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EFFECT OF DIFFERENT DOSES OF SACCHARIN ON SOME PHYSIOLOGICAL PARAMETERS OF LIVER IN MALE RATS

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Abstract

This study aimed to investigate the effects of different doses of saccharin on the liver of male rats. After 90 days of administration various doses of saccharin solution to male rats, in three treatment groups (G1,G2 and G3) in doses (250,500 and 750) mg/kg/day respectively with the control group treated by distilled 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 liver enzyme: Alanine aminotransferase (ALT), Aspartate transaminase (AST), Alkaline phosphatase (ALP), Gamma-GlutamylTransferase (GGT) by using spectrophotometer. The results were statistically analyzed to compare the data of individual treated group with the control group, showed that there was significant increase ($P< 0.05$) in liver enzyme (ALT,AST, ALP and GGT) concentration in all treated groups comparing with control group.

Key words : Rats, liver, ALP, ALT, AST, GGT.

Introduction

In the last decades, the rising concern about health and life quality have encouraged societies to exercise, eat healthy food and reduce the consumption of food rich in sugar, salt and fat with increased consumer interest in reducing sugar intake, food products made with sweeteners rather than sugar have become more common. These non-nutritive sweeteners, better known as artificial, have no calories and typically exceed the sweetness of sucrose by a factor of 300 to 500 times (Helal *et al*., 2019).

Saccharin is anon nutritive, non-caloric intense artificial sweetener it has 300-500 times the sweetness of sucrose, but has a slight bitter aftertaste. 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).

There are different forms of saccharin including sodium saccharin, calcium saccharin, potassium and acid saccharin. Sodium saccharin is more palatable and commonly used (Amin and Almuzafar, 2015).

Saccharin is known under the E number (additive code) E954. The acceptable daily intake (ADI) recommended by the food and drug administration (FDA) is 5 mg / kg body weight / day (Aruomaren *et al.*, 2017).

Many studies reported that there are different side effects associated with saccharin consumption including: 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).

Materials and Methods

This study was performed in the animal house of the College of Veterinary Medicine/ AL-Qasim Green University, for the period between 1 October 2019 to 1 January 2020. In this study 60 male Wister rats are used, they have been purchased from the animal house of the department of biology- College of Science / University of Babylon.. Animal ages between 60-65 days, weights

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ranged 110-130 g, 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. Experimental animals underwent to the laboratory conditions and suitable temperatures (18 - 20 ºC) with 12 hr. light and 12 hr. darkness. Allows animals to acclimation for a 20 days before the start of the experiment.

In this study, 60 male animals Wister rats were divided randomly into four groups each group included 15 animals with control group, the was dissolved in distilled water and each groups were drinking (according to weight) for 90 days about 1ml in day in morning between (10:30_12:30) by using oral gavage needle. The treatment groups (G1,G2 and G3) treated by saccharin solution in doses (250,500 and 750) mg/kg/day respectively, and the control group was treated by distal water, in a one ml for each rat. After 24 hours from last administration, all animals were anaesthetized by mix ketamine + xylzenas the following method:-

Ketamine-xylazine mix for rats : The following regimen will produce a surgical level of anesthesia for 15-30 minutes and sedation of 1-2 hours. Combine: [1.0ml] Ketamine (concentration: 100mg/ml) [0.5ml] Xylazine (concentration: 20mg/ml) had given in dose: 0.15ml per 100 gm of body weight so the delivered dose is Ketamine 100 mg/kg and Xylazine 10mg/kg The route of administration : Intraperitoneal injection using 1 ml syringe (Fish *et al.*, 2008).

The collection of blood sample was done directly from the heart by using the heart puncture by using sterile disposable syringe 5 ml of blood obtain serum of animals for assessment of biochemical parameters.

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

1. Effect of saccharin on Alanine aminotransferase (ALT)

The statistical analysis for the results of ALT in this study showed that there was a significant increase ($p<0.05$) in ALT concentration of G1(11.55 ±0.081)pg/ml compared with the GC (10.37 ±0.051)pg/ml, also showed that there was a significant increase ($p<0.05$) in ALT concentration of G2 (12.71 ±0.116)pg/ml compared with the GC (10.37 ±0.051)pg/ml. As in (Fig. 1).

The G3 saccharin concentration (750mg/kg/day) showed significant differences ($p<0.05$) with GC their values (21.67 ±0.129, 10.37 ±0.051)pg/ml respectively. Moreover, by comparing between treatment groups the results showed that there is significant increase ($p<0.05$) in ALT concentration in G3 (21.67 ±0.129) as compared with G1 and G2 their values are (11.55 969971, 12.71 ±0.116)pg/ml respectively.

In addition that the treatment G2 received saccharin at concentration (500mg/kg/day). Compared with G1 the result showed there was significant increase in G2 ($p<0.05$) their values (G2:12.71 ±0.116, G2: 12.71 ±0.116)pg/ml. As in (Fig. 2).

Previous study explained there was a highly significant increase in ALT enzymes after using saccharin (AL Kafafy 2015). Osfor& Elias (2003) reported that saccharin treated rats showed a significant increase in ALT activity after both 6 and 12 weeks of administration., where chronic saccharin intake reflects some abnormal changes in metabolic, hormonal and neural responses in males and female rats (Andreji *et al.,* 2013). The elevation in serum aminotransferase activities could be due to severe effects caused by free radicals that interact with cellular membranes or related to breakdown of liver parenchyma. The changes in liver function could be due to hepatocellular impairment which subsequently caused leakage and release of greater than normal levels of intracellular enzymes into the blood (Alsoufi *et al.,* 2017).

Low and high dose of Saccharin exhibited a significant increase in serum ALT activity when compared with control rats (Amin and AlMuzafar, 2015) the changes in liver function attributed to hepatocellular impairment which subsequently caused leakage and the release of greater than normal levels of intracellular enzymes into the blood. Elevation in the activities of aminotransferases indicated an early diagnosis of hepatotoxicity and considers as tissue damage biomarkers (Amin and AlMuzafar, 2015).

2. Effect of saccharin on Aspartate Transaminase (AST)

The result of this study showed that there was a significant increase ($p<0.05$) in AST concentration of G1(16.37 ±0.122)pg/ml compared with the GC (15.41 ±0.064)pg/ml, also showed that there was a significant increase ($p<0.05$) in AST concentration of G2 (17.99 ±0.151) pg/ml compared with the GC (15.41 ±0.064)pg/ml. As in (Fig. 2).

On other hand, there was significant inecrease ($p<0.05$) in AST concentration of the G3 (27.29 ±0.209)pg/ml compared with the GC (15.41 ±0.064)pg/

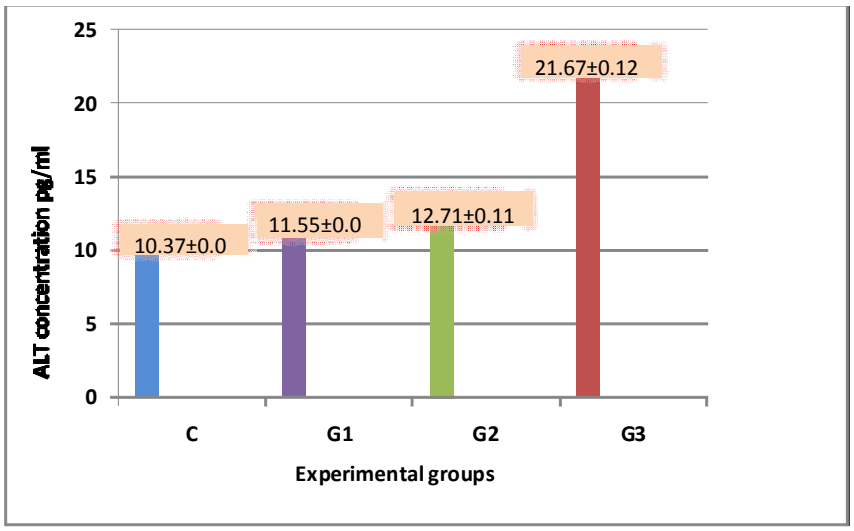

Fig. 1: Effect of different concentration of saccharin on ALT.

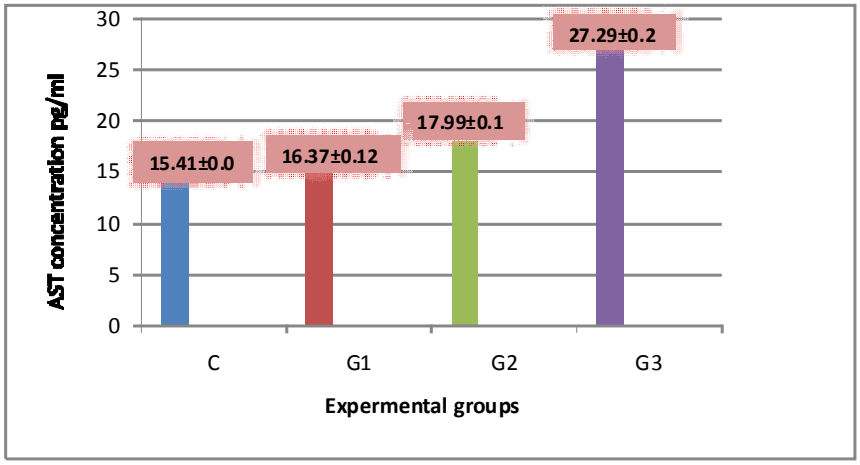

Fig. 2: Effect of different concentration of saccharin on AST.

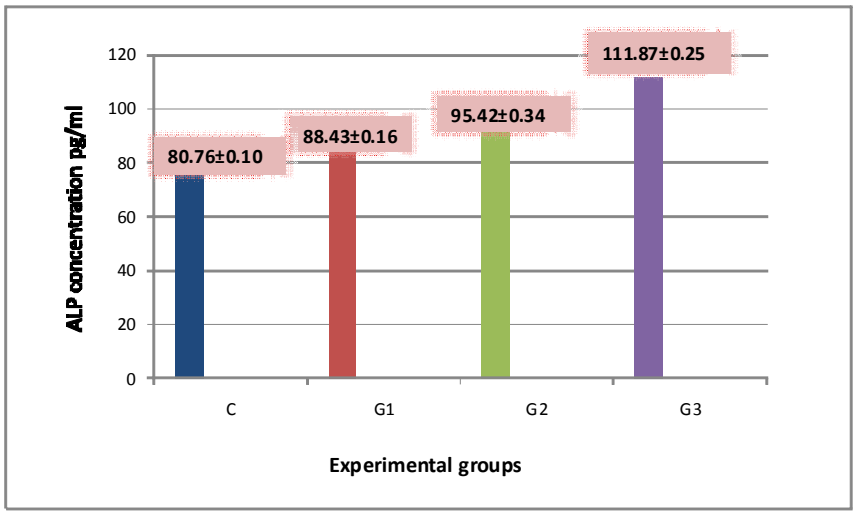

Fig. 3: Effect of different concentration of saccharin on ALP.

ml. A comparison between treatment groups of this study the results showed that there was a significant increase ($p<0.05$) in AST concentration of the G2 (17.99 ±0.151)pg/ml compared with the G1 (16.37 ±0.122)pg/ml, as well as, there was a significant increase ($p<0.05$) in AST concentration of the G3(27.29 ±0.209)pg/ml compared with the G1 (16.37 ±0.122)pg/ml, and the result showed that there were significant increase ($p<0.05$) in AST concentration in G3 (27.29 ±0.209) pg/ml compared with G2 (17.99 ±0.151)pg/ml. As in (Fig. 2).

The increase in AST activity significantly higher than normal which may be due to hepatocellular damage. Hepatocellular injury or metabolic disturbances cause alter hepatocellular membrane permeability which results in release of this soluble enzyme. Subsequent to an acute, diffuse injury, the magnitude of ALT increase in the plasma crudely reflects the number of affected hepatocytes. arise of plasma AST activity generally indicate hepatic pathology (AL-Edany 2011).

Azeezand *et al.,* in (2019) showed that the significant increase in the activity of AST serum markers of liver function. This alteration was suggested as a common sign of impaired liver function. The elevation in serum aminotransferase activity could be due to an effect caused by free radical interaction with cellular membranes or could be related to breakdown of liver parenchyma (Muriel,2009). The changes in liver function could be attributed to a hepatocellular impairment. Subsequently, this alteration would cause the release of abnormal levels of intracellular enzymes into the blood.

The elevation in the activity of aminotransferase indicates an early diagnosis of hepatotoxicity and is considered a biomarker of tissue damage (Amin *et al.,* 2016).

3. Effect of saccharin on alkaline phoshatase (ALP)

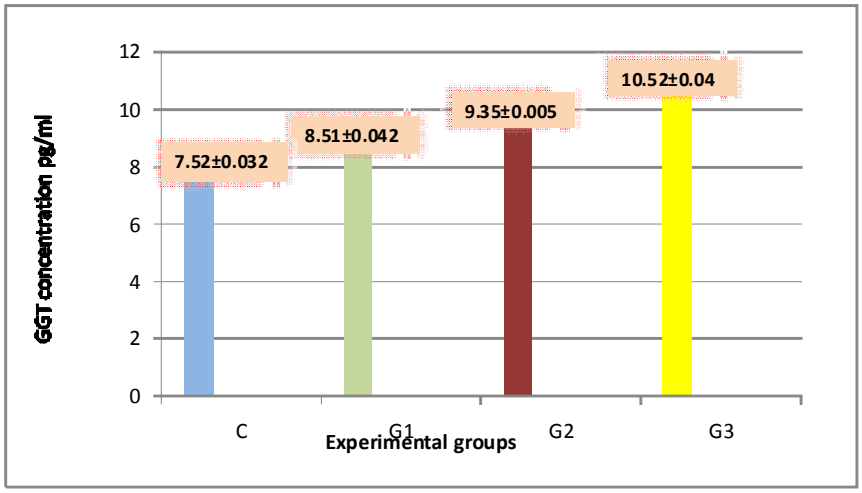

Fig. 4: Effect of different concentration of saccharin on GGT.

Results of the current study showed that there is significant increase ($p<0.05$) of ALP concentration in G1 compared with GC their values (88.43±0.16, 80.76 ±0.10) pg/ml respectively. The results study present in G2 (95.42 ±0.34)pg/ml saccharin concentration (500mg/kg/day) reach significant increased ($p<0.05$) of ALP concentration compared with GC their values (80.76 ±0.10)pg/ml. Besides that, the result showed that there is high significant increased ($p<0.05$) of ALP concentration in G3, when compared G3 saccharin concentration (750mg/kg/day) and GC their values (111.87 ±0.25, 80.76 ±0.10) pg/ml respectively.

Furthermore, when compared between treatment groups the results showed in G2 (95.42 ±0.34) pg/ml that there is significant increase ($p<0.05$) in ALP concentration as compared with G1 (88.43±0.16). The result study show that there is significant increase ($p<0.05$) of ALP concentration in G3 (111.87 ±0.25) when compared with G1 and G2, their values (88.43±0.16, 95.42 ±0.34±0.25) pg/ml respectively. As in (Fig. 3).

Amin *et al* in 2016 showed that the elevation in serum alkaline phosphatase (ALP) in low and high saccharin may be an evidence of obstructive damage in the liver tissue due to saccharin. The liver cells play an important role in both synthesis and secretion of ALP into the bile. Therefore, the alterations in ALP activity caused by saccharin may be attributed to early cholestatic liver damage which primarily affects the liver parenchyma, thus making ALP a sensitive index in the diagnosis of infiltrative diseases (Abdallah, 2002).

Azeez and *et al.,* in 2019 reported that a low dose of 10 mg/kg.b.w. and a high dose of 500 mg/kg.b.w. of saccharin exhibited a significant increase in the activity of ALT, AST, ALP, and serum markers of liver functions. This alteration was suggested as a common sign of impaired liver functions. The elevation in serum aminotransferase activity could be due to an effect caused by free radical interaction with cellular membranes or could be related to breakdown of liver parenchyma (Muriel, 2009). The changes in liver function could be attributed to a hepatocellular impairment. Subsequently, this alteration would cause the release of abnormal levels of intracellular enzymes into the blood. The elevation in the activity of aminotransferase indicates an early diagnosis of hepatotoxicity and is considered a biomarker of tissue damage (Amin *et al.,* in 2016).

4. Effect of saccharin on Gamma-Glutamyl Transferase (GGT)

Results of current study show that there was a significant increase ($p<0.05$) of GGT concentration of G1 (8.51 ±0.042) pg/ml compared with GC that found their value (7.52 ±0.032)pg/ml. The G2 saccharin concentration (500mg/kg /day) compared with GC the result showed significant increase ($p<0.05$) of GGT concentration of G2, their values (9.35 ±0.005, 7.52 ±0.032) pg/ml respectively, also the results G3 saccharin concentration (750mg/kg/day) showed there is a significant increase ($p<0.05$) compared with control group their values (10.52 ±0.04, 7.52 ±0.032) pg/ml respectively. As in (Fig. 4-4).

And when compared between treatment groups the results showed that there is significant increase ($p<0.05$) in GGT concentration of G3 (10.52 ±0.04) pg/ml compared with G1 (8.51 ±0.042) pg/ml and G2 (9.35 ±0.005)pg/ml. Besides that, the G2 saccharin concentration (500mg/kg/day) has been significant increase ($p<0.05$) compared with G1 their values (9.35 ±0.005, 8.51 ±0.042) pg/ml respectively. As in (Fig. 4).

Saccharin caused hepatotoxicity as clearly indicated by the significant increase in levels of ALT, AST and GGT. Serum levels of transaminases is generally considered as sensitive markers of liver function and their concentrations are increased in the serum because of their cytoplasmic nature and are thus released into blood due to changes in the permeability of hepatocyte membranes (Ruokonen and Järvinen 2014).

Increased serum GGT activity has long been used in clinical practice as a marker of liver dysfunction (Ruokonen and Järvinen 2014).

Conclusion

Saccharin had harmful effects on liver by increasing effect on liver enzymes: Alanine aminotransferase (ALT), Aspartate Transaminase (AST), alkaline phoshatase (ALP) and Gamma-Glutamyl Transferase (GGT).

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