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FULL ARTICLE

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Comparative effects of commonly used commercially available non-nutritive sweeteners on diabetes-related parameters in non-diabetic rats

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Abstract

Studies of non-nutritive sweeteners (NNS) in diabetes models have been limited to their pure forms or NNS-sweetened products. Hence, we conducted a comparative study on the effects of commercial table-top NNS on diabetes-related parameters in non-diabetic rats. Normal animals were fed for 5 weeks with aqueous solutions of aspartame-, sucralose-, stevia-, sodium cyclamate- and saccharin-based commercial NNS at concentrations equivalent to the sweetness of 10% sucrose solution and thereafter food intake, blood glucose, lipid profile, and biochemical parameters were measured. Aspartame adversely affected blood cholesterols, while cyclamate increased food intake and weight gain. Stevia reduced weight gain and exhibited insulinotropic effects. These data in normal rats hypothetically suggest that stevia-based NNS may help in glycemic control and body weight management, while cyclamate- and aspartame-based NNS may increase body weight and risk of cardiovascular diseases. Further clinical studies are, however, required to confirm the results of this study.

Practical applications

The use of NNS is becoming more popular, especially for individuals with diabetes. However, while there are several commercial table-top NNS available in the market, little is known about how they affect most diabetes-related parameters of consumers, as most of the previous studies on NNS have been limited to their pure forms or NNS-sweetened products. Therefore, we comparatively studied the effects of some commercially available table-top forms of the different NNS (aspartame, sucralose, cyclamate, saccharin, and stevia) on diabetes-related parameters in normal rats. These findings in normal rats suggested that some commercially available NNSs like stevia-based NNS may be suitable for glycemic control and body weight management, while cyclamate- and aspartame-based NNS may increase body weight and risk of cardiovascular diseases. However, these finding in normal rats is subject to additional corroborative clinical studies.

Abbreviations: ALP, alkaline phosphatase; ALT, alanine transaminase; ASP, aspartame; AST, aspartate transaminase; AUC, area under the curve; CLM, cyclamate; CON, control; GLP-1, glucagon-like peptide-1; HDL, high-density lipoprotein; LDH, lactate dehydrogenase; LDL, low-density lipoprotein; NFBG, non-fasting blood glucose; NNN, non-nutritive sweeteners; OGTT, oral glucose tolerance test; SAC, saccharin; SCL, sucralose; STV, stevia; SUC, sucrose.

KEYWORDS
blood glucose, commercially available, diabetes, lipid profile, non-nutritive sweeteners, rats, weight gain

1 | INTRODUCTION

The consumption of non-nutritive sweeteners (NNS) is increasing globally, because of their presence in various food products. While overweight and obese individuals consume NNS to reduce their calorie intake from refined sugar, the individuals with diabetes consume these to curtail postprandial hyperglycemia (Brown, de Banate, & Rother, 2010; Burke & Small, 2015). Others consume NNS as a part of NNS-sweetened food products such as beverages, bakery food, confectionaries, sweets and candies, pharmaceuticals, etc. (Malik et al., 2010). The commonly used NNS include saccharin, aspartame, sucralose, acesulfame potassium (Ace-k), cyclamate, and Stevia etc. Most of them are used alone or in combination with other sweeteners.

Despite the controversies surrounding the use of some NNS, due to potential side effects on chronic consumers (Sharma, Amarnath, Thulasimani, & Ramaswamy, 2016), some reviews suggest that consumption of some NNS-sweetened beverages may pose lower risk of obesity than consumption of sugar-sweetened beverages (Periera, 2013) and moderate consumption of NNS may be helpful in the management of body weight and diabetes (Morris et al., 1993). However, according to the reports published in some reviews, the effects of several NNS on endocrine glucose and lipid metabolism, calorie intake, and weight gain remain controversial (Brown & Rother, 2012; Gardner, 2014; Shankar, Ahuja, & Sriram, 2013). Thus, it is still unclear whether the consumption of NNS is helpful in the management of obesity and diabetes and related diseases.

Some studies in healthy individuals and individuals with type 2 diabetes have demonstrated that sucralose does not affect appetite, glucose homeostasis, glucagon-like peptide-1 (GLP-1), and serum insulin (Brown, Brown, Onken, & Beitz, 2011; Ford et al., 2011; Grotz et al., 2003), while a review report and a study in diabetic rat suggest that sucralose consumption alters serum glucose, insulin, GLP-1 and lipid profile (Saada, Mekky, Eldawy, & Abdelaal, 2013; Schiffman & Rother, 2013). Studies conducted in mice suggested that NNS, including saccharine, aspartame, and sucralose induced glucose intolerance by altering gut microbiota (Suez et al., 2014). Furthermore, 3 weeks supplementation of 0.1% saccharin to juvenile rats did not significantly affect food intake and weight gain (Park et al., 2010). However, female rats fed for 35 days with 35 mg/kg BW of saccharin showed a significant reduction in blood glucose, serum triglycerides and serum cholesterol (Abdelaziz & Ashour, 2011), while normal rats supplemented with 0.0005% saccharin in drinking water showed increased blood glucose and body weight but reduced food intake (Andrejić et al., 2013).

According to a review report (Brown & Rother, 2012), major in vitro studies on enteroendocrine or islet cells revealed that NNS can elicit gut hormones, GLP-1, and glucose-dependent insulinotropic peptide. However, in rodents, NNS did not affect gut hormone secretion but accelerated intestinal glucose absorption. In human studies, both gut hormone secretion and intestinal glucose absorption remained unaltered following the consumption of NNS (Brown & Rother, 2012).

Although the reported inconsistent physiological effects (particularly on diabetes-related parameters) of most NNS may be attributed to various factors such as type of experimental model, dose of NNS, route of administration, duration of study and length of intervention, type experimental subjects, etc., it is also worthy to note that studies on most NNS have been limited to their pure forms or NNS-sweetened products, when these NNS are not consumed in their pure forms. In fact, they are usually commercially available as "table-top" NNS and in different processed foods and drinks. The commercially available NNS, contain other ingredients and additives like sodium carboxymethyl cellulose (stabilizer or emulsifier), anticaking agents, dextrose, lactose, phenylalanine (for the aspartame-based sweetener) and flavoring or a combination with Acesulfame K (for the cyclamate and aspartame-based sweeteners). Acesulfame K has been reported to increase food intake, weight gain, blood cholesterol in rats and/or mice (Bian et al., 2017; Cong et al., 2013; Roy, Davidson, & Swithers, 2007), while phenylalanine has been reported to stimulate body fat oxidation in healthy males, when supplemented before exercise (Ueda et al., 2017). These additives or ingredients may influence the physiological effects of commercial NNS. However, the physiological effects (particularly on diabetes-related parameters) of these commercially available NNS remain unknown. Therefore, the present study was conducted to comparatively investigate the effects of commonly consumed commercially available NNS on diabetes-related parameters in normal rats.

2 | MATERIALS AND METHODS

2.1 | Commercial NNS and other chemicals reagents

Aspartame-based, sucralose-based, sodium cyclamate-based, saccharin-based, and stevia-based NNS used in this study, as well as sucrose, were purchased from a South African medicine store in Durban. Sodium chloride (NaCl), potassium hydroxide (KOH), D-glucose, sodium sulphate (Na_2SO_4), phenol, glycogen from Oyster, sulphuric acid (H_2SO_4), formalin, and ethanol were purchased from Sigma Aldrich, Johannesburg, South Africa. Ultrasensitive rat insulin ELISA kit was purchased from Mercodia, Uppsala, Sweden.

2.2 | Animals

Sprague-Dawley rats (49 days old) were obtained from the Biomedical Resource Unit located at the University of KwaZulu-Natal (Westville Campus), Durban, South Africa. The rats were randomly divided into the following groups: Normal control (CON) as well as normal rats fed with sucrose (SUC), aspartame (ASP), sucralose (SCL), cyclamate (CLM), saccharin (SAC), and stevia (STV) based sweeteners. The rats were kept in medium-sized polycarbonated cages, with two animals per cage. Temperature and humidity were controlled, and the housing unit was maintained at 12 hr light-dark cycle. The rats had ad libitum access to commercial rat chow. Animal maintenance and protocols were in accordance with the ethical guidelines of the Experimental Animal Ethics Committee of the University of KwaZulu-Natal, South Africa (Ethics number 003/13/Animal).

2.3 | Feeding of non-nutritive sweeteners

Rats were acclimatized for 1 week. Thereafter rats belonging to the SUC group had ad libitum access to either 10% sucrose solution, while rats belonging to the ASP, SCL, CLM, SAC, and STV groups had ad libitum access to their respective commercial NNS solutions (dissolved in drinking water) at concentrations equivalent to the sweetness of 10% sucrose. Treatment continued for a 5-weeks experimental period, during which the normal control rats (CON) received normal water ad libitum.

2.4 | Food and fluid intake, body weight, and blood glucose

Food and fluid intake of all animals were measured daily, while body weight change was measured weekly. Weekly "tail-tip" 3 hr fasting blood glucose was measured throughout the experimental period using GlucoPlus Glucometer (Saint-Laurent, Quebec, Canada).

2.5 | Oral glucose tolerance test

In the last week of the experiment, the oral glucose tolerance test (OGTT) was performed. Blood glucose of overnight fasted animals was measured, before orally administering 2 g/kg body weight glucose. Subsequently, blood glucose was measured at 30, 60, 90, and 120 min after the glucose ingestion using a Glucometer. The area under the curve (AUC) of the different animal groups was calculated using the following formula (Sakaguchi et al., 2016):

$$\text{Area under curve(mmol h/L)} = \frac{\{BG(0)\}+\{BG(30)\times 2\}+\{BG(60)\times 3\}+\{BG(120)\times 2\}}{4}$$

where BG(0), BG(30), BG(60), and BG(120) represents blood glucose at 0 (just before glucose administration), 30, 60, and 120 min, respectively after the administration of glucose.

2.6 | Animal euthanasia and blood collection

At the end of 5 weeks experimental period, animals were fasted overnight, euthanized with halothane anesthesia and blood was collected via cardiac puncture in non-heparinized tubes. Serum was obtained from the blood after centrifuging at 3,000 rpm for 15 min and preserved at −30°C for biochemical analysis.

2.7 | Serum biochemical analysis

Urea, uric acid, total protein, albumin, total cholesterol, high-density lipoprotein (HDL) cholesterol, triacylglyceride (TG), creatinine, alanine transaminase (ALT), aspartate transaminase (AST), lactate dehydrogenase (LDH), and alkaline phosphatase (ALP) were measured in the serum using an Automated Chemistry Analyzer (LabMax Plenno, Lagoa Santa, Brazil). Serum low-density lipoprotein (LDL) cholesterol was calculated using the following formula:

$$\text{LDL}-\text{cholesterol} = \left[\text{Total cholesterol}-(\text{HDL}-\text{cholesterol}+\text{TG}/5)\right]$$

where TG/5 is equivalent to very low-density lipoprotein (VLDL) cholesterol.

Serum insulin concentration was measured by an enzyme-linked immunosorbent assay (ELISA) method in a plate reader (Synergy HTX Multi-mode reader, BioTek Instruments Inc, Winooski, USA) using an ultrasensitive rat insulin ELISA kit (Mercodia, Uppsala, Sweden) according to manufacturer's protocols.

2.8 | Statistical analysis

One-way analysis of variance (ANOVA) was used in analyzing the data and presented as mean ± *SD*. Significant differences between means at $p < .05$ were obtained with the Tukey's HSD-multiple range post hoc test. Statistical analyses were performed using IBM Statistical Package for the Social Sciences (SPSS) for Windows, version 18.0 (IBM Corp., Armonk, NY, USA).

3 | RESULTS

3.1 | Effects of NNS on food and fluid intake and body weight

Data are presented in Figure 1. All treatment groups, except ASP and STV groups, showed significantly higher fluid intake ($p < .05$) than

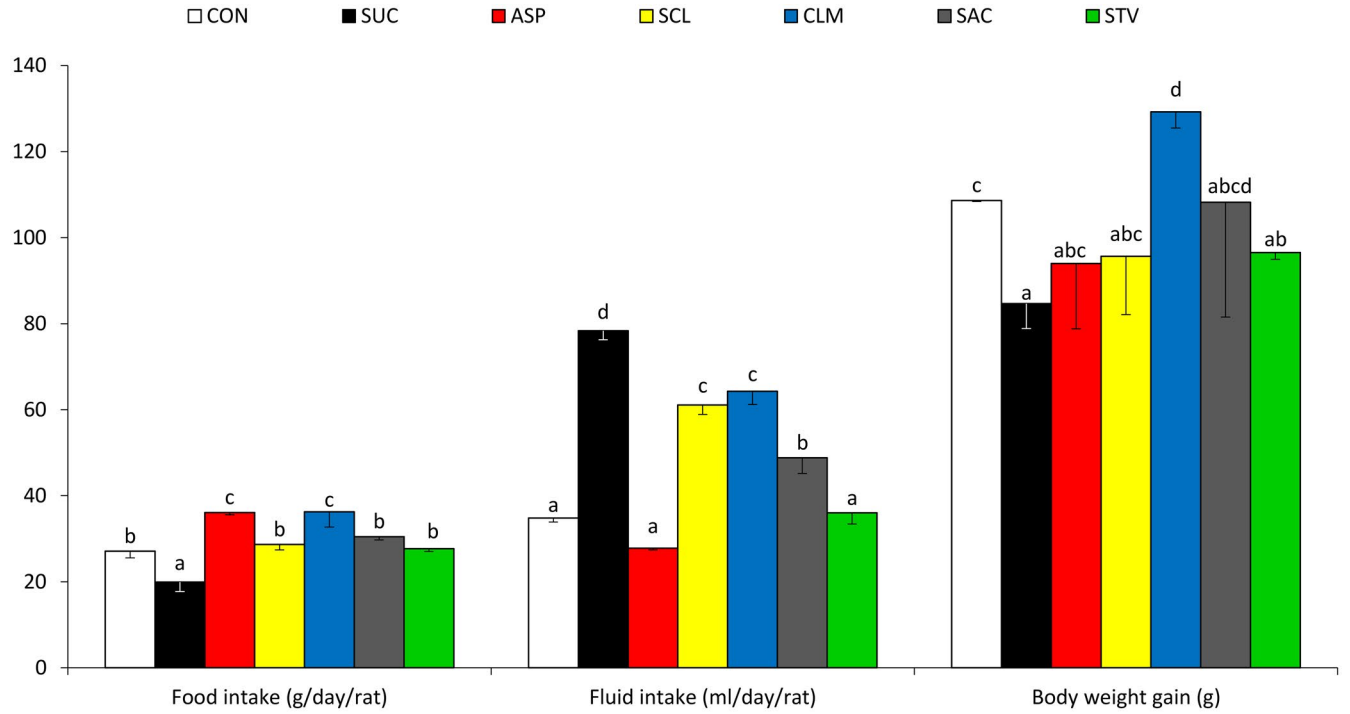

FIGURE 1 Mean food and fluid intake and body weight gain during the 5-week experimental period. Data are presented as mean ± standard deviation. "a–d" data labels indicate significant difference ($p < .05$), when comparing data values. ASP, aspartame; CLM, cyclamate; CON, control; SAC, saccharin; SCL, sucralose; STV, stevia; SUC, sucrose

the CON group, with the SUC group showing the highest ($p < .05$) fluid intake. Fluid intake of ASP and STV groups did not differ significantly from that of the CON group. The food intake of sucrose-fed rats was significantly lower ($p < .05$) than that of control and NNS-fed rats. Aspartame- and cyclamate-based NNS feeding led to significant ($p < .05$) elevation of food intake, while there was no significant change in food intake of rats fed with sucralose-, saccharin-, and stevia-based NNS, relative to the control animals (Figure 1). Sucrose and commercially available NNS, excluding cyclamate- and saccharin-based NNS reduced animal body weight gain, with significant reduction observed in animals fed with sucrose and stevia-based NNS (Figure 1). Saccharin-based NNS hardly influenced animal body weight gain, while treatment with cyclamate-based NNS lead to a significant increase ($p < .05$) in animal weight gain. The score table suggest that cyclamate-based NNS had the most detrimental effect on body weight gain (Table 1).

3.2 | Effects of NNS on glycemic and insulin profile of animals

Neither feeding of sucrose nor commercially available NNS leads to a significant change in weekly NFBG (Figure 2). However, significant differences in oral glucose tolerance were observed between the treatment groups at the 60th and 120th min after glucose ingestion (Figure 3a). SUC and ASP groups consistently showed the higher blood glucose during OGTT, particularly at the 60th and 120th min after glucose ingestion compared to other groups. Blood glucose of the ASP group was significantly higher ($p < .05$) than that of the CON group at the 60th min, while blood glucose of the SUC group was significantly higher ($p < .05$) than that of the SCL group at the 120th min. AUC data showed that sucrose, aspartame-, and cyclamate-based NNS notably exacerbated glucose tolerance in animals, with aspartame-based NNS showing significant ($p < .05$) effect (Figure 3b). Except for stevia-based NNS, which lead to significant ($p < .05$) serum insulin elevation, commercially available NNS and sucrose did not significantly alter serum insulin level after a 5-weeks administration (Figure 4).

3.3 | Effect of NNS on blood lipids

Data are presented in Figure 5. Sucrose and commercially available NNS, excluding aspartame-based NNS, significantly reduced ($p < .05$) serum triglyceride. Aspartame-based NNS feeding led to a slight, but an insignificant elevation of serum triglyceride. Sucrose and commercially available NNS, excluding stevia-based NNS, increased serum total cholesterol, with significant increment shown by sucralose- and cyclamate-based NNS (Figure 5). Aspartame- and stevia-based NNS significantly ($p < .05$) decreased and increased serum HDL-cholesterol, respectively, while sucrose and other commercially available NNS did not show any significant impact on serum HDL-cholesterol. Serum LDL-cholesterol was increased in all the treatment groups, with significant ($p < .05$) increment shown in SUC, ASP, SCL, and CLM groups (Figure 5). The score table suggests that STV had the most beneficial effect on the lipid profile (Table 1).

TABLE 1 Scoring value of sucrose and NNSs for different diabetes-related parameters in normal rats

Diabetes-related parameters		Score value for effects of treatment groups compared to CON					
Category	Assay or measurement	SUC	ASP	SCL	CLM	SAC	STV
Calorie and weight gain	Food intake	+1	−1	0	−1	0	0
	Weight gain	+1	0	0	−1	0	+1
	Net effect score	**+2**	**−1**	**0**	**−2**	**0**	**+1**
Glycemic and insulin profile	NFBG at week 5	0	0	0	0	0	0
	AUC of OGTT	0	0	0	0	0	0
	Serum insulin	0	0	0	0	0	+1
	Net effect score	**0**	**0**	**0**	**0**	**0**	**+1**
Serum lipid profile	Triglyceride	+1	0	+2	+1	+1	+2
	Total cholesterol	0	0	−1	−1	0	0
	HDL-cholesterol	0	−1	0	0	0	+1
	LDL-cholesterol	−1	−2	−2	−2	0	0
	Net effect score	**0**	**−3**	**−1**	**−2**	**+1**	**+3**
Total net effect score		***+2***	***−4***	***−1***	***−4***	***+1***	***+5***

Note: Values 0 to 3 means either no significant difference (0) or first to third level (1 to 3) of significant difference ($p < .05$) of treatment groups compared to the diabetic control groups. Positive (+) and negative (−) score values, respectively, mean that the effect of treatment groups was potentially beneficial (+) or detrimental (−) relative to control or untreated group (CON).

Abbreviations: ASP, Aspartame; AUC, area under the curve; CLM, cyclamate; CON, control; HDL, high density lipo-proteins; LDL, low density lipo-proteins; NFBG, non-fasting blood glucose; NNS, non-nutritive sweeteners; OGTT, oral glucose tolerance test; SAC, saccharin; SCL, sucralose; STV, stevia; SUC, sucrose.

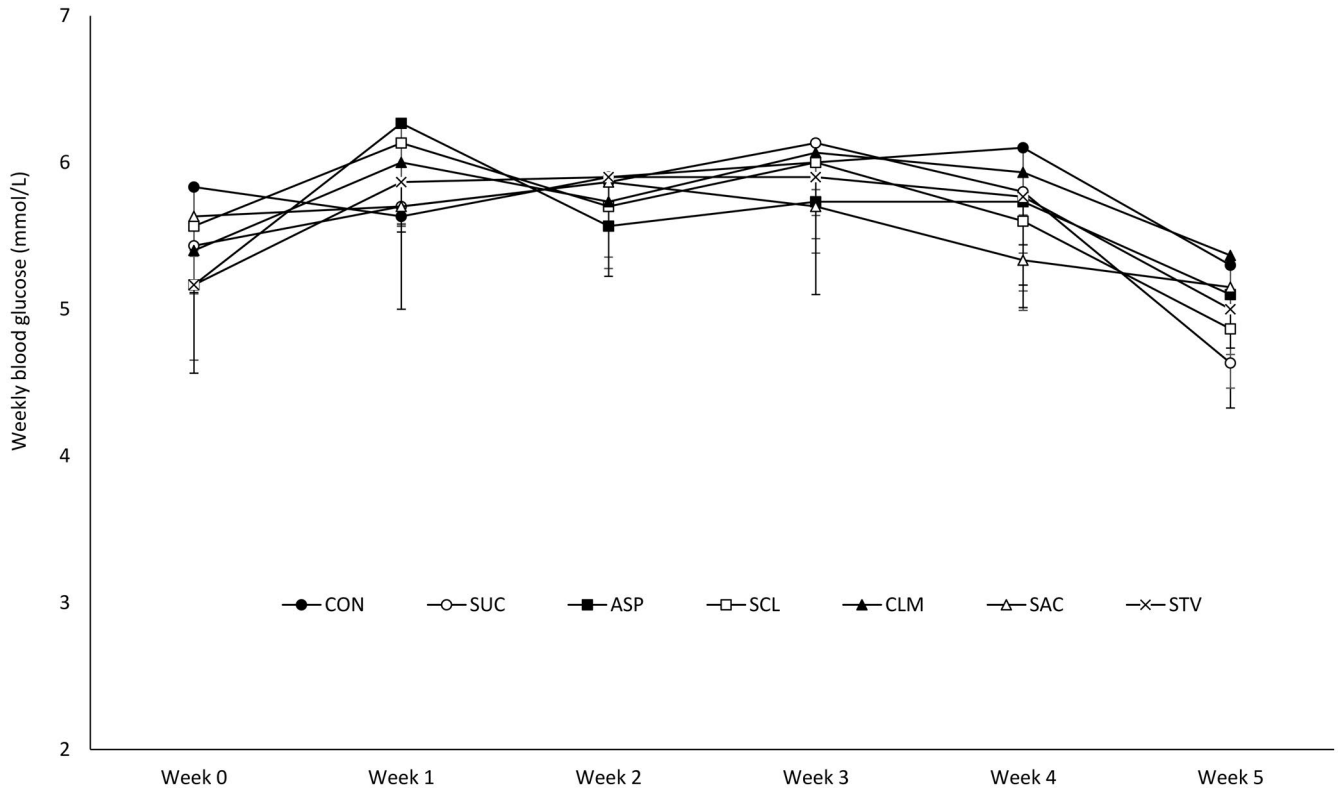

FIGURE 2 Mean weekly 3 hr fasting blood glucose in different animal groups over the 5 weeks experimental period. Data are presented as mean ± standard deviation. ASP, aspartame; CLM, cyclamate; CON, control; SAC, saccharin; SCL, sucralose; STV, stevia; SUC, sucrose

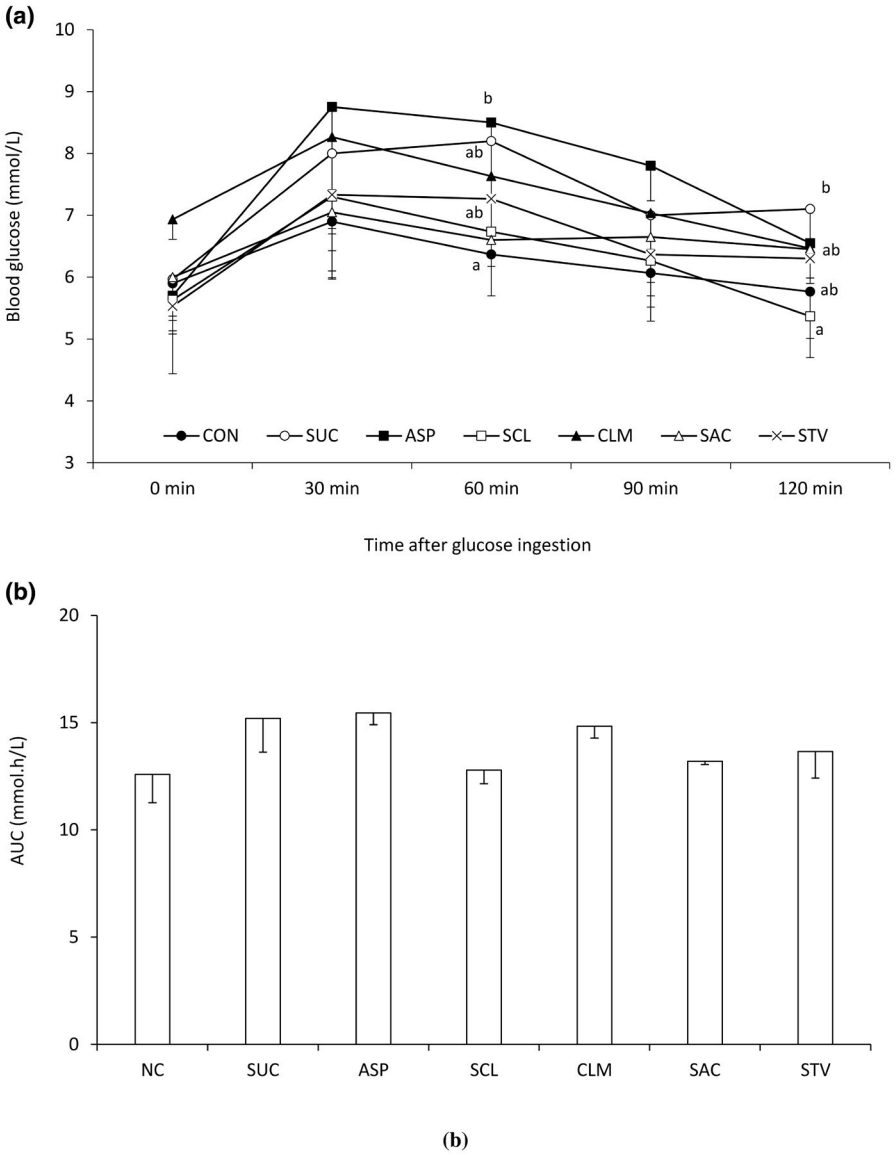

FIGURE 3 (a) Oral glucose tolerance test (OGTT) and (b) respective area under curve (AUC) of different animal groups at the last week of the experimental period. Data are presented as mean ± standard deviation. "a and b" data labels indicate significant difference ($p < .05$), when comparing data values. ASP, aspartame; CLM, cyclamate; CON, control; SAC, saccharin; SCL, sucralose; STV, stevia; SUC, sucrose

3.4 | Effects of NNS on blood biochemical parameters

Data are presented in Table 2. Blood biochemical parameters, which include serum AST, ALT, ALP, urea, uric acid, creatinine, LDH, and albumin were not significantly altered after 5-weeks feeding of sucrose and commercially available NNS.

4 | DISCUSSION

Most NNS are not consumed in their pure forms. They are mostly commercially available as branded products containing other ingredients. However, studies on NNS regarding their glycemic control potentials have mostly been carried out on the pure forms or their sweetened products, but not on the commercially available ones consumed by people. Hence, the present study investigated the effects of commercially available NNS on diabetes-related biochemical parameters in normal rats. Data suggest that different commercially available NNSs possess varying effects, which could influence the use of these sweeteners.

Reducing high caloric food intake like carbohydrates has been one of the recommendations for managing weight gain, obesity, metabolic syndrome, and type 2 diabetes (SEMDSA, 2017). The use of sugar substitutes, such as NNS is increasingly becoming a welcomed approach to curtail detrimental calorie intake (Brown et al., 2011; Brown et al., 2010; Burke & Small, 2015). However, most consumers are still bewildered concerning the effects of NNS on appetite, food intake, and body weight, perhaps due to controversial reports emanating from various studies

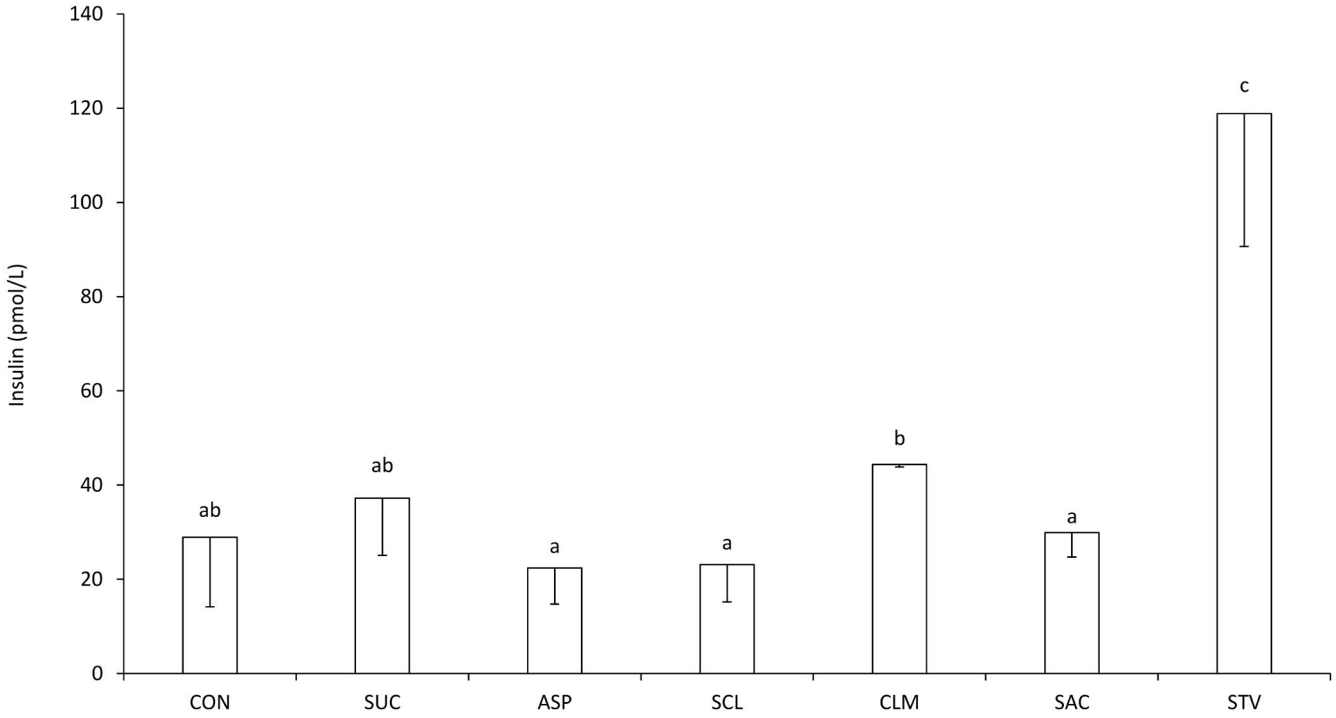

FIGURE 4 Serum insulin concentration of overnight fasted animal at the end of the 5-week experimental period. Data are presented as mean ± standard deviation. "a–c" data labels indicate significant difference ($p < .05$), when comparing data values. ASP, aspartame; CLM, cyclamate; CON, control; SAC, saccharin; SCL, sucralose; STV, stevia; SUC, sucrose

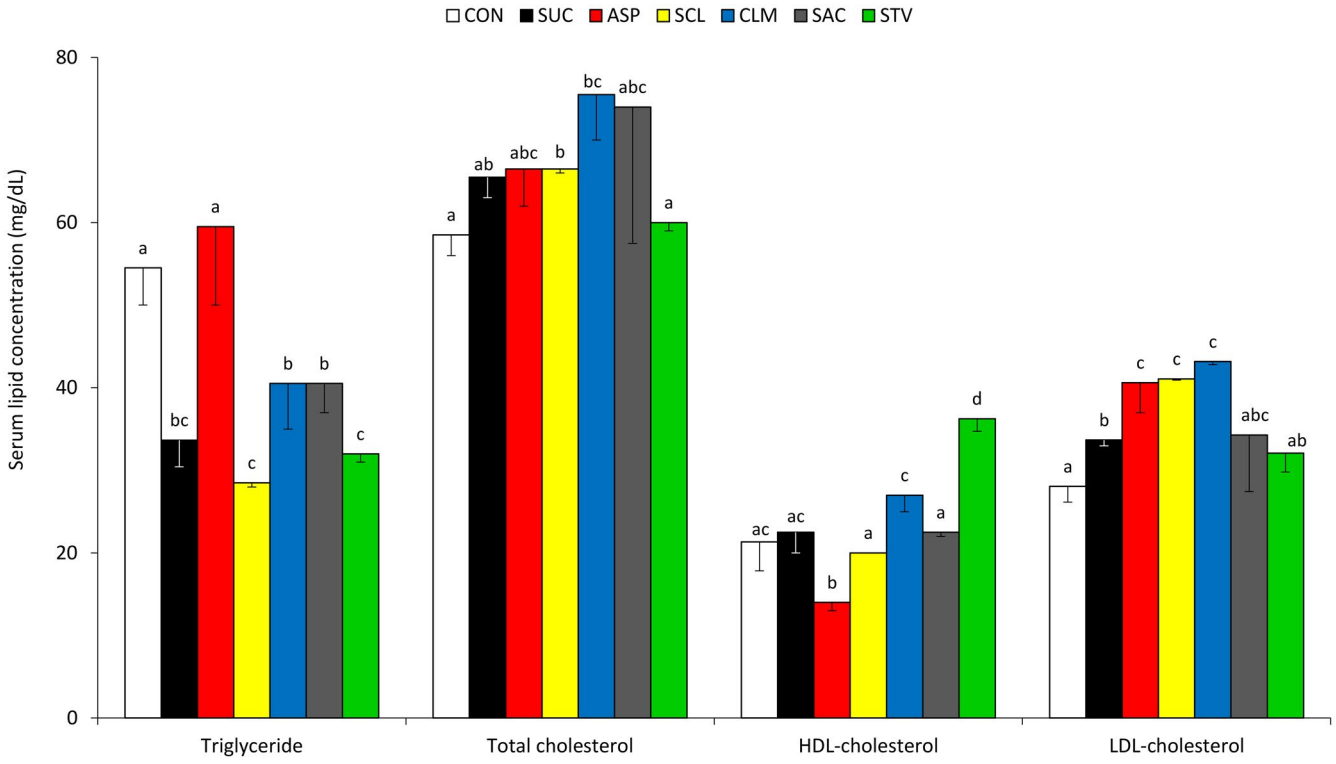

FIGURE 5 Serum lipid profile in the different animal groups at the end of the experimental period. Data are presented as mean ± standard deviation. "a–c" data labels indicate significant difference ($p < .05$), when comparing data values. ASP, aspartame; CLM, cyclamate; CON, control; SAC, saccharin; SCL, sucralose; STV, stevia; SUC, sucrose

TABLE 2 Data for serum biochemical parameters of different animal groups at the end of the 5-week experimental period

	CON	SUC	ASP	SCL	CLM	SAC	STV
AST (U/L)	85.00 ± 2.00	96.00 ± 9.17	80.00 ± 2.00	86.00 ± 3.00	89.00 ± 3.00	82.00 ± 12.00	92.00 ± 2.00
ALT (U/L)	42.50 ± 0.50	45.00 ± 1.00	46.50 ± 1.50	46.67 ± 8.74	45.00 ± 2.65	45.50 ± 11.50	51.00 ± 10.15
ALP (U/L)	25.00 ± 2.00^{a}	29.33 ± 6.66^{a}	22.50 ± 1.50^{ab}	19.33 ± 2.31^{ab}	16.00 ± 3.00^{b}	23.00 ± 1.00^{ab}	17.00 ± 1.00^{b}
Urea (mg/dl)	40.67 ± 2.08^{ab}	31.00 ± 3.46^{a}	47.50 ± 4.50^{b}	49.00 ± 1.00^{b}	44.67 ± 3.51^{ab}	45.50 ± 4.50^{ab}	38.00 ± 12.00^{ab}
Uric acid (mg/dl)	4.54 ± 1.91	3.94 ± 0.95	5.99 ± 0.95	4.38 ± 1.08	4.69 ± 1.82	4.00 ± 1.70	5.45 ± 1.70
Creatinine (mg/dl)	0.35 ± 0.08	0.41 ± 0.04	0.46 ± 0.02	0.44 ± 0.10	0.39 ± 0.04	0.41 ± 0.07	0.41 ± 0.05
LDH (U/L)	226.33 ± 6.51	239.00 ± 28.00	242.50 ± 15.50	364.00 ± 101.00	357.50 ± 5.50	561.00 ± 137.00	265.33 ± 94.73
Albumin (g/dl)	3.21 ± 0.16	3.09 ± 0.08	3.15 ± 0.02	3.14 ± 0.10	3.11 ± 0.33	3.10 ± 0.05	3.22 ± 0.40
Total protein (g/dl)	6.91 ± 0.37	6.88 ± 0.10	7.40 ± 0.10	7.08 ± 0.04	7.41 ± 0.54	6.87 ± 0.04	7.21 ± 0.67

Note: Data are presented as mean ± *SD* . "a-b" data labels indicate signficant difference ($p < .05$), when comparing data values.

ALP, alkaline phosphatase; ALT, alanine transaminase; ASP, aspartame; AST, aspartate transaminase; CLM, cyclamate; CON, control; LDH, lactate dehydrogenase; NNS, non-nutritive sweetener; SAC, saccharin; SCL, sucralose; STV, stevia; SUC, sucrose.

(Purohit & Mishra, 2018; Romo-Romo et al., 2016; Silva, Brasiel, & Luquetti, 2019).

In a previous study, it has been reported that NNS-sweetened (saccharin and aspartame) drinks did not increase hunger or food intake in normal human subjects (Canty & Chan, 1991). However, when compared with sucrose-sweetened diet/beverage in overweight subjects, NNS-sweetened (54% aspartame, 23% cyclamate, 22% acesulfame K, and 1% saccharin) diet/beverage reduced hunger, energy intake, and body weight, while sucrose increased these parameters (Sørensen, Vasilaras, Astrup, & Raben, 2014). Moreover, Rolls (1991) showed that NNS (aspartame, saccharin, and acesulfame-K) can increase hunger or appetite, especially when used as unflavored solutions. However, it is important to note that the appetite-related physiological effects of different NNSs may vary and cannot be generalized (Roll, 1991).

In the present study, animals consumed different commercial-based NNS solutions to varying levels, possibly due to the perceived tastes of the NNS (some may taste more pleasant than others) (Figure 1). It is important to note that our experiment did not control the amount of sweetener consumed by the animal groups, since the sweeteners were supplied ad libitum as drinking solutions. The reason for this was to mimic how these commercial sweeteners are consumed by users. However, this may be considered as a limitation of our study, since it is a comparative study. The fluid intake of sucrose was significantly higher than that of the NNS, which could be due to the more pleasant and clean taste of sucrose compared to the NNS (O'Donnell & Kearsley, 2012). However, food intake did not follow a similar trend. Sucrose significantly reduced food intake, while the NNS either increased food intake (aspartame- and cyclamate-based NNS) or did not significantly alter it (sucralose-, saccharin- and stevia-based NNS) (Figure 1); and perhaps, may contribute to the lower weight gain observed in sucrose-fed animals compared to the NNS-fed animals (Figure 1). This data corroborates with several studies that have reported lower calorie intake and weight gain in caloric sweetener-fed experimental subjects compared to their non-caloric sweetener-fed counterparts (Pinto, Foletto, Dal Lago, Barcos, & Bertoluci, 2015; Rogers & Blundell, 1989; Roy et al., 2007). Additionally, it has been reported that caloric sweeteners, like sugars, stimulate satiety and reduced food intake (Anderson & Woodend, 2003; Islam, 2011).

Comparing among the NNS, varying effects on food intake and weight gain was observed, but in a somewhat consistent pattern (Figure 1). For example, although aspartame- and cyclamate-based NNS significantly increased food intake in normal animals, the animals in the CLM group consistently showed the highest food intake and weight gain among all the NNS groups (Figure 1). The higher food intake in the animals fed with cyclamate- and aspartame-based NNS compared to the other commercial NNS may be partly attributed to the presence of acesulfame K in the cyclamate- and aspartame-based NNS. Studies have reported that acesulfame K consumption in rats and mice increased food intake and weight gain (Bian et al., 2017; Roy et al., 2007). However, unlike the commercial cyclamate-fed rats, increased food intake did not result in increased weight gain in commercial aspartame-fed rats. This may be attributed to the presence of phenylalanine the aspartame-based commercial NNS, which has been reported to stimulate whole-body fat oxidation in healthy males when supplemented before exercise (Ueda et al., 2017). Data suggest that commercial cyclamate-based NNS may not be suitable for weight gain management, especially in overweight or obese individuals. The other commercial NNS did not significantly alter body weight gain, while stevia-based NNS significantly reduced ($p < .05$) body weight gain (Figure 1) in normal rats, thus may be useful in weight gain control. Further clinical studies, are, however, needed to confirm this effect in humans.

Non-caloric or NNS are known not to elicit postprandial glycemic and insulin responses, because they provide negligible calorie compared to caloric sweeteners, such as sugars (O'Donnell & Kearsley, 2012). Thus, one would expect a momentary elevation in blood glucose and insulin levels following the feeding of sucrose but not NNS, as reported previously (Anton et al., 2010). Interestingly, in the present study, sucrose did not show significant influence on the weekly 3 hr fasting blood glucose and serum insulin of rat (Figures 2 and 4), possibly because, this effect represents a sub-chronic outcome on 3 hr-fasted (for weekly blood glucose) or overnight fasted (for serum insulin level) rats, rather than momentary postprandial glycemic or insulin response. However, the oral glucose tolerance test, which represents momentary postprandial glucose response, showed that sucrose fed-rats had worse glucose tolerance than normal rats (Figure 3a and b); perhaps continuous consumption of sucrose overs several weeks affected insulin action. The correlation between sugars, such as sucrose and fructose and the risk of insulin resistance has been well documented in a previous study by Macdonald (2016).

Most of the tested commercial NNS did not significantly alter weekly NFBG, oral glucose tolerance (except aspertame), and serum insulin (except stevia) of rat (Figures 2–4), which corroborates with the non-glycemic and insulinemic response of NNS (O'Donnell & Kearsley, 2012). Significnatly lower glucose tolerance was observed after feeding aspertame-based NNS (Fig. 3A) when stevia-based sweetener caused significant serum insulin elevation in rats (Figure 4). Being a NNS, it is most probable that the elevated insulin shown by the stevia-based sweetener is due to an insulinotropic potential rather than the momentary postprandial insulin response. Recent studies corroborate with this effect of stevia in normal mice (Rosales-Gómez et al., 2018) and diabetic rats (Ahmad & Ahmad, 2018). Moreover, insulin was measured in overnight fasted rats, suggesting that elevated serum insulin in stevia-fed rats may not be due to the modulation of intestinal sodium-glucose transport protein expression (SGLT) and diet-related glucose absorption (Moran et al., 2020). Data suggest that stevia-based commercial NNS may be not worsen glycemic control, at least in normal rats. Further clinical studies, are, however, needed to confirm this effect in humans.

High levels of triglyceride and cholesterol in the blood have been associated with the risk of several cardiovascular diseases, which is aggravated by elevated LDL-cholesterol to HDL-cholesterol ratio (Harchaoui, Visser, Kastelein, Stroes, & Dallinga-Thie, 2009; Nordestgaard & Varbo, 2014). This is because, elevated LDL-cholesterol, also referred as "bad" cholesterol, could lead to build-up of cholesterol in the arteries, which may result in atherosclerosis and increase the risk of heart diseases and stroke (Badimon & Vilahur, 2012). The perception of how artificial sweeteners affect lipid metabolism and/or profile seems to be inconsistent in different studies. A previous study reported that some NNS (Saccharin, AceK, and sucralose) can stimulate adipogenesis and suppress lipolysis (Simon et al., 2013). Another study suggested that some NNS (aspartame, acesulfame K, and saccharin) may promote atherosclerosis via structural and functional impairment of apolipoprotein-A1 and HDL-cholesterol (Jang, Jeoung, & Cho, 2011), which corroborates with the significant HDL-cholesterol reduction in normal rats after a 30-day administration of 500 mg/kg bw saccharin (Amin & AlMuzafar, 2015). In different studies, administration of saccharin (Jang et al., 2011) and stevia (Elnaga, Massoud, Yousef, & Mohamed, 2016) sweeteners significantly reduced ($p < .05$) serum total cholesterol, triglycerides, and LDL-Cholesterol, while administration of stevia (Elnaga et al., 2016) markedly increased ($p < .05$) serum HDL-cholesterol levels in overweight female rats.

Most of the tested commercially available NNS did not significantly alter total, HDL, and LDL cholesterol levels ($p < .05$) but markedly reduced serum triglyceride levels (Figure 5), which suggests that some of these NNS, in their commercially available forms, may not promote cardiovascular events. Stevia-based NNS significantly ($p < .05$) elevated serum HDL-cholesterol level, without significantly affecting serum LDL-cholesterol (Figure 5), which corroborates with the data of a previous study (Elnaga et al., 2016). In contrast, aspartame-, sucralose-, and cyclamate-based commercial NNS adversely affected serum cholesterol by elevating serum LDL-cholesterol levels (Figure 5). In fact, aspartame-based NNS appear to show the most detrimental effect on serum cholesterol by concomitantly decreasing ($p < .05$) serum HDL-cholesterol (Figure 5). The detrimental effect of the aspartame-based NNS on blood lipids may be partly attributed to the presence of acesulfame K, which has been reported to increase blood cholesterols in normal mice (Cong et al., 2013). These data in normal rats suggest that while stevia-based commercial NNS may not adversely affect blood lipids, the chronic consumption of aspartame-based commercial NNS may increase the risk of atherosclerotic and cardiovascular events. Further clinical studies, are, however, needed to confirm this effect in humans.

Alteration of several blood metabolites are diagnostic indexes of physiological derangement and tissue/organ damage associated with several disease conditions. For example, elevated blood levels of liver enzymes like ALT and AST are indicators of liver damage, while elevated blood urea could suggest renal malfunction. Although there have been some controversies about the safety of several NNS (Sharma et al., 2016), reports have shown that most NNS that have been approved for consumption by reputable regulatory bodies, like the Food and Drug Administration, are safe when consumed within accepted or estimated daily intakes (Fitch & Keim, 2012; Qurrat-ul-Ain & Khan, 2015). Findings of the present study showed that the tested commercially available NNS did not significantly and adversely alter the measured blood metabolites and metabolic makers of organ/tissue damage at least in normoglycemic condition (Table 2). Data suggest that the tested NNS may not initiate organ/tissue toxicity and/or damage in their commercially available forms, thus supports their safety.

5 | CONCLUSION

Findings of this study suggest that stevia-based commercially available NNS showed the most favorable effects on some diabetes-related parameters in non-diabetic rats, and thus may be useful in glycemic and body weight management. Moreover, aspartame- and

cyclamate-based commercially available NNS appear to show the detrimental effects on weight gain and blood cholesterols, thus may increase body weight and risk of cardiovascular diseases. Further clinical studies, are, however, warranted to confirm these effects in humans.

CONFLICT OF INTEREST

There is no conflict of interest within this study and manuscript.

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