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The Study of Different Doses of Saccharin on Biochemical Parameters in Male Wister Rats

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Abstract

Background: Saccharin is one of the world's most frequently used chemical sweeteners.

Objectives:This study aims to investigate the effect ofSaccharin on certain parameters of biochemistry.

Methodology: Sixty mature male Wistar rats were divided randomly into four groups, 15 for each group. The first control group dosed by distal water orally and the three treatment experimental groups (T1, T2, and T3) treated by saccharin solution orally at doses of (250, 500, and 750) mg/kg per day, respectively, for each rat, in one ml. All these classes were viewed on a regular basis for 90 days. After the end of experiment , blood sample was collected from for estimation body weight and blood glucose concentration. also the oxidative stress investigation was done by measuring catalase, peroxidase and glutathione.

Results : The results of this study showed that there were significant increase (P< 0.05) in glucoseconcentration of (T1, T2 and T3) groups when compared with control group. When compared between treated groups T3 was more effected as compared with (T1 and T2).On the other hand, The results showed there were significant decrease (P<0.05) in glutathione level and catalase of (T1, T2 and T3) groups when compared with control group. On the other hand (T3) group treated was more effected as compared with (T1 and T2) groups. The current study showed there was significant increase (P< 0.05) in peroxidase concentration of (T1, T2 and T3) groups when compared with control group However, (T3) group was more effected as compared with (T1 and T2) groups.

Conclusions : The statistical analysis for the results of body weight in this study showed that there was a significant decrease (p<0.05) in body weight of all treated groups compared with control group. (T3) group was more effected as compared with (T1 and T2) groups. From this study we concluded that Saccharin has a harmful effect on biochemical parameters.

Keywords: Saccharin, glucose concentration, oxidative stress, body weight.

INTRODUCTION

Alternative sweeteners, also known as intense, artificial, non-nutritive, or low-calorie sweeteners, are used in a wide range of food, beverage, confectionary, and pharmaceutical products around the world. To prevent the effects of sugar on certain medical conditions such as diabetes and hyperglycemia, consumers select foods with artificial sweeteners.(Ni Y, Xiao W, Kokot S, 2009). Non-nutritive sweeteners were discovered in the last century, beginning with saccharin which is the oldest non-nutritive sweetener. It is over 125 years old and has a history with great vicissitudes. Saccharin is suitable for use in cooking and baking for its high stability and non-existence of calories. It is absorbed almost completely but is excreted unchanged in the urine (Abdelaziz and Ashour, 2011). Saccharin is found in various types, including sodium saccharin, calcium saccharin, potassium, and saccharin acid. More palatable and regularly used is sodium saccharin (Amin and Almuzafar, 2015).Saccharin(1,2-benzisothiazol-3(2H)-one-1,1-dioxide) is a sulfonamide that is made from a coal tar compound. It used to sweeten various products like soft drinks, baked goods, jams, chewing gum, canned fruit, candy, dessert toppings and salad dressings, as well as cosmetic products (e.g., toothpaste, mouthwash, and lip gloss), vitamins, and medications (Whitehouse *et al*, 2008). The level of use depends on the intensity of sweetness desired. In higher concentration, the compound has a light bitter after taste. It is about 300 times sweeter than sucrose in concentrations up to the equivalent of a 10 % sucrose solution. The

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present stipulated Acceptable Daily Intake (ADI) value is 15 mg / kg of body weight (Karl *et al*., 2020).Consumption of saccharin has been associated with side effect such as :-carcinogenicity (Azeez*et al* , 2019), genotoxicity (Ylmaz and Ucer,2015). Hepatotoxicity (Amin and AlMuzafar, 2015). Nephrotoxicity (Mourad , 2011). And disturbance in the clotting system (Iroghama*et al*., 2017). Chronic consumption of saccharin affects biochemical parameters and the results recorded indicate different metabolic, hormonal and neural responses in male and female rats as a result of repeated use of this sweetener after a single dose in drinking water (Andrejic*et al*, 2013).

MATERIALS AND METHODS

Experimental animals:-

The present study has been conducted in the animal house of the College of Veterinary Medicine, AL-Qasim green University.sixty mature male Wistar rats (ages between 65-70 days and weighted 150±10 g). Male rats were allowed to acclimatize to the animal house environment before beginning of the experiment. Animals were housed in polypropylene cages inside a well-ventilated room. Each cage contain five rats or less. Male rats were fed on the standard chow (appendix 1) and drinking water *ad libitum* throughout the experiment. Room temperature was maintained at 23±2ºC, the light-dark cycle was on a 12 hr light/dark cycle with light on at 06:00 a.m. and off at 06:00 p.m. during the experimental periods.

Experimental design:-

Sixty male animals Wister rats were divided randomly into four groups each group included 15 animals with control group, saccharin was dissolved in distilled water and each groups were drenching (according to weight) for 90 days about 1ml in day in morning between (10:30_12:30) by using oral gavage needle (Jasim*et al*.,2019 b).. Groups of experiment as flows: Control group: administration of distilled water 1ml daily along period of experiment for 90 day.Group 1: administration of saccharin concentration 250mg/ k.g. per day. About 1ml daily for 90 day. Group 2: administration of saccharin concentration 500mg/ k.g. per day. About 1ml daily for 90 day.Group 3: administration of saccharin concentration 750mg / k.g. per day. About 1ml daily for 90 day.

Plasma and Serum Preparation

Blood was collected in test tubes with cap and allowed to clot (for 20 minutes), then serum was separated by centrifugation at (4000 rpm, 0.894xg) for 10 minutes (Laessig*et al*., 1976). The separated serum of each animal was subdivided nearly into (6) samples using appendroff tubes (0.5ml) and kept at deep freezer until using for assessment of the biochemical parameters . Glucose measured by using a glucose machine manufactured by the company HOT Germany.Determination of Serum peroxidase Concentration: according to (Guide and Shah, 1989).Determination of Serum Glutathione Concentration: according to (Burtis&Ashwood, 1999).Determination of Serum Catalase: according to (Aebi, 1974).

Statistical Analysis:

Results were expressed as mean ± standard error of the mean (SEM). Comparisons were performed using two way analysis of variance (ANOVA2) and newman- keuls to test all groups unpaired values. Differences were considered to be significant at the level of $P<0.05$. All statistical analysis were carried out using the SPSS (2010, USA).(Khashi Mahmoud, 2000).

RESULTS AND DISCUSSION :-

Glucose concentration:-

The result illustrated in figure (1) recorded significant increase ($p<0.05$) in Glucose of (T1, T2 and T3) (145±0.42, 166.06 ±0.35 and 185.8 ±0.24) pg/ml respectively compared with control group (118±0.55) pg/ml. Moreover, by comparing between treatment groups the results showed that there is significant increase ($p<0.05$) in Glucose concentration in T3 compared with (T1 & T2).

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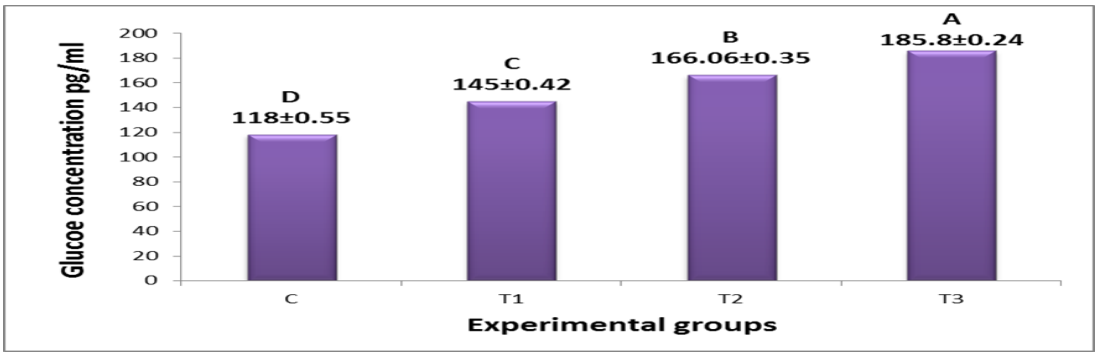


Figure (1): Effect of Saccharin on blood glucose concentration.

Glutathione (GSH):-

The result illustrated in figure (2) recorded significant decrease ($p<0.05$) in Glutathione of (T1, T2 and T3) (1.74±0.008, 1.52±0.006 and 1.04±0.005) pg/ml respectively compared with control group (2.32±0.067) pg/ml. Moreover, by comparing between treatment groups the results showed that there is significant decrease ($p<0.05$) in Glutathione concentration in T3 compared with (T1 & T2).

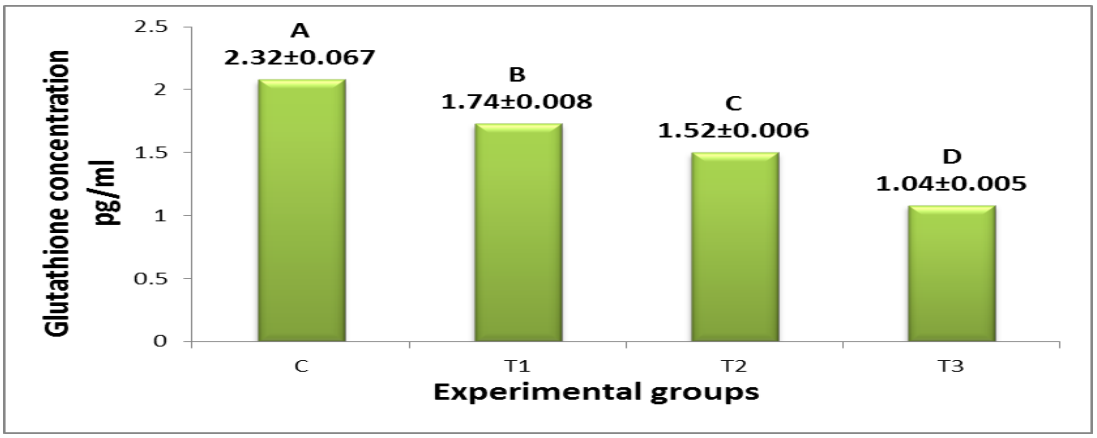
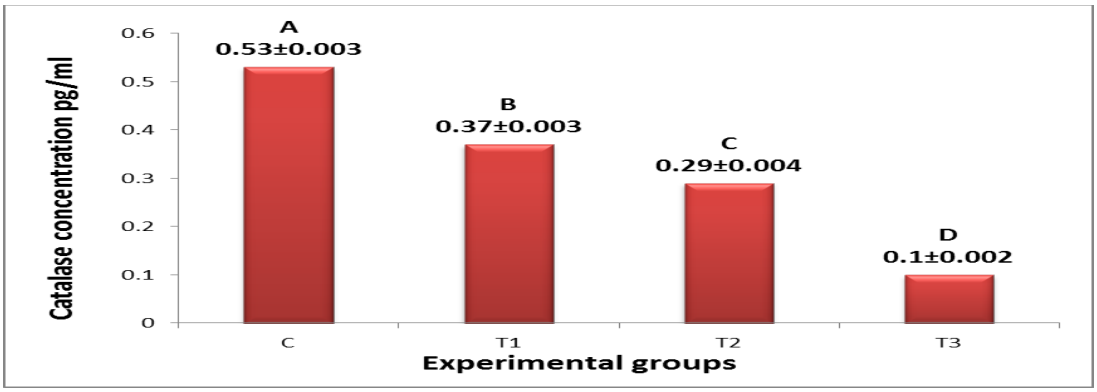


Figure (2): Effect of Saccharin on glutathione concentration.

Catalase (CAT) :-

The result illustrated in figure (3) recorded significant decrease ($p<0.05$) in Catalase of (T1, T2 and T3) (0.37±0.003, 0.29±0.004 and 0.1±0.002) pg/ml respectively compared with control group (0.53±0.003) pg/ml. Moreover, by comparing between treatment groups the results showed that there is significant decrease ($p<0.05$) in Catalase concentration in T3 compared with (T1 & T2).



Figure(3): Effect of Saccharin on catalase concentration.

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peroxidase (Pox) :-

The result illustrated in figure (4) recorded significant increase ($p<0.05$) in POX of (T1, T2 and T3) (4.29±0.023, 6.53±0.037 and 8.45±0.032) pg/ml respectively compared with control group (1.54±0.015) pg/ml. Moreover, by comparing between treatment groups the results showed that there is significant increase ($p<0.05$) in POX concentration in T3 compared with (T1 & T2).

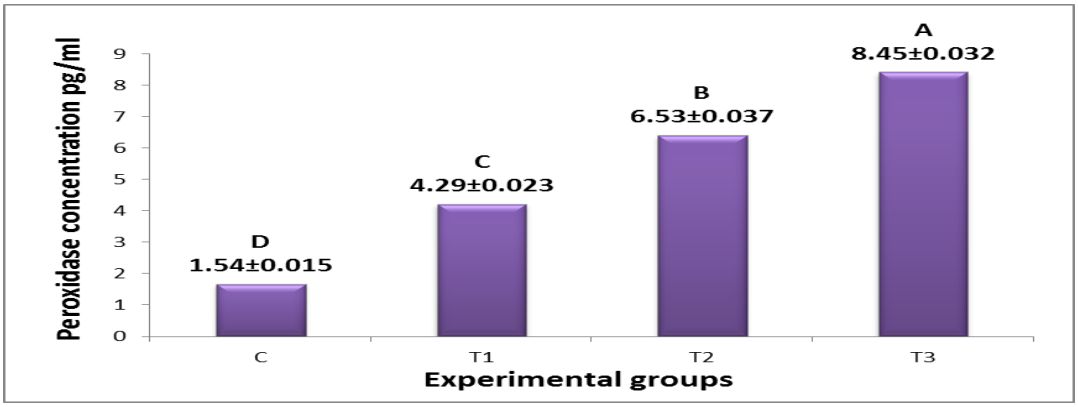

Figure (4): Effect of Saccharin on peroxidase concentration.

Body weight:-

The result illustrated in figure (5) recorded significant decrease ($p<0.05$) in body weight of (T1, T2 and T3) (216.2±1.07g, 176.33±0.45 and 154.86±0.55) g respectively compared with control group (293.66±0.83) g. Moreover, by comparing between treatment groups the results showed that there is significant decrease ($p<0.05$) in body weight level T3 compared with (T1 & T2).

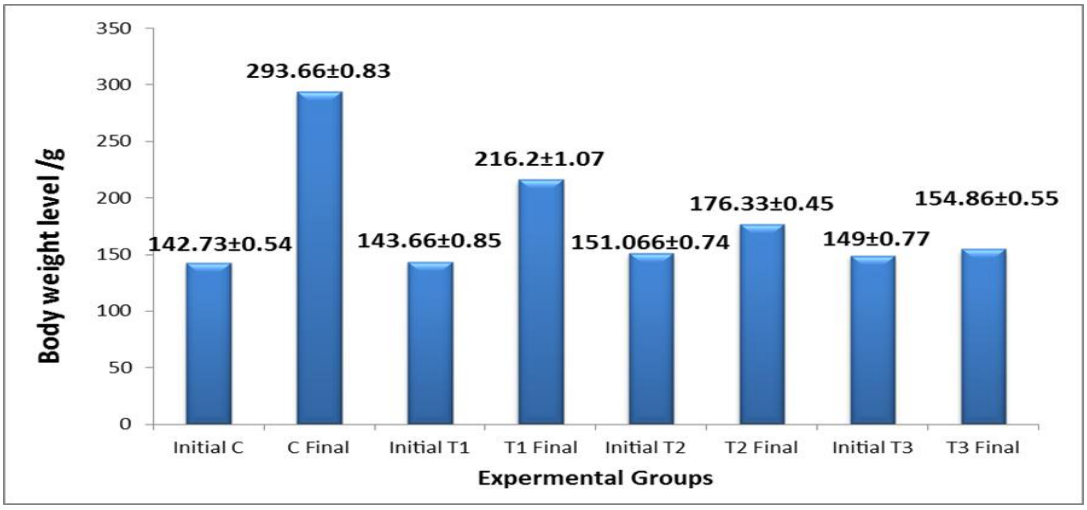

Figure (5): Effect of Saccharin on body weight.

exposure to high-intensity sweeteners is hypothesized to interfere with a predictive relationship between sweet tastes and calories that could influence the energy balance, which in turn would change glucose homeostasis and decrease satiety(Swithers*et al*, 2012). In this study the results showed that the use of different level of Saccharin for laboratory rats there will increase ($p<0.05$) in glucose concentration compared with control group, the results was agreement with (Adnan *et al*.,2015; Azeez*et al*, 2019) and (Leibowitz*et al*, 2018) found that when they consumed saccharin, there was a substantial rise in glucose levels relative to the control group.Prokic, *et al* in(2015) also refers to a substantial rise ($p<0.05$) in the glucose level relative to the control group when treated with Saccharin.While this study was disagree with Cooper *et al* (1988) showed no considerable rise in fasting blood glucose concentration ($p > 0.251$). Amin *et al* (2016) recorded that rats ingested low and high doses of saccharin and showed a substantial decrease in glucose levels.a portal infiltration of mononuclear

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inflammatory cells, primarily lymphocytes and macrophages, attributed to the oxidative stress caused by high doses of saccharin to the inflammation initiated in the liver cells. Inflammatory processes thus cause oxidative stress, producing excess reactive oxygen (ROS) species and reactive nitrogen species. A respiratory burst is experienced by stimulated inflammatory cells and ROS is released, such as superoxide anion, hydrogen peroxide and various secondary oxidants(Jasim*et al*.,2019 a). Thus, as the release of ROS by inflammatory cells induced a decrease in GSH, these free radicals may also lead to hepatic cell damage and increase the release of AST and ALT enzymes into the serum, as we observed in rats treated with saccharin, particularly those consuming high doses (Azeez*et al*, 2019).In our study the results showed that the use of different level of Saccharin will decrease ($p<0.05$) in glutathione concentration compared with control group, the results was agreement with (Amin and AlMuzafar, 2015) The outcome of their analysis was a decrease in GSH activity relative to the control group. In this study the results showed that the use of different level of Saccharin for laboratory rats there was a significant decrease ($p<0.05$) in catalase concentration compared with control group, the results was agreement with(ALKafafy, 2015), which showed that high saccharin doses induced a substantial decrease in liver catalase relative to control rats and attributed high saccharin dose-induced oxidative stress to inflammation of liver cells.(Szaleczky*et al*, 1999) showed that elevated serum lipid peroxidation, indicating that the NNS has a strong ameliorative effect against diabetic oxidative stress. The actions of these enzymes have further increased most of the NNS. The increased activity of antioxidant enzymes in diabetic conditions may be due to a adaptive physiological response in the serum of diabetic animals to elevated oxidative stress markers.Saccharin increased the activity of hepatic peroxidase in diabetic animals and caused significant increases in renal antioxidant enzymes ($p<0.05$) and significant increases in the activity of cardiac peroxidase enzymes ($p<0.05$) (Mchunu et al, 2019).(Abdallah, 2002) showed highly significant reduction in body weight gain percent in saccharin groups when compared to the control group. In agreement with this result Dib et al. (1996) reported a significant reduction in body weight of rats (50%) after administration of sodium saccharin for 14 days. Garland et al. (1991 b) also noted that body weights of saccharin treated male and female rats were significantly lower than the controls after 12 weeks treatment. They attributed this weigh loss to reduce food consumption per day.While this study was disagree with (Iroghama*et al*, 2017) reported the highest increase in weight gain, This could be due to the inhibition of neurotransmitter serotonin blunts the sensation of carving carbohydrate and this is part of the body's feedback system that helps limit consumption of carbohydrate to appropriate levels. A substantial increase in body weight during the time of saccharin intake was noticed in comparison with the control group (Azeez et al, 2019).

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