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EFFECTS OF ARTIFICIAL SWEETENER(ASPARTAME) ON THE WEIGHT OF IMMUNE ORGANS AND GRANULOCYTES CELLS IN RATS

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ABSTRACT

This study aimed to determine the effect of aspartame on the white blood cells. Used for this study 24 adult male rats and 24 adult female rats, with age of 65-70 day, almost on an average live weight between (185-200) g. The experiment has concentrated on the daily dose, where the animals were randomly divided into two groups, each group contains 12animals females. the first, control groupdosed with 1ml physiologic solution daily, the second group gavage with concentrated aspartame (54 mg/kg/per day). The all groups had their daily dosages through the 75 day, and the experiment continued from October 2015-January 2016. After the dosage's period has been completed, hematological samples have been taken from the rats to examine the count of neutrophils cells and isolation of spleen, thymus and lymph nodes to estimate the weight of it. The results showed there was significant decrease($P<0.05$) in theneutrophil count, however no significant differences in monocyte and eosinophil count among groups, compared with the control group. Also showed that the weights of spleen, thymus and lymph nodes in aspartame treated animals were significantly decreased compared to control animals.

KEYWORDS: Aspartame, rats neutrophils, immune organ, artificial sweetener effects.

1. INTRODUCTION

Aspartame -APM is a dipeptide artificial sweetener that is widely used in between all ages as a non-nutritive sweetener in foods and drinks. Aspartame a high intensity sweetener most commonly found in low calorie beverages, chewable multi-vitamin, breakfast cereals, dessert mixes, dietSoda, table top sweeteners added to tea or coffeeand used in food products, pharmaceutically it has been approved as a sweetener for liquid carbonated beverages (Oyama*et al*., 2002; Rencuzogullari*et al*., 2004). APM 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, which 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®, Canderel® & others, it may appear on behalf of (E951), After ingestion, aspartame is immediately absorbed from the intestinal lumen and metabolized to phenylalanine, aspartic acid and methanol (Ranney*et al*., 1976). Chemical engineering has led to develop artificial sweeteners, used as this 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 hydrolyzed to its components (Butchko*et al*., 2002). It is slightly soluble in water (about 1% at 25°C), sparingly soluble in alcohol and insoluble in fats and oils. Aspartame in dry products is fairly stable even at high temperatures. However, in solution, its stability is a function of time, temperature, pH and available moisture. Aspartame is most stable between pH values of 3 and 5 even with increasing temperature. However, it breaks down and loses its sweetness in normal cooking or baking (Trocho*et al*., 1998).

W.B.Cs are an important component of the host defense system, responsible for defense against bacteria, fungi, viruses, and attacking parasites, the granulocytes are the most numerous, the new granulocytes have horseshoe-shaped nuclei that become multiplied as the cells grow adult (Kime*et al.,* 2010). The number of leukocytes in the blood is often an indicator of disease, one of the major components of inflammatory process, it is an important subset of the complete blood count, however, this 1% of the blood makes a large difference to health, because immunity depends on it (Lafleur, 2008). WBC count plays an important role in pathogenesis of insulin resistance and cardiovascular disease (Nakanishi *et al*., 2002).

Neutrophils are a type of phagocyte and are normally found in the bloodstream. During the beginning (acute) phase of inflammation, particularly as a result of bacterial infection, environmental exposure and some cancers, neutrophils are one of the first-responders of inflammatory cells to migrate towards the site of inflammation (Jacobs *et al*., 2010).Neutrophils are recruited to the site of injury within minutes following trauma, and are the hallmark of acute inflammation, however, due to some pathogens being indigestible, they can be unable to resolve certain infections without the assistance of other types of immune cell (Cohen*et al*., 2002).

The thymus provides an inductive environment for development of T cells from hematopoietic progenitor cells. In addition, thymic stromal cells allow for the selection of a functional and self-tolerant T cell repertoire. Therefore, one of the most important roles of the thymus is the induction of central tolerance.

2. MATERIALS AND METHODS

In this study, I have taken 48 blood sample, 24 samples from male and 24 samples from female Wister rats. All the rats were housed under controlled temperature (26 ± 2ºC) condition with 12:12 h light: dark cycle.

2.1. MATERIALS

2.1.1. Instrument and tools

Table (2-1) shows instruments and tools that are used in this study.

NO.	Instruments & tools	Source
1	Balance of weight	Sartorus _ Germany
2	Centrifuge	Heraeus -Christ Gumby_ Germany
3	Disposable syringe	Medical company _ Syria
4	EDTA tubes	Medical company -Syria
5	Eppendrof tubes	Medical company -Syria
6	Hematological analyzer	Blood Analyze -Poland
7	Micropipettes-automatic	SLamed -Germany
8	Dissociation set	Elfar- Germany
8	Plain tubes	Afma. Dispo. -Jordan
9	Refrigerator	Ishtar -Japan
10	Sensitive balance	Sartouris Meter AE 200 -Germany

2.1.2. Chemicals

Table (2-2) shows chemicals and kit that used in this study.

No.	Chemicals	Origin
1	Aspartame	NutraSweet –Monsanto company_ America
2	Chloroform	BDH_ England
3	Formalin 10%	Difco_ U.S.A
4	Normal saline	BDH _ England

Experimental animals underwent to the laboratory conditions and suitable temperatures. In this study used 24 animal female Wister rats were divided randomly into two groups each group included 12 animals, the animals gavages for 75 days about 1ml in day in morning about (9:30_10:30) by used oral gavage needle. Groups of experiment: Group control: administration by normal saline 1ml daily along period of experiment. Group A administration by aspartame concentration 54mg/kg/day about 1ml daily.

2.2. METHOD

2.2.1. Sample collection

Blood samples and isolation of spleen, thymus and lymph nodes. Stress-free blood samples were collected as per the technique described by Feldman and Conforti (1980). At the end of experimental period all the animals were exposed to mild anesthesia and blood samples were collected from the heart, plasma and serum were separated by centrifugation at 3000 rpm at 4ºC for 15 min. Later all the animals were sacrificed under deep anesthesia using chloroform. The spleen, thymus and lymph nodes were excised, washed in ice cold saline and blotted to dryness. Quickly after weighed, the spleen, thymus and lymph node samples were homogenized by using Teflon glass homogenizers. 10% homogenate of these tissues were prepared in phosphate buffer (0.1 M, pH 7) and centrifuged at 3000g at 4 C for 15 min to remove cell debris and the clear supernatant was used for further biochemical assays.

2.2.2. Hematological examination

After measuring the count of white blood cells WBC as well as lymphocytes percentage by putting sample blood In EDTA tube in automatic hematological analyzer (according protocol manufacturing company) as flowing method:

1- Route the system, if blank needed appears on the screensaver insert the blank (Deionized distal water) in adapter then press the measurement analysis key.
2- Placed the sample in the special adapter.
3- Pushed start key the system is ready for analysis.

2.2.3. Immune organ weight

The method according (Cross *et al*., 1982).

3. RESULTS AND DISSECTION

3.1. Effect of aspartame on neutrophils count

In animals treated with aspartame concentration (54mg/kg/day) the results were showed significant decrease (P<0.05) in the neutrophil count, when compared to control animals. However there were no significant differences in monocyte and eosinophil count among groups. The results are summarized in Table 3.

Table 3: Effect of aspartame on granulocytes cells count.

Cell type	Control	Aspartame
Neutrophil	20.82 ± 0.37	13.40 ± 1.3*
Eosinophil	6.73 ± 1.06	5.25 ± 1.02
Monocytes	2.14 ± 0.2	2.8 ± 0.7

*P < 0.05 compared with control

In aspartame treated animals, there was a decrease in Neutrophils count. This decrease appears to have a linear relationship as duration of oxidative stress increased. This reduced blood leucocyte number during stress reflects a dynamic redistribution of cells rather than loss of cells. Glucocorticoids mediate the trafficking of leucocyte out of the blood and among tissues during stress (Dhabar and McEwen, 1997) Based on this the redistribution of the leucocytes may be due to circulating corticosteroids level as suggested by Seyle. The neutrophils and lymphocytes vary in opposite direction. The decrease in neutrophils can be attributed due to their margination, an abnormal distribution due to local chemotaxis that causes the cell retention in several organs (Berner*et al*., 2005).

3.2. Effect of aspartame on organs weight

The weight of spleen, thymus and lymph nodes immune organs in aspartame treated animals G A were significantly decreasedP < 0.05 compared to control animals. The data are presented in Table 4.

Immune organs	Control	Aspartame
Spleen	4.52 ± 1.03	1.92 ± 0.36*
Thymus	2.41 ± 0.1 3	0.8 ± 1.10*
Lymph node	0.3 ± 0.04	0.051 ± 0.004*

*P < 0.05 compared with control

The present study clearly confirms that aspartame can be act as chemical stressor as indicated by the elevated corticosteroid level in the aspartame-treated group. Increased corticosterone has been shown to decrease the size and weight of the spleen and thymus (Franco *et al*., 1990). The significant reduction in organ weight and organ cell count may be due to oxidative damage which was studied by Skrzydlewksa and Szynakain (1997) who reported that oxidative damage caused marked organ weight loss in rats upon methanol intoxication. This is also reported by Parthasarathy*et al* (2006) Formaldehyde the first metabolite of methanol, increases the population of shrunken cells, dead cells and hydolipid cells (Nakao*et al*., 2003). which might be the reason for decreased cellularity (reduction in organ weight and cell count) within our observation.

4. CONCLUSIONS

Conclude from this study that

1. Aspartame not effected on total leucocyte count.
2. High dose of aspartame effect on immunity of body by decrease the percentage of Neutrophils cell.
3. disturbance of vital function of immune organs which finally decreased their cellularity (reduction in organ weight.

5. Recommendations

1. Study about effects of aspartame on other body systems.
2. Also study the effect of aspartame by use quantities PCR and immunohistochemistry.
3. Study the effect of different doses of aspartame on differential count of WBCs, cortisol and on RBCs.
4. Necessary to be careful when using the food and beverages as a sweetener.

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