EFFECTS OF ASPARTAME ON DOPAMINE CONCENTRATION IN RATS BRAING

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ABSTRACT

This study aimed to determine the effect of aspartame on the dopamine concentration in brain adult rats. For this study 24 adult male rats and 24 adult female rats were used, with age of 60-65 day, and an average live weight between (165-185) gm. The experiment has designed depend on the concentration on the daily dose, where the animals randomly divided into three groups, each group contains 12 animals (six males & six females). And the control group included six males and six females as well. The first group G A gavaged with aspartame concentration of (40 mg / kg/ per day), the second group G B gavage with concentrated aspartame (54 mg/kg/per day) and the third group G C gavage aspartame dosed of (70 mg/ kg/ per day), while the control group dosed with 1ml physiologic solution daily. All groups had their daily dosages through the mouth, and the experiment continued from October 2015- January 2016. After the dosage's period of time completed, samples of blood were taken to study the concentration of the dopamine in the brain. The results of the study showed that; rats not have significant differences (P> 0.05) in dopamine concentration in animals' brain from the first group G A comparing with control group. The second group G B, which dosed with concentration dose (54 mg/ kg/ per day), did not show significant differences (P >0.05) when it compared with control group for both sexes. As well the third group dosed with concentration dose (70 mg/ kg/ per day), showed highly significant decrease (P <0.01) in the level of dopamine in the brain comparing with control group and other groups . conclude from study that the high concentration of aspartame lead to decrease the dopamine concentration in brain rats, also conclude that female rats were more sensitive to aspartame than male .

Key words: Aspartame, dopamine , Astrocyte, artificial sweetener, phenylalanine.

تأثير الاسبارتام على تركيز الدوبامين في دماغ الجرذان

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الملخص

هدفت هذه الدراسة الى تحديد تأثير مادة الاسبارتام على انسجة الدماغ في الفئران البالغة , استخدمت في هذه الدراسة (24) ذكر و(24) انثى من الجرذان البالغة بعمر (60- 65) يوم وبمعدل وزن حي تراوح بين (165-185) غم تقريبا , وتم تصميم التجربة على اساس تركيز الجرعة اليومية حيث قسمت الحيوانات بصورة عشوائية الى 3 مجاميع كل مجموعة تضم 12 حيوان 6 ذكور و6 اناث , بالإضافة الى مجموعة السيطرة التي ايضا تضمنت 6 ذكور و6 اناث, المجموعة الاولى G A جرعت الاسبارتام بتركيز(40 ملغم/كغم /اليوم) , والمجموعة الثانية G B جرعة بتركيز (54 ملغم /كغم /اليوم) , اما المجموعة الثالثة G B جرعت بتركيز(70ملغم/كغم/اليوم), اما مجموعة السيطرة فقد جرعت 1ml من المحلول الفسلجي. المجاميع جرعت بطريقة التجريع الفموي اليومي واستمرت التجربة للفترة من تشرين الاول 2015_ كانون الثاني 2016. بعد الانتهاء من مدة التجريع اخذت نماذج من الدم لدراسة تركيز الدوبامين في الدماغ . وقد أظهرت نتائج الدراسة ان تجريع الجرذان بالاسبارتام ادى الى عدم ظهور فروق معنوية (P>0.05) في تركيز الدوبامين في الدماغ في حيوانات المجموعة الاولى التي جرعت بتركيز(40mg/kg/day) مقارنة مع مجموعة السيطرة, وكذلك المجموعة الثانية التي جرعت بتركيز(54mg/kg/day) لم تظهر

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أي فروق معنوية (P>0.05) عند مقارنتها مع مجموعة السيطرة في كلا الجنسين, بينما حيوانات المجموعة الثالثة بتركيز (70mg/kg/day) فقد اظهرت النتائج حدوث انخفاض عالي المعنوية (P<0.01) في مستوى الدوبامين في الدماغ مقارنة مع مجموعة السيطرة والمجاميع الاخرى. لذلك نستنتج ان التراكيز العالية للاسبارتام تؤدي الى انخفاض في تركيز الدوبامين في دماغ الجرذان وايضا نستنتج ان الاناث اكثر حساسية للاسبارتام من الذكور.

كلمات مفتاحية: اسبارتام, دوبامين ,الخلايا النجمية, المحليات الصناعية, فنيل انيلين

Introduction

A natural sweeteners have become more popular and used in more products sugar-free products, because their use to sweetener is assumed to allow a cut in sugar used, and decrease in caloric intake, can keep the desirable palatability of food and soft drinks. Sweeteners are also gaining importance as part of nutritional guidance for diabetes, a disease with increasing incidence in developed and developing countries (Humphries *et al*.,2008). Aspartame - APM is a dipeptide artificial sweetener that is widely used in all ages as a non-nutritive sweetener in foods and drinks, high intensity sweetener are most commonly found in low calorie beverages, chewable multi-vitamin, breakfast cereals, dessert mixes, diet Soda, table top sweeteners added to tea or coffee and used in food products, and pharmaceuticals which has been approved as sweeteners for liquid carbonated beverages (Fry,1999 ;Oyama *et al*.,2002; Rencuzogullari *et al*.,2004). It's one of the most widely used artificial sweeteners in over 90 countries worldwide in over 6000 products (Magnuson *et al*., 2007). The sweetener aspartame is the industrial sugar, it is known in the European Union under the E number (additive code) E951 and shopping this article sweetened under many brand names as NutraSweet®, Equal® , Furasweet®, Nutrasweet®,Canderel® & others (Arcella *et al*., 2004). After ingestion, aspartame is immediately absorbed from the intestinal lumen and metabolized to phenylalanine, as aspartic acid, and methanol (Ranney *et al*., 1976). Chemical engineering has led to develop artificial sweeteners, as alternatives to sugar, the LD50 of aspartame in mice and rats is >5 g/kg (Kotsonis and Hjelle, 1996). Aspartame is metabolized by digestive esterase and peptidases in the intestinal lumen to methanol and to its constituent amino acids phenylalanine and aspartic acid or absorbed by intestinal mucosal cells where its hydrolyzed to its components, phenylalanine is taken up across the blood-brain barrier into the central nervous system and it is partly transform editor tyrosine by phenylalanine hydroxylase and then to L-3,4-dihydroxyphenylalanine (DOPA), dopamine (DA), noradrenaline (NA) and adrenaline (A) in the liver (Butchko *et al*., 2002). Most articles about harmful effects of the artificial sweetener are focused primarily on its harmful influence on the nerve cells (Butchko *et al* ,.2002; Ekong,2009; Mourad & Noor, 2011). Some scientists claims that aspartame damages the nervous system and that some scientists consider aspartame to be a neurotoxin, thus placing the general population at risk for neurological damage, alleged harmful effects include seizures and change in level of dopamine (brain neurotransmitter), and then lupus, multiple sclerosis, and Alzheimer's disease, some studies have recommended further investigation into possible connections between aspartame and negative effects such as headaches, brain tumors, brain lesions, and lymphoma (Roberts 1991; Olney *et al*., 1996; Soffritti, 2006). In the brain, dopamine roles as a neurotransmitter—a chemical released by neurons to send signals to other nerve cells, the brain contains several different dopamine pathways, one of which plays a major role in reward-motivated behavior and others pathways are involved in motor control and in controlling the release of various hormones, these pathways and cell groups form a dopamine system which is neuromodulatory (Schultz, 2015). The higher the level of dopamine activity, the lower the impetus required to evoke a given behavior, such as a significance, high levels of dopamine lead to high levels of motor activity and impulsive

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behavior; low levels of dopamine lead to torpor and slowed reactions (Chakravarthy *et al*.,2010). The degeneration of nerves in the course nigrostriatal lead to Parkinson's disease and the imbalance between cholinergic activity dopaminergic in the brain because decay nerves dopaminergic or lack secretion dopamine or excess Colin, this disease with difficulty movement and speech and also tremors muscle , aspartame caused dose-dependent many effects on the level of brain serotonin, noradrenaline and dopamine (Waugh & Grant, 2006).

Materials and Methods

The study was performed in the animal house of the Collage of Science Department of Biology/ Babylon University, for the period between October 2015 to the last of January 2016. In this study used 48 male and female Wister rats that have been purchased from the animal house of the Biology Science Collage / University of Babylon , animal ages between 60-65 days , weights ranged 165_185 , placed in plastic cages especially designed for this purpose and strung with metal hoods, equipped singled to drink water system and furnished sawdust and has clean cages and sterilized with disinfectant care. Has been provided with water and the bush animals that have been manufactured according to the formula described by (ward ,1970). Which allows animals to adaptation for a week before the start of the experiment.

Experimental animals were adapted to the laboratory conditions and suitable temperatures. This study used 48 animal male and female Wister rats, divided randomly into three groups each group included 12 animals six males and six females with control group also six males and six females. The animals gavages for 75 days about 1ml day in the morning between (9:30_10:30) by using oral gavage needle. The experiment groups were described as follow: Control group dosed normal saline 1ml daily during the experiment. Group A gavaged by aspartame concentration 40mg/kg/day about 1ml daily. Group B dosed by aspartame concentration 54mg/kg/day about 1ml daily. Group C dosed by aspartame concentration 70mg/kg/day about 1ml daily. At the end of the experiment 5 ml of blood was drawn in morning from the heart after animals anesthesia by chloroform, by using the heart puncture directly and using 5 ml sterile disposable syringe. Then 1 ml of blood was placed in test tube container on the substance EDTA anticoagulant for conducting analyzes of blood standards. While placing 3 ml of the remaining blood in clean plain test tubes free from relevant anti-coagulant, and left for 15_20 minutes at the laboratory temperature and then samples placed inside the centrifuged at 3000 cycles / minute for 15 minutes for the purpose of separation of serum. Isolated by suction micropipette minute mechanical pipette and put in new piping plastic for the purpose of testing dopamine concentration, the serum was keep at -5 C° degree until using. All reagent and samples were brought to room temperature before use centrifuge. The samples were centrifuged again after thawing and before the assay (according protocol manufacturing company).

Table (2-3) shows the components of the diet given during the study (Ward,1970).

Sequence	Article bush	Ratio	Per 10 kg
1	Full Cream Milk dryer	20.0	2.00
2	Buckwheat groats	17.0	1.7
3	Wheat flour	17.0	1.70
4	Barley groats	20.0	2.00
5	Corn groats	25.5	2.50
6	Food salt	1.0	0.10

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Statistical analysis

The Statistical analysis was carried out for data according to factorial experiment (2×4), it was the comparison between the averages by using the Duncan test (Duncan ,1955). At the level of probability of 5% or 1% to test the significant differences between the averages of traits and applying the statistical program (Sas , 2010).

Results and dissections

The results of this study showed that no significant difference (P>0.05) between group A of aspartame concentration (40mg/kg/day) compared with control group and the value were (95.13 ±10.55, 97.848±10.69) pg/ml respectively, also group A and group B didn't found to be significantly differences (P>0.05) in the dopamine consented were (95.13±10.55, 91.58±10.59) pg/ml respectively, however, the results showed that there is significant decrease (P<0.01) in dopamine concentration between group A when compared with group C aspartame concentration (70mg/kg/day), there value were (95.13±10.55, 68.95±8.75) pg/ml respectively. In addition, group B was aspartame concentration (54mg/kg/day), comparing with control group which not reach to significant difference (P>0.05) in brain dopamine concentration with value (91.580±10.59, 97.84±10.69) pg/ml, also group B compare with group A not significant difference (P>0.05) was found between them. While group B with C, the results showed significant decrease (P<0.01) in dopamine concentration in brain rats between these groups and the value were (91.580±10.59, 68.95±8.75) pg/ml respectively. Therefore, group C aspartame concentration (70mg/kg/day) showed significant decrease (P<0.01) in dopamine concentration in compared with control group, its values were (64.44±11.43, 97.84±10.69) pg/ml respectively. Also the results showed significant depression (P<0.01) in the dopamine concentration in brain in group C compared with group A and B, and the value were (64.44 ±8.75, 95.13±10.55, 91.58±10.59) respectively. As in figure (3-1)

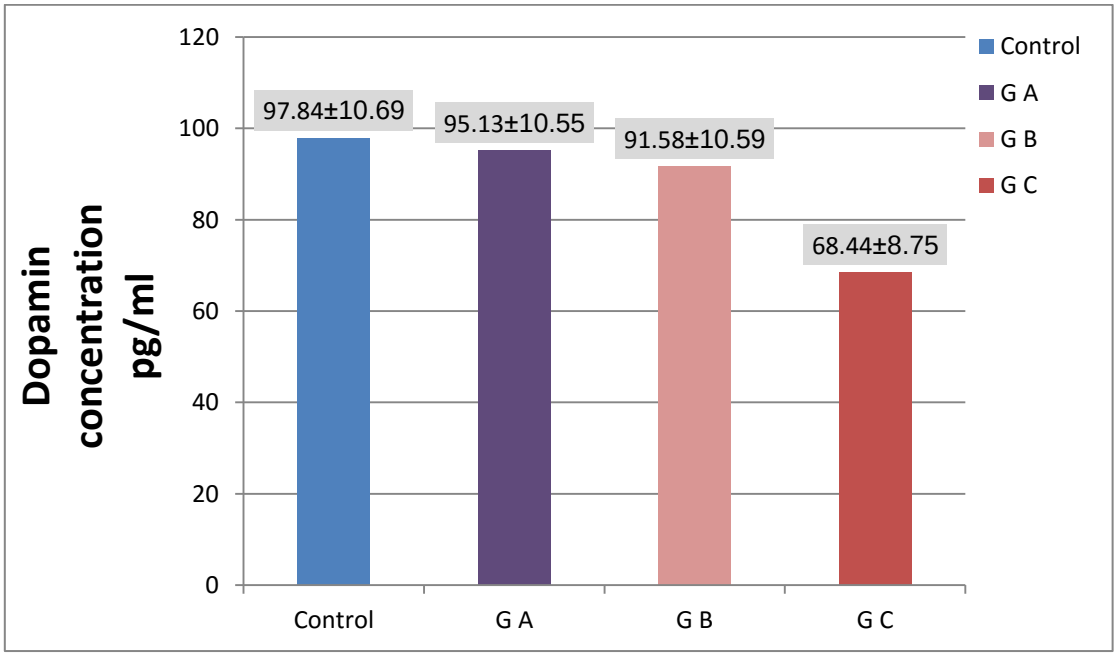

Fig. (3-1): Effect of aspartame on dopamine concentration in brain.

Glial cells are Astrocytes cells , its functions and presence are closely connected with the central nervous system (CNS), the artificial sweetener is broken down into

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phenylalanine, aspartic and methanol during metabolism in the body (Torii *et al*., 1985). The excess of phenylalanine blocks the transport of important amino acids to the brain contributing to reduce levels of dopamine and serotonin, astrocytes directly affect the transport of this amino acid and also indirectly by modulation of carriers in the endothelium (Karol & Jadwiga, 2013). Aspartic acid at high concentrations is a toxin that causes hyper excitability of neurons and is also a precursor of other excitatory amino acid – glutamates, their excess in quantity and lack of astrocytic uptake induces excitotoxicity and leads to the degeneration of astrocytes and neurons (Schultz *et al*.,2000). The methanol metabolites cause CNS depression, vision disorders and other symptoms leading ultimately to metabolic acidosis and coma, astrocytes do not play a significant role in methanol poisoning due to a permanent consumption of large amounts of aspartame, despite intense speculations about the carcinogenicity of aspartame, the latest studies show that its metabolite diketopiperazine is cancirogenic in the CNS, it contributes to the formation of tumors in the CNS such as gliomas, medulloblastomas and meningiomas (Monte, 1984). Glial cells are the main source of tumors, which can be caused inter alia by the sweetener in the brain and this information lead to damage effect to the cellular brain by aspartame (Perego *et al*.,1988).Oxidative stress is defined as a breach in the balance between free radical production and antioxidant defense mechanisms, that the dietary sweetener aspartame increases oxidative stress in the brain, the reduced form of glutathione, a tri-peptide of glycine, glutamic acid and cysteine, and a major antioxidant in tissue defense against oxidative stress, is decreased in brain tissue by the administration of aspartame, therefor it decreased lightly in brain suffering from a number of neurological diseases (Schulz *et al*., 2000). The implication of GSH consumption by free radicals in the pathogenesis of these disorders, the free radicals oxidation of polyunsaturated fatty acids in biological systems is known as lipid peroxidation, the recognition and measurement of lipid peroxidation is the evidence most frequently cited to support the involvement of free radical reactions in toxicology and disease (Gutteridge, 1995). Lipid peroxidation evaluated by the measurement of thiobarbituric acid-reactive substances (TBARS) was increased in brain after aspartame dosage, aspartame itself impairs cellular antioxidant status because of the decreased brain levels of GSH, and glucose, therefore, aspartame increases oxidative stress in brain which could have important effects in view of the fact that oxidative stress is implicated in various brain pathologies and that the agent is one of the most widely used artificial sweeteners in human foods and drinks (Dickinson *et al*., 2003). However, in contrast to the effects of the dietary sweetener on oxidative stress in the brain, aspartame is devoid of such effects in the liver, the vulnerability of the brain tissue to oxidative insults and the relative inadequacy of brain antioxidant mechanisms particularly glutathione in contrast to the liver is the most likely explanation, the finding that aspartame increases brain oxidative stress because of mild systemic inflammatory response evoked by bacterial endotoxin implies that the agent should be avoided in circumstances such as bacterial infection, psychiatric illness, or brain trauma (Abhilash *et al*., 2013).

Many researchers agree with our results in the present study like this Studies on the effect of aspartame on brain monoamines have yielded inconsistent results with increased norepinephrine and dopamine in various brain regions (Coulombe and Sharma 1986). Increased striatal serotonin (Goerss *et al*. 2000).Decreased serotonin (Yokogoshi *et al*. 1984; Torii *et al*. 1985). Decreased dopamine in the striatum (Bergstrom *et al*. 2007). Or even no effect on brain dopamine and norepinephrine levels (Torii *et al*. 1985; Perego *et al*. 1988; Dailey *et al*. 1991) being reported. Dopaminergic cell groups and pathways make up the dopamine system which is neuromodulator, its plays important roles in executive functions, motor control, motivation, arousal, reinforcement, and reward, besides that lower-level functions including lactation, and nausea (Schultz, 2007). Moreover it plays

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a role in pain, abnormalities in dopaminergic neurotransmission occur in several painful clinical conditions, including burning mouth syndrome, fibromyalgia, and restless legs syndrome (Wood, 2008). Decrease of dopamine concentration in brain in high dose of aspartame and this results agreed with (Hawkins *et al*.,2006; Bergstrom *et al*., 2007). They suggest that the reason go back to phenylalanine one of aspartame metabolism products it works to block the neutral amino acid transporters NAAT and then prevent essential amino acid tyrosine that necessary to synthesis dopamine from crossing blood brain barrier, also (Humphries *et al*.,2008) suggest that astrocyte are directly involved in the transport of phenylalanine and in the Astroytosis (proliferation of astrocyte) which increase Phe entering to the brain after amount used of aspartame, but this differ with what reach it (Coulombe and Sharma,1986; Goerss *et al*.,2000) reported that, increase in dopamine in various regions after 3 hours from ASP dosing. However Dailey *et al*., in (1991) concluded that aspartame failed to induce significant changes in brain serotonin or dopamine concentration.

In the other side, the influence the sex of animal on dopamine concentration in brain, the results showed that group A males didn't have significant difference (P>0.05) with control group (129.75± 3.09, 139.78± 3.83) pg/ml respectively, however, females showed significant decrease (P <0.01) compared with control, also group A with group B males and females the results show not found significant differences (P>0.05) between them. However, group A males and females comparing with group C they are significant depression (P <0.01) in dopamine concentration. While group B the males not reach to significant differences (P>0.05) with control, however the females with control showed significant decrease (P<0.01) and values were (57.06±1.27, 145.61±3.42) pg/ml respectively, and group B males and females comparing with group C showed significant decrease (P <0.01) in dopamine concentration. In addition the results of group C males and females demonstrate that's significant decrease (P < 0.01) in dopamine concentration than control group, the male value (56.73±3.46, 139.78±3.83) and females (39.50± 4.03, 145.61 ± 3.42) respectively. As figure (3-2).

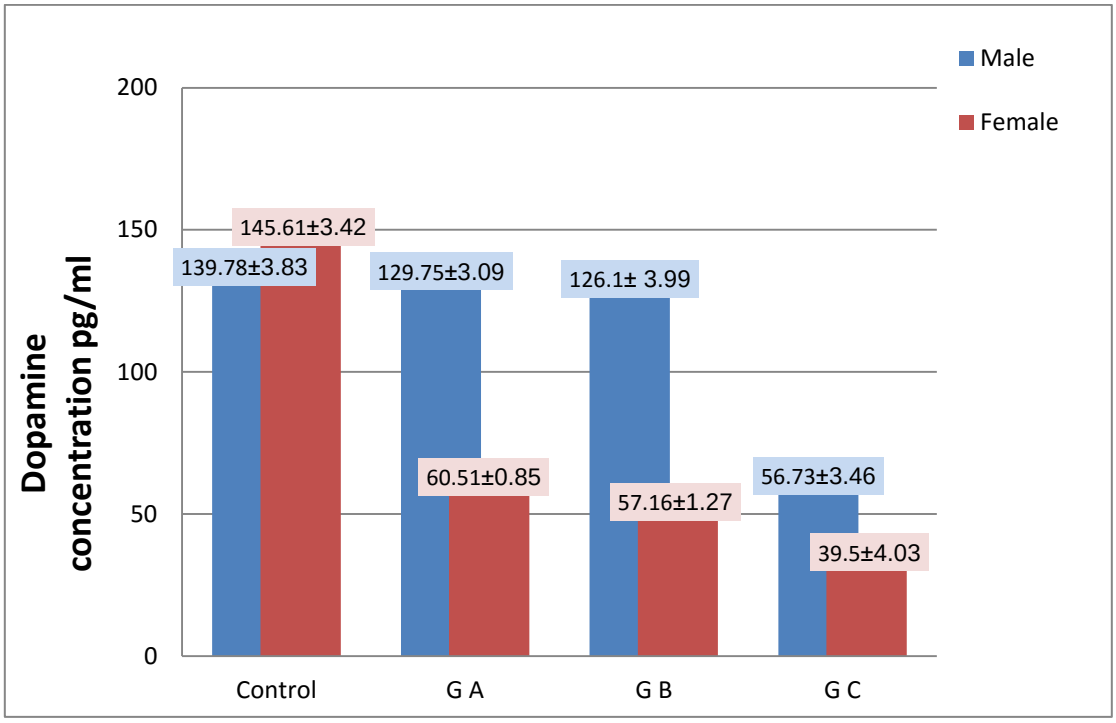

Fig. (3-2): Effect of sex on dopamine concentration in brain.

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Both males and females studies recommend that the rate of brain dopamine and serotonin metabolism and activity of the system that transports large neutral amino acids into brain is higher in females than males, because of the incidence of major depression is higher in females (Nishizawa *et al*., 1997). This study agreed with Walker *et al*., in (2000) when concluded that the concentration of dopamine released per stimulus pulse and the maximal velocity of dopamine uptake were greater in female rats than males, therefor according to results of this study the dopamine in females brain showed more sensitive than in males brain, its decrease when rise aspartame concentrations compared with control group. Nnevertheless, the other cause may be due to estrogen hormone and estrus cycle which always effects on many mechanisms in females body than males, in addition some neurotransmitters more presence in females than males that may be due to more of dopamine neurotransmitter make females more sensitive to aspartame than males (Vincent *et al*., 2005).

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