج في التركيزين (3.5،35 ملغم / كلغم) من ناحية أخرى، سبب الأسبارتام زياده معنويه في تشووهات رؤوس النطف في التركيزين (350،35 ملغم / كلغم). ووفقا لهذه النتائج، يمكن الاستنتاج بان الأسبارتام يملك مخاطر السمية. لذا فمن الضروري توخي الحذر عند استخدامه في الأغذية والمشروبات كمادة للتحلية.

الكلمات الدالة: الموت المبرمج، الاسباراتام، السميه الخلويه، الاضافات الغذائية

Introduction

Aspartame is a nonnutritive sweetener that first allowed by the FDA in 1981 for use in dry foods and permitted for beverages in 1983. It is consumed by tens of millions of people in beverages, instant breakfasts, desserts, breath mints, sugar free chewing gum, vitamins, pharmaceuticals, and numerous other products. It is an alternative sweetening choice to diabetics, dieters, and others who intake limits sugar [1]. The suitable daily intake of aspartame, reputable by Food and Drug Administration (FDA) is 50 mg/kg. Department of Agriculture found some people in the U.S. devoted more than 16mg/kg/day [2].The Ministry of Agricultural of Turkey has recommended that Antimicrobial Stewardship Program (ASP) uses a maximum dose of 5500 mg/kg [3]. Aspartame is a dipeptide methyl ester, built up of a phenylalanine molecule, aspartate molecule and methyl group esterified to the carbonic acid group of the phenylalanine. In the upper part of the small intestine, methanol is released by hydrolysis of the methyl ester by pancreatic chymotrypsin this is immediately captivated in the small intestine [4]. Trocho (1998) [5] Conclusion that methanol developing from aspartame plays a function in adduct formation caused by formaldehyde formation the DNA-protein crosslink, on the other hand [6] reviewed a study on safety of ASP and reported that ASP is harmless, and reported that ASP did not produce DNA damage in rat hepatocytes and was not clastogenic in mice when it was given orally [7]. There are multiple genotoxicity studies of aspartame, these studies have recommended addicted to possible connection between aspartame and diseases like brain tumors, brain lesions, and lymphoma [8].The questions have been asked about brain cancer, lymphoma and genotoxic effects like DNA-protein crosslink's, chromosome aberrations.There is debate in the scientific and medical community as to whether these symptoms are caused by instant or lasting exposure to aspartame. In human research of aspartame, it is usually provided in slow-dissolving capsules, but the concentration using slow-dissolving capsules of aspartate in blood

from ingesting aspartame is much lower than from ingesting liquid aspartame such as in carbonated beverages [9]. The purpose of the present study is to determine whether and to what extent aspartame would induce cytotoxic effect like apoptosis and sperm head abnormality in mature male mice.

Materials and Methods

The study was carried out on male Swiss albino mice aged 8–10wk, weighed 20–25g. each experimental group consists of five animals for each treatment in addition to the control. They were maintained under circumstances of ambient room temperature and relative humidity. The aspartame substance was obtained from Furat pharmaceutical industries, Baghdad-Iraq. Aspartame was dissolved in distilled water. Three concentrations were prepared 3.5mg/kg body weight, 35 mg/kg body weight, and 350 mg/kg body weight were administered orally, these concentrations were selected according to cytotoxicity of aspartame [10]. Control animals were treated with the distilled water only. The animals were scarified after 7 days, blood samples were taken from mouse's heart for apoptosis analyses, for sperm morphology test the cauda epididymis was dissected after 1 week of treatment with aspartame.

Apoptosis

The apoptosis assay kit-FITC (ExBio, Czech) was intended of early apoptotic cells by flow cytometry. Annexin V binding buffer was concentrated 10X and was diluted with deionized water prior to use in order to prepare 1X. Cells were harvested by centrifuge at 2000rpm for 5min., the pellets resuspended in cold PBS and washed by gentle shaking, re-centrifuge washed cells and supernatant was discarded. Cells resuspended with 1X binding buffer and adjustment cell to 5×10^5 cells/ml preparing sufficient volume of cell suspension (100µl/assay) then cells were stained with 5µl of Annexin V-FITC and PI, incubate 15 mints in dark at room temperature, after incubation period cells were harvested by centrifugation at 2000 rpm for 5 min. Pellets were resuspended in 100µl with binding buffer and then analyzed by flowcytometry.

Sperm Morphology test

Aspartame was administered daily for a period of one week. After one week the animals were sacrificed by cervical dislocation, the cauda epididymis was isolated and placed in a watch glass containing 1ml of phosphate buffered saline (PBS). Both the cauda epididymis were minced in the PBS using a wire mesh and forceps. The suspension obtained was filtered through layers of muslin cloth, to remove the tissue remains. The filtered suspension was stained with 1% aqueous eosin Y (10:1). After 30 min a drop of the suspension was taken on a clean slide and smear was made. The slides were air dried and observed under the microscope to score different types of abnormal head of sperms. 1000 sperms per animal were examined for each treatment and control groups [11].

Statistical analysis

The data were analyzed using the one-way analysis of variance (ANOVA) followed by LSD analysis to compare various groups with each other. Results were expressed as mean ± standard deviation (SD).

Results and discussion

The differentiation between apoptosis and necrosis cells was done by using Annexin V-FITC kit, as shown in Table (1) considerable and significant increases were detected in three doses respectively (15.2% ,20.5% and 6.5%) ($p\leq0.05$) compared to control (0.9%); on the other hand the percentage of necrosis was significantly different ($p\leq0.05$) in 350, 35 mg/kg doses (1.02%, 5.4%) as compared to control (0.3%). In late apoptosis there was remarkable significant increases in 35, 3.5 mg/kg doses (3.2%, 4.9%) but higher dose indicate insignificant differences (0.3%) compared to the control (0.00). In Table (2) the results have revealed significant ($p\leq0.05$) increases in the number of sperm head abnormality in 350, 35 mg/kg doses (17.9%±2.1, 15.1±2.7) respectively compared with control group (4.7±1.5).

Table (1): The percentage of apoptosis in the lymphocyte of mice blood treated with different doses of aspartame.

Treatment Groups	Apoptosis (%)	Necrosis (%)	Late apoptosis (%)
Negative control(D.W)	0.9±0.3a	0.3±0.1a	0.00±0.00a
Aspartame350mg/kg	15.2±1.5b	1.02±0.3b	0.3±0.1 a N.S
Aspartame35mg/kg	20.5±2.6c	5.4±1.3c	3.2±0.7b
Aspartame3.5mg/kg	6.5±2.6d	0.29±1.4a	4.9±1.5c

Values were expressed as mean± SD.

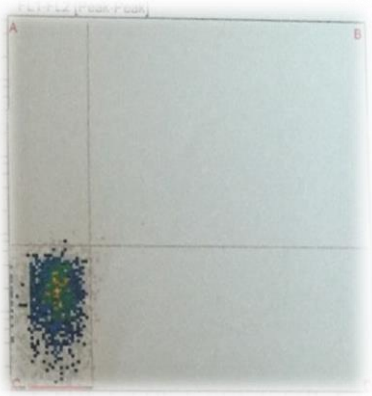
Different letters means significant differences at p≤0.05.

Table (2): Percentage of abnormal sperms heads induced in the treatment groups with aspartame and controls groups.

Treatment Groups	Time in weeks	Percentage of abnormal sperm (%)
Negative control(D.W)	1	4.7±1.5 a
Asparatame350mg/kg	1	17.9±2.1 b
Asparatame35mg/kg	1	15.1±2.7 b
Asparatame3.5mg/kg	1	5.8±2.3 a

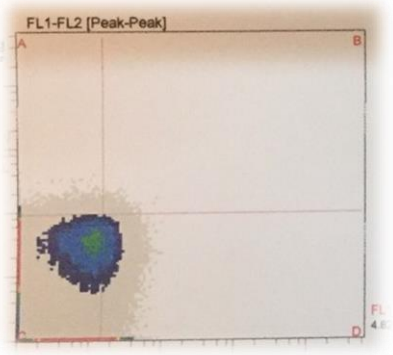
Values were expressed as mean± SD.

Different letters means significant differences at p≤0.05.



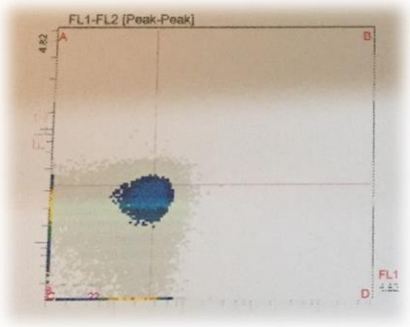
Control

FL1-FL2-A 0.3%
FL1-FL2-B 0.00%
FL1-FL2-C 98.8%
FL1-FL2-D 0.9%



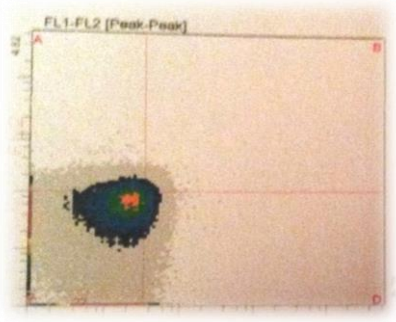
Treated with asparatame (350mg/kg)

FL1-FL2-A 1.02 %
FL1-FL2-B 0.3 %
FL1-FL2-C 83.4 %
FL1-FL2-D 15.2 %



Treated with asparatame (35mg/kg)

FL1-FL2-A 5.4 %
FL1-FL2-B 3.2 %
FL1-FL2-C 70.9 %
FL1-FL2-D 20.5 %



Treated with asparatame (3.5mg/kg)

FL1-FL2-A 0.29 %
FL1-FL2-B 4.9 %
FL1-FL2-C 88.31 %
FL1-FL2-D 6.5 %

Figure (1): Annexin V expression for mice induce apoptosis the label inside quadrants indicate the percentage of A: necrotic cells (PI +/Annexin V -) B: late apoptosis (PI+/Annexin V+) C: Viable cells (PI-/annexin V -) D: apoptotic cells (PI-/Annexin V+).

In this study Aspartame significantly induces apoptosis and sperm head abnormality and shows the cytotoxicity in different doses, Aspartame was composed of phenylalanine (50%), aspartic acid (40%) and methanol (10%) [12]. Thomas [13] reported that the metabolic products of aspartame such as phenylalanine and methanol have genotoxic risk for humans, recent research has shown it to be a multipotential carcinogenic agent for laboratory animals, at a daily dose of 20 mg/kg of body weight, a level that is much less than the current suitable daily intake [14], and Bandyopadhyay [15] evaluated the genotoxic potential of the low-dose range (7–37) mg/kg of aspartame by comet assay test in the bone marrow cells of Swiss Albino Mice, these parameters of DNA were enhanced in the bone marrow cells

due to the sweetener-induced DNA strand breaks by increased comet-tail level and the percent of DNA in the tail. Sushant (2010) [16] showed the genotoxic and carcinogenic effect of ASP on human lymphocyte and chromosome .The result agreements with [17] that revealed that ASP induced the sperm head abnormality, chromosomal aberration and micronucleus formation in different doses. Walaa (2014) [18] demonstrated that aspartame induce rat testicular toxicity at dose 1000mg/kg when gave three time per week for 12 weeks. Horio (2014) [19] showed that aspartame induced apoptosis predominantly by the use mitochondrial pathway involved in apoptosis proper to oxygen toxicity and increased the expressions of caspases 8 and 9 and cytochrome C. Also [20] reported that the administration of aspartame for 90 days was imbalance in a neutrophil and lymphocyte normal white blood cell homeostasis, a significant increase in the lipid peroxidation with nitric oxide level, and an alteration of membrane bound ATPase activities, which finally decreased the cellularity of immune organs. Azza (2012) [21] approved the consumption of aspartame leads to alterations in the genetic system of liver histopathological by formation lesions in the liver and bone marrow of mother albino rats and their off spring.

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