

Artificial Sweeteners Perturbed Liver Enzymes in Rat Model

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ABSTRACT

In the recent time there has been an increased demand of low-fat or low calorie diet universally. In the mean while the availability of low calorie products has also increased like sugar free drinks, beverages, biscuits, jams and jellies. On contrary to this, some studies suggest that the prolong use of non-nutritive sweeteners alters the homeostasis of glucose and insulin. It results in fluctuation of glucose level in blood and increase in bodyweight. This study intends to evaluate the effect of non-nutritive sweeteners on the liver function test and record the alteration in the levels of ALP, AST and ALT. Seventy rats were divided into seven equal groups, controlled group received distilled water and the rest six were given NNS Sucrose, Stevia, Sucralose, Saccharine Aspartame and Acesulfame-k, respectively. On the evaluation of Alanine aminotransferase ALT, saccharine and aspartame markedly increased the level of ALT from 40U/L to 80 U/L. Both of these NNS have shown the most raised level of Alanine aminotransferase. This represents the stress on the liver associated with the use of NNS and suggests the use to be controlled in humans.

Key words: Artificial Sweeteners, Liver Enzyme, Bilirubin, Albumin.

INTRODUCTION

Obesity is defined as the accumulation of excess fats in the body; it has been the one of the major causes of premature mortality and increased morbidity.¹ It results into diseases like diabetes, hypertension, cardiovascular diseases, stroke. In the recent time there has been an increased demand of low-fat or low calorie diet universally. In the mean while the availability of low calorie products has also increased like sugar free drinks, beverages, biscuits, jams, and jellies. These products consume the alternative to sugar such as sucralose, cyclamate, saccharin, and aspartame all of which are commercially available in the market.² All of the following sweeteners are frequently being used which have the sweet taste resembling sugar but they have less or no additional calories. Also, they do not stimulate the appetite, resulting in to no increase in calorie intake and degrading weight gain.

Some evident benefits of Non-nutritive sweeteners include the possibility of consuming less sugar which further helps in managing glycaemia, blood cholesterol and obesity.³ Supported by the evidence, a meta-analysis on multiple randomized control trials in the impact of nonnutritive sweeteners on the body weight stated that on the comparison of sucrose vs. the NNS, a significant difference in BMI was recorded. data suggested that in obese/overweight adults, the results of NNS were favorable and the replacement of sugar with NNS contributes to reducing weight.⁴ Another study evaluated the blood sugar level of adults who have been using NNS considering their weight, age and other diseases, if any. It concluded that the intake of artificial sweeteners does not elevate the blood glucose level.⁵

On contrary to this, some studies suggest that the prolong use of non-nutritive sweeteners alters the homeostasis of glucose and insulin.⁶ It results in

fluctuation of glucose level in blood and increase in bodyweight. Other reported adverse effects regarding the use of artificial sweeteners includes the metabolic syndrome,⁷ alteration of neuroendocrine system.⁸ This study intends to evaluate the effect of non-nutritive sweeteners on the liver function test and record the alteration in the levels of ALP, AST and ALT.

MATERIAL AND METHODOLOGY

Animals

Albino rats of 3 to 4 weeks were used as animals in the study. Each rat weighted in the range of 250 to 350 grams and were kept under the controlled environment at the set temperature of 20 to 24°C with 12 hours' light and 12 hours' dark cycle. Food and water was supplied to the rats as per need. All animals were kept under the protocols of animal's ethics committee.⁹⁻¹¹

Chemicals

In order to measure the serum albumin (g/dl), Total bilirubin concentration (mg/dl), levels of ALP, AST and ALT we have used the colorimetric assay kits from Biolabo (France). The experimental group received the following NNS Sucrose, Stevia, Sucralose, Saccharine Aspartame and Acesulfame-k.

Experimental design

Seventy rats were divided into seven equal groups, controlled group received distilled water and the rest six were given Sucrose 10% solution, Stevia 200mg/kg/day, Sucralose 3g/kg/day, Saccharine, Aspartame 250mg/kg/day and Acesulfame-k 250mg/kg/day respectively. Normal saline was used as a placebo in the controlled group.

Collection of sample

For the lab analysis the sample of blood was taken under the ether anesthesia, through the capillary

tubes from the retro orbital sinus of the rats. It was set for 1 hour to clot at the average room temperature and further centrifuged at 3000 rpm for almost fifteen to twenty minutes. The serum was separately analyzed from each rat to examine ALT, AST, ALP, Urea and Creatinine.

Statistical analysis

All the result was presented in bar graphs with respect to each group individually. The differences were studied using one-way analysis of variance (ANOVA) and Fisher test (Stat View). All the values were statistically significant with $p \leq 0.05$.

RESULTS

On the evaluation of Alanine aminotransferase ALT, the results of controlled group remained constant at 40 U/L. in the sucrose group, the level of ALT raised slightly from 40 U/L to 45 U/L. this increase in the level is too less to be considered. In the third group stevia was administered, on the results we observed marked decrease of 10 U/L i.e. from 40 U/L to 30 U/L. Sucralose increased the level from 40 U/L to 50 U/L while saccharine and aspartame markedly increased the level of ALT from 40U/L to 80 U/L. Both of these NNS have shown the most raised level of Alanine aminotransferase. Lastly, Acesulfame showed an inconsiderable increase from 40 U/L to 45 U/L (Figure 1).

In Case of (AST) aspartate transaminase, the controlled group values remained constant at 120 U/L as seen previously. Simple sucrose slightly increased the level for 120 U/L to 130 U/L. in Case of the stevia group the level of ALT remained maintained at 120 U/L. Sucralose increased the most i.e. from 120 U/L to 150 U/L. in case of saccharine and Acesulfame the levels before and after remained almost the same while in Aspartame the level of ALT decreased from 120 U/L to 80 U/L (Figure 1).

On the analysis of alkaline phosphate ALP, the level of controlled group and stevia group remained constant at 110 U/L. Sucrose and Aspartame slightly increased the level from 110 U/L to 140 U/L and 150 U/L, respectively. While saccharine and sucralose increased the level of ALP from 110 to 150 U/L (Figure1).

While assessing the concentration of bilirubin in all the seven groups we have found the similar results. Almost all the groups kept the level of bilirubin constant, however saccharine and Aspartame slightly decreased it. The recorded value of bilirubin was 0.7 mg/dl (Figure 2).

Similarly, the level of plasma albumin remained the same in all the groups at 4g/dl. However, only Acesulfame-k markedly decreased the value from 4 to 3 g/dl (Figure 3).

DISCUSSION

Liver enzymes are measured through liver function test. It is often evaluated to check and monitor the function of liver and its health. The most common liver enzymes also known as liner panel includes Aspartate transaminase (AST), alkaline phosphatase (ALP), Alanine transaminase (ALT) and Gamma-glutamyl transferase (GGT).¹² If the level of these enzymes are raised it could either be temporary due to the impact of any medication or alcohol etc. However, prolong and marked elevation shows the insult to the liver cells. It is a common indication for the diseases like hepatitis. Bilirubin is the byproduct of the reactions which occur in the liver. If the levels of bilirubin are increased, it shows the injury or the stress to the liver or the duct.

Various studies have been conducted regarding the use of non-nutritive sweetener and its relation with the function of liver. In a study which held a review on the evidence about the clinical and experimental outcomes of the NNS impact on the liver, it has highlighted the potential

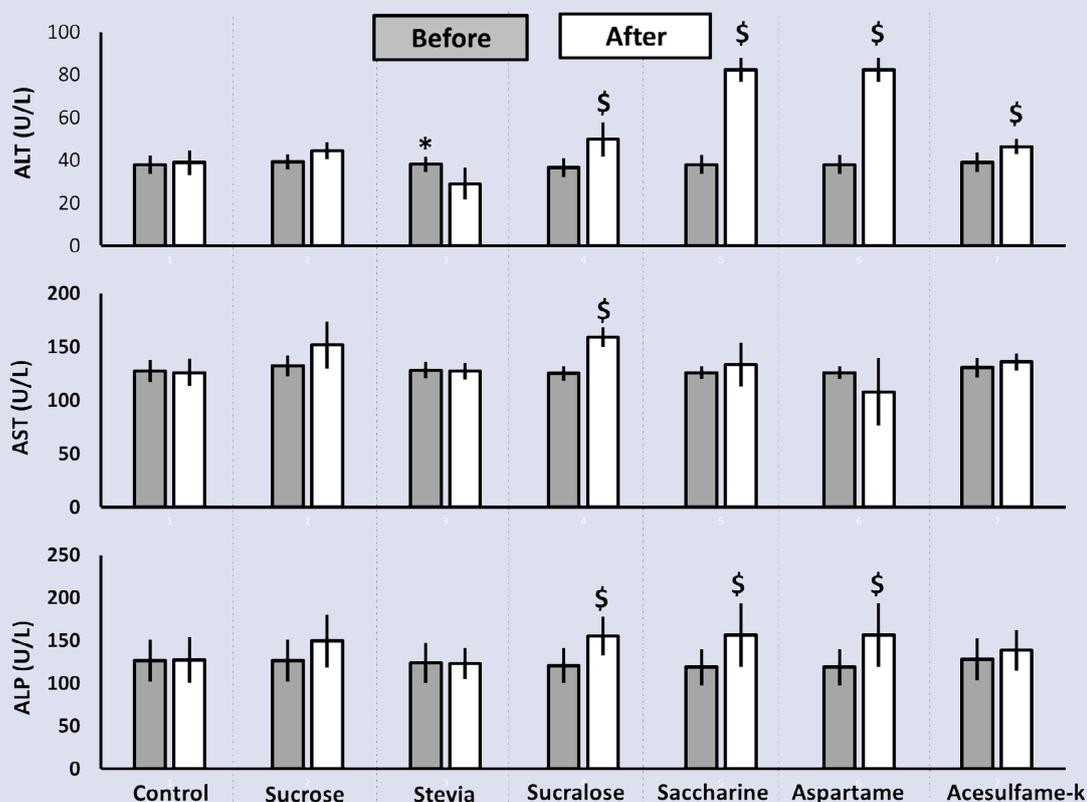


Figure 1: Liver function test (U/L) of plasma of experimental rats in agent-exposed group as compared to negative and positive. The data expressed as mean±SD, * <0.05 (before compared to after), \$ <0.05 (after compared to before). ALT=Alanine aminotransferase, AST=Aspartate transaminase, ALP=Alkaline Phosphatase.

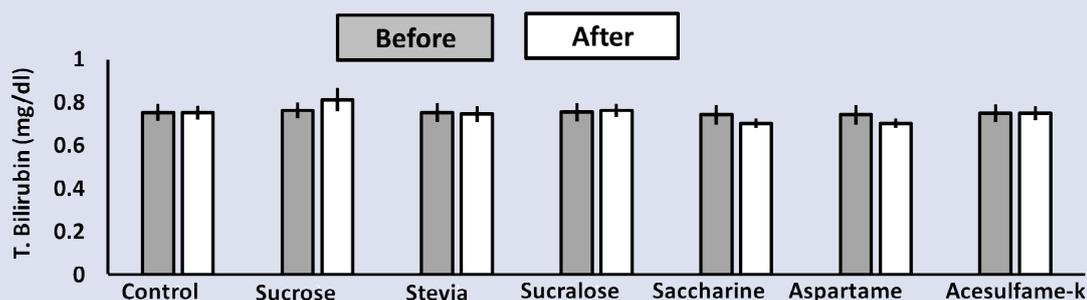


Figure 2: Total bilirubin concentration (mg/dl) in plasma of experimental rats in agent-exposed group as compared to negative and positive. The data expressed as mean±SD.

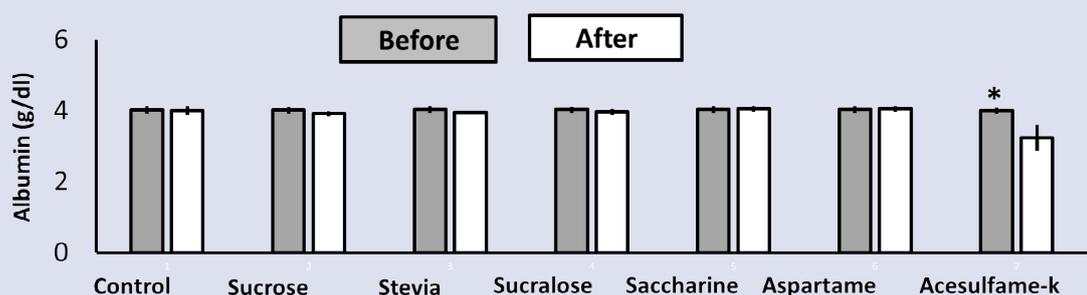


Figure 3: Plasma albumin (g/dl) of plasma of experimental rats in agent-exposed group as compared to negative and positive. The data expressed as mean±SD, * <0.05 (before compared to after).

of NSS causing alteration in metabolism and resulting in to metabolic syndrome and non-alcoholic fatty liver.¹³ Some of the other adverse effects were also stated which included, an increase in the appetite leading to increased consumption of calories. A major adverse effect which has been stated is the intolerance towards glucose, a number of studies have supported this point.¹⁴⁻¹⁶ It has also been associated with the increase in weight, however the results from the Randomized control trials have been conflicting the findings.

Our study has shown that the major increase in Alanine aminotransferase (ALT) was seen in the saccharine and aspartame. On a study which was conducted on male rats to evaluate the liver enzymes at different dosage of saccharine has given the following results.¹⁷ The rats were divided into four groups, one controlled group with the administration of distilled water. The rest three groups, G1, G2 and G3, received saccharine at different doses of 250, 500 and 750 mg/kg/ per day. After the experimental duration of 90 days, the results showed that there was an increase in Alanine aminotransferase, aspartate transaminase, Gamma-glutamyl transferase and alkaline phosphate in all the three groups as compared to the controlled group.

Another study evaluated the impact of long term use of saccharine, in the three made groups the dose of saccharine administered was 2.5 mg/kg, 5 mg/kg, and 10 mg/kg respectively.¹⁸ Over a period of 120 days the results demonstrated oxidative stress and hepatotoxicity. The study concluded with the statement that saccharine is harmful and should not be used in diet.

Various other studies have also highlighted the similar results and stated saccharine as hepatotoxic substance which is not safe for the use.^{19,20} The increased level of Alanine aminotransferase in the aspartame group raises the same questions on the hepatotoxicity of the sweetener. The studies regarding this states as follows. On the evaluation of the long term use of aspartame in the diet to reduce the sugar intake and avoid

gaining weight, serious adverse effects have been seen. In the trial which was held on the three groups of mice evaluated its long term effect. Aspartame was administered orally at a dose of 80 mg/kg. after 12 weeks, liver fibrosis, elevated liver enzymes and reduced enzyme antioxidant activity was proven.²¹ Many other researches²²⁻²⁴ have highlighted and supported our results regarding the hepatotoxicity, nephrotoxicity and oxidative stress on liver caused by aspartame. Liver protection trial has been commenced against drug deleterious effects using herbal remedies,²⁵ propolis,²⁶ vitamins,^{27,28} and zinc as a mineral.²⁹

CONCLUSION

The following results and support from all the relevant studies suggest that the use Saccharine and aspartame as NNS in diet must be avoided until more authentic work is done to prove the hepatotoxic effects wrong.

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ADHERENCE TO ETHICAL STANDARDS

The study was approved and registered in College of Medicine, University of Mosul.

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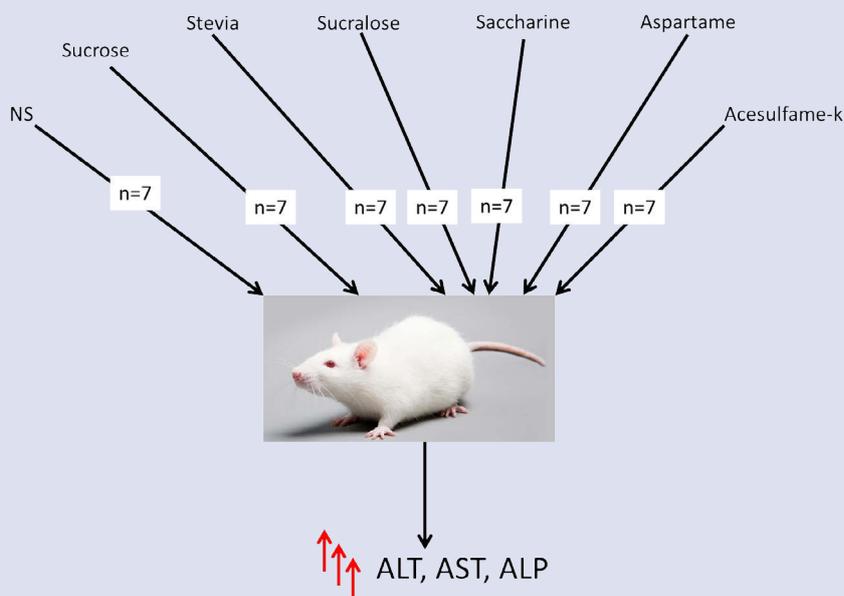
CONFLICTS OF INTEREST

The authors declare no conflicts of interest concerned in the present study.

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GRAPHICAL ABSTRACT



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