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EFFECT OF DIFFERENT DOSES OF ASPARTAME ON THE MALE REPRODUCTION HORMONES CONCENTRATION IN RATS

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Abstract

This study aimed to investigate the effects of different doses of aspartame on the male reproductive system of rats. After 90 days of administration various doses of aspartame solution to mature male rats, in three treatment groups (T1, T2, and T3) in doses (250, 500, and 750)mg/kg/day respectively with the control group treated by tap water, one ml for each rat. After 24 hours of the last dosage, the blood sample was collected and prepared to measure the concentration of the hormones (LH, FSH, and T) by using ELISA kits of Elabscience Company. The results were statistically analyzed to compare the data of individual treated group with the control group, showed that there was significant decrease ($p < 0.05$) in the LH and FSH concentrations of treatment groups in comparison with the control group, and there was significant decrease ($p < 0.05$) in the testosterone hormone concentration of T2 and T3 in comparison with the control group and T1.
***Keywords*:** Aspartame, Hormones, Rats

Introduction

In the last two decades, growing concern about health and life quality has encouraged people to exercise, eat healthy food, and decrease the consumption of food rich in sugar, salt, and fat (Butchko *et al.,* 2002; Appleton and Blundell, 2007) with growing concern to use the artificial sweeteners or nonnutritive sweetener (NNS), they are synthetic sugar intense sweeteners more than sucrose with a low calorie (Chattopadhyay *et al*., 2014).

Aspartame (APM) is one of the most nonnutritive sweeteners widely used, it is widespread in over 90 countries found in about 6000 products (Magnuson *et al*., 2007). It was discovered in 1965 by James Schlatter a chemist (Mazur *et al.,* 1970). The sweetener aspartame is known in the European Union under the E number (additive code) E951 and shopping this sweetener article under many brand names as NutraSweet®, Equal®, Furasweet®, Canderel®, E951 and others (Grenby, 1991; Arcella *et al*., 2004). The risk of APM due to its metabolite components, when aspartame ingested it's completely metabolized by the gut enzymes (peptidase and esterase) into three main components phenylalanine (50%), aspartic acid (40%), and methanol (10%) (Singh *et al.,* 2013).

The status of aspartame is still controversial, it underwent many studies, the safety of aspartame has been evaluated by various regulatory agencies like Food and drug administration (FDA) and others. Also, it underwent repeated tests to ensure it's safe for use (Butcko *et al.,* 2002; Mitchell, 2006). There were many studies refer the aspartame consumption related to many adverse effects such as seizures, headaches, allergies as well as impairment in behavioral and cognitive function. In the united states, the acceptable daily intake of APM is 50 mg/kg body weight. The oral lethal dose 50 (LD50) is more than 5000 mg/kg (Butchko *et al.,* 2002; Whitehouse *et al.,* 2008).

Materials and Methods

The study was performed in the animal house of the College of Veterinary Medicine / AL_Qasim Green University, for the period from (9 October 2018) to (7 January 2019). In this study used 60 animals of male Wistar rats ages (80-90) days, weights ranged (300-350)gm. They were placed in the 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).

Then the animals were divided randomly into four groups control (C), treatment 1(T1), treatment 2 (T2), and treatment 3 (T3). 15 male rat animals for each group. Allows animals to adaptation for a two weeks before the start of the experiment. Aspartame solution was prepared by dissolved aspartame pure powder in the tap water. The animals were dosage in the morning between (10:30_11:30)A.M by using oral gavage needle. The treatment groups (T1, T2, and T3) treated by aspartame solution in doses (250, 500, and 750)mg/kg/day respectively, and the control group was treated by tap water, in a one ml for each rat. After 24 hours of the last dosage, all animals was anaesthetized by Ketamine-Xylazine mixture 1.0 ml Ketamine (concentration: 100 mg/ml) and 0.5 ml Xylazine (concentration: 20mg/ml) respectively / IP (Dobrek *et al.,* 2017). The blood sample was collected directly by heart puncture at 5 ml and prepared to measure the concentration of the hormones (LH, FSH, and T) by using ELISA kits of Elabscience company.

The procedure methods depend on the manual leaflet which was accompanying with each kit.

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 error (SE).

Results and Discussion

The statistical analysis for the results of hormone (LH) in this study showed that there was a significant decrease ($p < 0.05$) in the concentration of LH in T1 (23.6±2.30)mIU/ml in a comparative with the control group (33.9±1.77)mIU/ml. Also, showed that there was a significant decrease ($p <0.05$) in the concentration of LH in T2 (21.3±1.06)mIU/ml in a comparative with the control group (33.9±1.77)mIU/ml, as well as, there was a significant decrease ($p < 0.05$) in the concentration of LH in T3 (19.4±0.71)mIU/ml in a

comparative with the control group (33.9±1.77)mIU/ml. While when comparing results of treatment groups (T1, T2, and T3) showed that there were non-significant differences (p‧ 0.05) between them their values are (23.6±2.30, 21.3±1.06, and 19.4±0.71)mIU/ml respectively. Table (1).

Table 1 : Effect of aspartame on reproduction male hormones concentration.

Experimental groups	LH (mIU/ml)	FSH (ng/ml)	Testosterone (ng/ml)
Control	33.9±1.77a	128.8±1.09a	15.4±0.83a
T1	23.6±2.3b	96.7±2.66b	14.1±0.78a
T2	21.3±1.06b	93.6±2.23b	9.7±0.46b
T3	19.4±0.71b	91.6±2.19b	9.5±0.53b

The result of FSH concentration showed that there was a significant decrease ($p < 0.05$) in the concentration of FSH in T1 (96.7±2.66)ng/ml in a comparative with the control group (128.8±1.09)ng/ml. Also, there was a significant decrease ($p < 0.05$) in the concentration of FSH of T2 (93.6±2.23)ng/ml in a comparative with the control group (128.8±1.09)ng/ml.

As well as, there was a significant decrease ($p < 0.05$) in the concentration of FSH of T3 (91.6±2.19)ng/ml in a comparative with the control group (128.8±1.09)ng/ml. When compared between treatment groups (T1, T2, and T3) showed that there were non-significant differences ($p < 0.05$) between them their values are (96.7±2.66, 93.6±2.23, and 91.6±2.19)ng/ml respectively. Table (1).

The statistical analysis of the results of testosterone hormone concentration for this study showed that there were non-significant differences ($p < 0.05$) between T1(14.1±0.78) ng/ml and the control group (15.4±0.83)ng/ml. While, in compared T2 and control group showed that there was a significant decrease ($p < 0.05$) between them, their values are (9.7±0.46, 15.4±0.83) ng/ml respectively. Also, there was a significant decrease ($p < 0.05$) in the concentration of testosterone hormone of T3 (9.5±0.53) ng/ml in a comparative with the control group (15.4±0.83) ng/ml.

In addition, there was a significant decrease ($p <0.05$) between T2 and T1 their values are (9.7±0.46 and 14.1±0.78)ng/ml respectively. Also, there was a significant decrease ($p < 0.05$) of T3 in a comparative with T1 their values are (9.5±0.53 and 14.1±0.78)ng/ml respectively. While the result showed that there were non-significant differences ($p < 0.05$) in testosterone hormone concentration between T2 and T3, their values are (9.7±0.46, 9.5±0.53) ng/ml respectively Table (3).

The causes of the affected concentration of hormones in this study may because aspartame (APM) metabolites products: phenylalanine (Phe), aspartic acid (aspartate) and methanol (MeOH) this agreed with (Choudhary and Devi, 2014; Ashok and Sheeladevi, 2015) who were attributed the harmful effects and toxicity of APM due to its metabolites products. Aspartame consumption has been shown an increase in the concentration of Phe, aspartate, and MeOH in the blood (Filer and Stegink, 1988; Bowen and Evangelista, 2002; Humphries *et al.,* 2008; Degani, 2010). Excess Phe level in the plasma can be toxic to the brain, it will interfere with the tyrosine and tryptophan, leads to lowers the concentration of the brain catecholamine, serotonin, and dopamine. that causes upsets the balance of neurotransmitters and lead to neurological, behavioral, and hormonal changes (Humphries *et al.,* 2008).

In addition, aspartate play an important role in neurotransmitter balance in the central nervous system and the excess level may induce neuroendocrine disturbances (Watkins, 1984; Stone and Burton, 1988). Abdel-Salam *et al.* in (2012) state in his study the APM intake associated with decreased the levels of several important brain neurotransmitters like serotonin, dopamine, and noradrenaline, this impacts the balance of hormones secretion.

Parthasarathy *et al.* in (2006) and El-Haliem, in (2013) pointed that the aspartame administration leads to hypertrophy of the most cells in pars distalis and induce histological changes in the pituitary-thyroid axis with reducing pituitary hormones secretion of adult male albino rats. Also, Stanley, in (2013) referred to aspartame induce a change in pituitary hormones prolactin, FSH, and LH.

The result of this study agreed with other previous studies, Hozayen *et al.* in (2014) who remembers, the aspartame administration to the male rats induced decrease concentration of serum FSH, LH, and testosterone. Also, Morovvati *et al.* in (2019) referred to that the aspartame administration to the adult mice causes decrease concentration of the testosterone hormone.

Moreover, Puica *et al.* in (2008) referred to the chronic administration of aspartame at the pre-pubertal stage on juvenile rabbits induced neurodegenerative effects especially in the circum ventricular organ (CVO) of the hypothalamus; and severe structural and functional alterations in hypothalamic-pituitary axis. Also, Puica *et al.* in (2009) recorded the chronic administration of aspartame at the pre-pubertal stage on white Wistar rats induced neurodegenerative effects especially in the CVO of the hypothalamus, and severe structural and functional alterations in the hypothalamic-pituitary axis. This affected hormones concentration and lead to low secretion of gonadotropin-releasing hormone (GnRH), luteinizing hormone (LH), and follicle stimulating hormone (FSH), as well as, the inhibition of the synthesis and the secretion of testosterone hormone, that causes the diminution of the reproductive capacity.

Conclusion

The conclusion which resulted of this study are the aspartame has harmful effects on the male reproductive system because of their an adverse effect on the concentration of the hormones which regulate the male reproductive system (LH, FSH, and Testosterone).

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