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Artificial Sweeteners Connoted Vitiation of Rat Metabolic Biomarkers

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Abstract

The consumption of non-nutritive sweeteners (NNS) has markedly enlarged in recent years universally, due to multiple factors including the increased prevalence of obesity and hyperglycemia. Studies have inconclusive results about NNS use, some evidence states that it contributes to weight gain and an increase in appetite, however, others say that it plays a significant role in reducing weight and controlling diabetes. In this study we aim to examine the effects of multiple non-nutritive sweeteners on body weight, fasting blood sugar, and total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) using an in vivo rat model. In the results, it was found that stevia reduced the weight of the rat by 50 grams after eight weeks and in the rest of the groups, the weight remained almost the same except for sucrose followed by acesulfame-k which represented a marked increase in weight. There was an overall increase in total cholesterol and LDL and a reduction in HDL in all groups. Stevia, aspartame, and saccharin were found to be most beneficial with respect to reducing weight and controlling the level of fasting blood sugar. Additionally, the results from multiple studies have contradicting conclusions which highlight the uncertainty in the effects of NSS.

Keywords: Artificial sweeteners, Lipid profile, Fasting blood sugar, Metabolism.

Introduction

The prevalence of hyperglycemia and obesity have greatly necessitated the use of non-nutritive sweeteners (NNS) (Mbambo *et al.*, 2020). As recommended by the diabetic association of America, the use of NNS must be encouraged in order to promote a healthier diet (American dietetic association, 2004). Overweight and diabetes are the risk factors for hyperlipidemia, cardiovascular diseases, and metabolic syndrome (Althanoon and Merkhan, 2021, Almukhtar *et al.*, 2021, Merkhan *et al.*, 2021), therefore, NNS is often taken by the overweight population to reduce their calorie intake in the form of refined sugar, while diabetic patients use it to control blood sugar. Nonnutritive sweeteners are biologically inactive compounds and their chemical composition is completely different from that of carbohydrates (Skokan *et al.*, 2007). Sugar sweeteners are used in many synthetic products including beverages, confectioneries, bakery items, energy drinks, etc. Some of the most commonly used NNS are sucralose, aspartame, cyclamate, saccharin, and acesulfame-k.

Studies have inconclusive results about NNS use, some evidence states that it contributes to weight gain and an increase in appetite, however, others say that it plays a significant role in the reduction of weight and control of diabetes (Feijó *et al.*, 2013). Despite the mixed perceptions and evidence towards these products, the use of NNS is also associated with several adverse effects like increasing body weight, impairment of insulin and glucose metabolism, kidney injury, neurogenic disorders, and also cancers (Alsunni, 2020).

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The long-term use of non-nutritive sweeteners has reportedly altered the glucose homeostasis by an understood mechanism that degrades the reward response from the central nervous system on the intake of sugar and the alteration of secretion of insulin and increment induces the disturbances (Ramos-García *et al.*, 2021).

Different sweeteners have been produced with varying chemical compositions sucralose possesses a similar structure as that of carbohydrates and is conveniently used because of its stability under different temperatures and pH (Brown *et al.*, 2011), while stevia contains sweetness as well as it holds very beneficial antioxidant effects too (Šic Žlabur *et al.*, 2018) Additionally, saccharin is a highly polar compound. Its absorption in the gut is quite slow and it is rapidly excreted through urination (Azeez *et al.*, 2019).

In this study we aim to examine the effects of multiple non-nutritive sweeteners including Sucrose, Stevia, Sucralose, Saccharin, Aspartame, and Acesulfame-k on the body weight, fasting blood sugar, and total cholesterol, triglycerides, HDL, and LDL.

Methodology

Animals: Albino rats of 3 to 4 weeks were used in the study (7 rats for each group; n=49 in total), they were assigned into 7 groups by physical randomisation method. Each rat weighed in the range of 250 to 350 grams and was kept under a controlled environment at the set temperature of 22 ± 2 ◦C with 12 hours light and 12 hours dark cycle. Food and water were provided to the rats as per need. All animals were kept under the protocols of the animal's ethics committee.

Biochemical Assays

Chemicals: In order to measure the glucose concentration, low-density lipoprotein (LDL), high-density lipoproteins (HDL), total cholesterol (TC), total proteins (TP), triglycerides (TG), and fasting blood sugar (FBS) we have used the colourimetric assay kits from Biolabo (France). Sucrose (refined sugar), Stevia (MicroIngredients, USA), Sucralose (BulkSupplements, USA), Saccharine (Heartland, USA) Aspartame (Heartland, USA), and Acesulfame-k (Nutricost, USA)were purchased locally.

Experimental design: Rats were divided into seven equal groups, the controlled group received distilled water and the rest six were given Sucrose 10% solution, Stevia 200mg/kg/day, Sucralose 3g/kg/day, Saccharine 25mg/kg/day, Aspartame 250mg/kg/day and Acesulfame-k 250mg/kg/day.

Collection of samples: From each group; both controlled and experimental, 10 blood samples were collected 3ml each through the orbital venous plexus. Each sample was taken in a plain tube without anticoagulant and was set to clot at room temperature for 30 to 60 minutes. Sera were isolated by centrifugation at 3000 rpm for around fifteen minutes which was then used to measure the biochemical parameters.

The fasting blood sugar in the serum was calculated by evaluating the amount of red quinoneimine released from the oxidation of glucose substrate. To evaluate the total cholesterol (TC), total proteins (TP), and triglycerides (TG) enzymatic methods were used. The concentration of low-density Lipoproteins was calculated according to the Friedewald formula (Friedewald *et al.,* 2013),

LDL-C (mg/dl) = TC- (HDL-C+ VLDL-C)

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Statistical analysis: All the results were 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) followed by Kruskal–Wallis to determine the different groups, the results were further confirmed by a series of t-tests to identify the difference between groups. All the values were statistically significant with $p \leq 0.05$.

Results

On the detailed weight analysis in all seven groups after 8 weeks we have found that in the controlled group there was a slight increase in mean weight (grams) from 214 to 256, meanwhile, sucrose markedly increased the weight by 130grams in eight weeks followed by Acesulfame-k which marginally increased the weight. We only found the weight reduction effect from stevia, which reduced the weight of the rat by 27 grams, and in the rest of the groups, the weight remained almost the same (Figure 1A).

As expected, sucrose markedly increased the fasting blood sugar from 98 mg/dl to 120 mg/dl followed by sucralose and Acesulfame-k, both responsible for the slight non-significant increase in blood sugar level. In the case of stevia, aspartame, and saccharin we found promising diabetes-controlling results (Figure 1B).

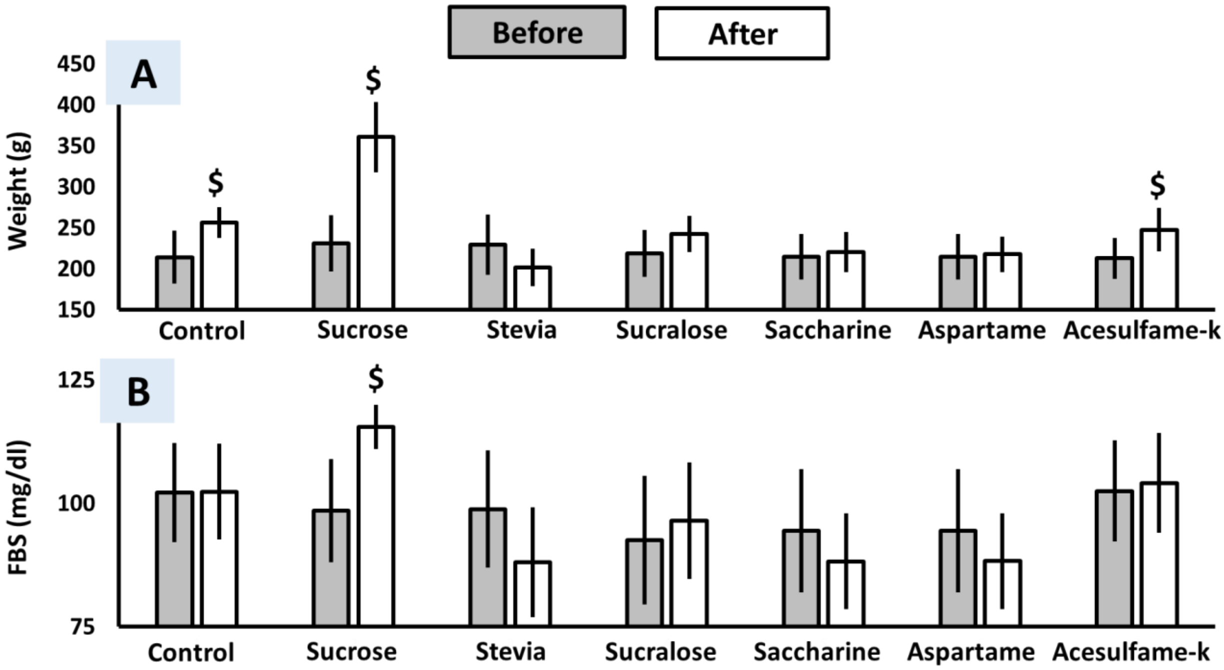

Figure 1. Weight and FBS of experimental rats in the NNS-exposed group as compared to negative and positive. The data expressed as mean±SD, *<0.05 (before compared to after), $<0.05 (after compared to before).

After an eight-week analysis of total cholesterol, each group presented the elevation in cholesterol however it was least in stevia and sucralose while the maximum increase was seen in Acesulfame-k i.e. from 116 mg/dl to 126 mg/dl. HDL level was decreased in almost every group with the most significant reduction by sucrose and acesulfame-k while LDL has evidently increased in all groups nevertheless stevia and sucralose showed a negligible increment of LDL (Figures 2 and 3).

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In the case of triglycerides, sucrose and acesulfame-k represented an obvious increase while in the rest of the groups the elevation was insignificant. Moreover, the level of TG was only reduced in the controlled group (Figure 2). Correspondingly, VLDL was similarly affected in the acesulfame-k group only (Figure3).

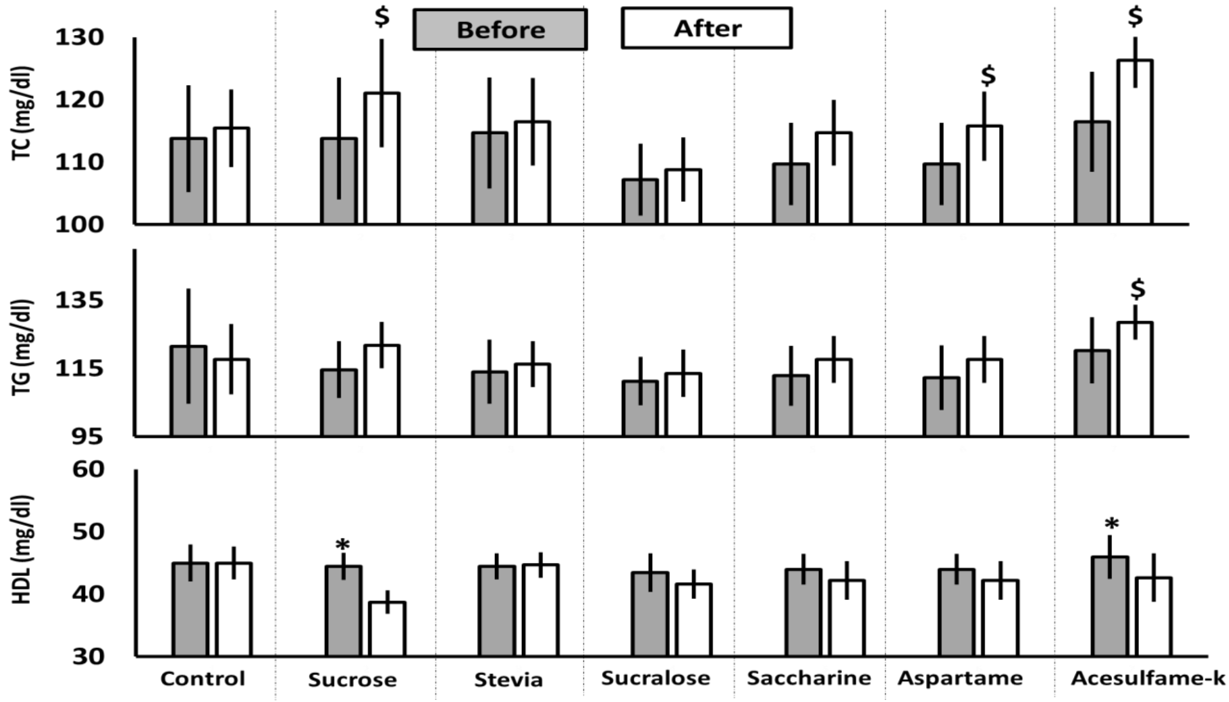

Figure 2. Lipid profile (mg/dl) of plasma of experimental rats in the NNS-exposed group as compared to negative and positive. The data expressed as mean±SD, *<0.05 (before compared to after), $<0.05 (after compared to before)

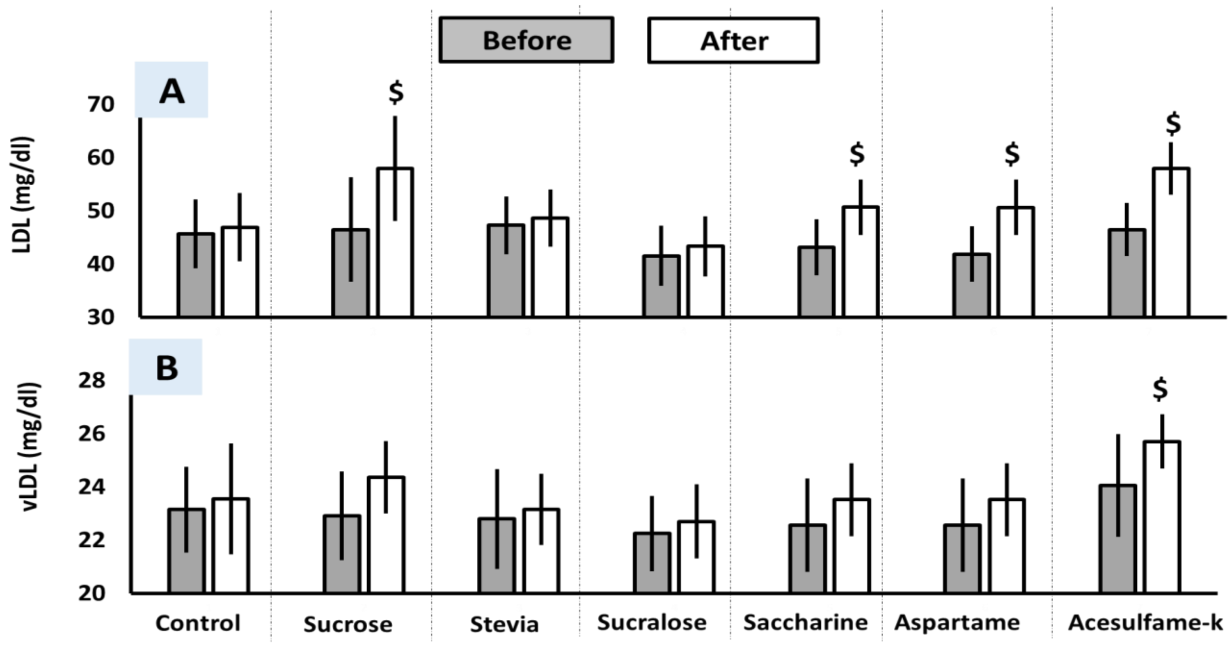

Figure 3. Calculated lipid parameters (mg/dl) of experimental rats in the NNS-exposed group as compared to negative and positive. The data expressed as mean±SD, *<0.05 (before compared to after), $<0.05 (after compared to before)

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Discussions

The use of non-nutritive sweeteners has markedly increased to control or reduce body weight and multiple studies have been done to evaluate these effects (Bian *et al.*, 2017), through our study we found that as compared to the increment in weight in the sucrose group and controlled group all the other groups have shown the maintained weight, meanwhile only stevia caused the weight to reduce. Similarly, a study held to highlight the impact of different sweeteners on body weight demonstrated that there was no increase found in body weight except in that of saccharin i.e. saccharin showed increased body weight in rats (Polyák *et al.*, 2010). These results display the difference in results obtained from the consumption of saccharin, however, the increase is still less as compared to the sucrose group.

NNS has successfully controlled the blood sugar level in comparison to sucrose consumption however the extent of control varies with respect to the sweetener used. A study compared the calculated dose of 2% stevia extracts in one cup of tea with one tablet of sucralose in the same amount of tea. On the fine evaluation of fasting blood sugar level, HbA1, and lipid profile after eight weeks it proved stevia extracts to be a potential alternative to sucralose with no increase in blood sugar level after two hours of intake and maintained HbA1c (Ajami *et al.*, 2020). Our study demonstrated more promising results of stevia in FBS, which was 98 mg/dl reduced to 88 mg/dl while sucralose showed a slight elevation from 92 mg/dl to 96 mg/dl. However, either of them has been beneficial when compared to the use of conventional sugar.

The effect of sweeteners on total cholesterol, HDL, and LDL is still uncertain. Our results present an overall increase in total cholesterol and LDL and controlled or reduced HDL. Following results are opposed by another study which showed the use of saccharine to be beneficial in reducing triglycerides, TC, high-density lipoproteins, and low-density lipoproteins however both high and low doses markedly increase liver enzymes and have hazardous renal and hepatic effects(Amin and Almuzafar, 2015).

Different treatment modalities have been used to control hyperglycemic and lipid parameters, including herbal remedies (Perera and Li, 2012, Modak *et al.*, 2007, Damnjanovic *et al.*, 2015), minerals (Younis *et al.*, 2022), vitamins (Merkhan and Abdullah, 2020), and dietary products (Mirmiran, 2014, Perera and Li, 2011) including artificial sweeteners. In practice, NNS is most commonly used by diabetic patients. Diabetes itself induced derangement of glucose and lipid profile, therefore, we conducted this study to avoid improper use of NNS in the diabetic and general population.

Conclusion

Stevia, aspartame, and saccharin are found to be most beneficial with respect to reducing weight and controlling the level of fasting blood sugar. Triglycerides were increased in all groups while total cholesterol remained controlled. It has been found that the use of NNS is quite beneficial compared to the use of sucrose however the results vary depending on the choice of sweetener, additionally the results from multiple studies have contradicting conclusions which highlights the uncertainty in the effects of NSS.

Conflict of interests: The authors declare no potential conflict of interests.

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