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Research Article
Digestive Enzyme Activities, Oxidative Status and Intestinal Histomorphometry of Rats Supplemented with Steviana, A Natural Sweetener

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Abstract

Background and Objective: Stevia (*Stevia rebaudiana*), often known as sweet leaf, is a perennial plant and a member of the Asteraceae family. The commercial version of stevia leaf powder, known as Steviana, is one of the most popular natural sweeteners in use today. The current study aimed to analyze the activities of gut digestive enzymes, intestinal oxidative state and intestinal histological structure in stevia-fed rats. **Materials and Methods:** In this study, male rats were given Steviana and the oxidative condition of the gastrointestinal tract (GIT), the activities of certain digestive enzymes and the histomorphology of the GIT were examined. Animals used in experiments were split into 2 groups: Control and treated groups. For four consecutive weeks, the treatment group received a daily oral intake of 5 mg kg^{-1} b.wt., of Steviana solution. At the end of the trial, serum, pancreas and intestinal tissue samples were taken. **Results:** The Steviana sweetener has a strong antioxidative effect on both blood and intestinal tissue. Both sample types showed a decrease in malondialdehyde levels and a marked increase in superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities compared to control samples. In samples of blood, pancreas and intestinal contents, the activities of digestive enzymes including α-amylase, lipase and protease were significantly elevated in Steviana-treated rats. In addition, histological analysis of the small intestine showed that in rats given Steviana sweetener, intestinal glandular crypt depth was more pronounced compared to the control animals. **Conclusion:** In summary, consumption of Steviana appears to enhance digestion and absorption processes through antioxidative effects, improving the health of the local GIT and subsequently accelerating the rate of synthesis and release of endogenous digestive enzymes. Another aspect that enhances the digestion and absorption process is the change in the histological structure of the intestinal glands, as shown by an increase in the crypt depth.

Key words: Stevia, natural sweetener, intestinal histomorphometry, oxidative status, digestive enzymes

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The overconsumption of sugar in foods and beverages is a risk factor for type II diabetes, obesity and cardiovascular disease[1,2]. Additionally, studies using animal models demonstrated that consuming large amounts of sucrose causes hyperinsulinaemia, hyperglycaemia, hypertension and insulin resistance[3]. The American Heart Association recommends a daily sugar intake limit of 30 g (100 calories) and 45 g (150 calories) for average-sized women and men, respectively[4]. To combat the health issues connected with obesity and diabetes, the number of food products containing non-caloric sweeteners has significantly increased over the past 10 years. Numerous research studies have focused on sweetener use in obese and diabetic individuals, with a primary goal of reducing calorie consumption in their regular diet[5].

Food additives that are used as sugar substitutes mimic the sweetness of sucrose while containing few or no calories. The artificial sweeteners sucralose, saccharine, aspartame and cyclamate are considered to belong to a different category than the natural sweetener stevia[6].

Artificial sweeteners are addictive, according to animal research, even in drug-sensitized and addicted rats. Lenoir *et al.*[7] found that preference for the extreme sweetness of saccharine may exceed that of cocaine. According to a study by Mathur *et al.*[8], type II diabetics who used artificial sweeteners showed worse insulin resistance than non-diabetics. Additionally, Bueno-Hernández *et al.*[9] discovered that artificial sweeteners, depending on dosage and usage frequency, may cause gastrointestinal problems and inflammation. They connected this to the increase in gastrointestinal hormones, which regulate gut motility. Artificial sweeteners can play a significant role in altering the gut microbiota and dysbiosis, according to Suez *et al.*[10].

Due to the harmful consequences of artificial sweeteners, people are turning to natural sweeteners such as stevia, which is obtained from the plant *Stevia rebaudiana*. Locals in South America have been using stevia extract as a sweetener and traditional remedy for hundreds of years. Steviol glycosides are sweet-tasting, non-caloric components of stevia extracts. Stevioside (5-10% of the total dry weight) and rebaudioside A (2-4% of the total dry weight) are the two main steviol glycosides that have been isolated from stevia leaves. Compared to sucrose, stevioside and rebaudioside A are 250-450 times sweeter[11].

The current study aimed to assess the effects of Steviana, a commercial preparation of stevia, on the activities of gut digestive enzymes, intestinal oxidative state and the histological structure of the intestinal wall in experimental male albino rats.

MATERIALS AND METHODS

Study area: The study was conducted in the Department of Biology at Jamoum University College at Umm Al-Qura University in the Kingdom of Saudi Arabia during the period from August, 2022 to December, 2022. The Umm Al-Qura University Biomedical Research Ethics Committee approved the study (HAPO-02-K-012-2023-06-1646). The trials were conducted in conformity with the rules and legislation of the country as well as those of the International Animal Ethics Committee.

Animal design and Steviana preparation: Twenty rats (6-8 weeks old, 120.0±20.0 g b.wt.) were obtained from the Laboratory Animal Production and Care Unit at the College of Applied Medical Sciences at Umm Al-Qura University and used in the study. The rats were kept in 2 groups of ten in the laboratory at 24°C with a 12/12 hrs cycle of light and dark. After one week of acclimation, Steviana was given orally every day at a dose of 0 mg kg^{-1} b.wt., (control rats) and 5 mg kg^{-1} b.wt., (treated rats) for four weeks using a rat stomach tube. Food and water were offered *ad libitum*. Steviana sweetener was obtained from a mega-hyper mall in the form of a jar containing powder (200 g). It was produced by Said Salim Bawazir Sons for Toothpaste and Sweeteners Ltd. Co., Jeddah, Saudi Arabia. The dose was calculated to comply with the daily ingestion limit set by the FDA in the United States (5 mg kg^{-1})[12]. The ST was diluted with distilled water to a final concentration of 10% (w/v) and then filtered using a 0.22 micron Millipore filter (Carrigtwohill, Co., Cork, Ireland). Taking into account increases in animal weight over the course of the study, weekly adjustments to the dosage were made to maintain the 5 mg kg^{-1} b.wt., level.

Samples of serum and intestinal digesta: At the end of the trial, 7 blood samples were taken from control and treated rats through orbital sinus puncture under mild anesthesia and serum was separated and stored frozen at -20°C for oxidative and digestive enzyme analyses. Each small intestine (SI) was taken after euthanasia and stored in 1 mL of phosphate-buffered saline (PBS) to keep them hydrated until processing.

To obtain uniform intestinal digesta samples, the method of Jin *et al.*[13] was used. The tract was massaged from the distal end of the duodenum to the ileocecal junction. Samples were immediately diluted 10-fold with ice-cold PBS (pH 7.0), homogenized for 1 min and then sonicated for 1 min in three 30 sec cycles. The samples were then centrifuged for 20 min at 4°C at 4500 rpm. The supernatants were divided into small aliquots and stored at -70°C for digestive enzyme analysis.

Samples of intestinal and pancreatic tissues: The SI lumen was washed thoroughly with PBS to completely remove the intestinal contents. The 5 tissue samples from the pancreas, proximal duodenum, middle jejunum and distal ileum were taken and stored at -70°C for further analysis. The samples were homogenized following the kit's instructions, using a sample to a diluent ratio of 1:10. The homogenate was centrifuged for 10 min at 4°C at 4500 rpm. The supernatant was analyzed for oxidative and digestive enzymatic activity. Other intestinal tissue samples were taken and treated with 4% paraformaldehyde for histomorphological analysis.

Analysis of antioxidative and digestive enzymes: The homogenates of the intestinal tissue segments and contents were used for the analysis of oxidative and digestive enzymes. Catalase (CAT) activity was measured using the ammonium molybdate method[14], superoxide dismutase (SOD) activity was measured using the hydroxylamine method[15], glutathione peroxidase (GPx) activity was measured using the dithiodinitrobenzoic acid method[16] and malondialdehyde (MDA) content was measured using the TBA method[17]. The UV colorimetric method[18] was used to measure protease activity, the starch-iodine colorimetric technique[19] was used to measure α-amylase activity and the colorimetric method[20] was used to measure lipase activity. The kits were obtained from the Biodiagnostic Company, Jeddah, Saudi Arabia. Sample protein concentrations were measured using a commercial kit from Thermo Fisher Scientific, Waltham, Massachusetts, United States of America and are stated as mg of protein[21].

Histomorphometry: The Liu *et al.*[22] approach was used to examine the intestinal morphology of intestinal tissue segments. In summary, tissue samples were embedded in paraffin, fixed and then sectioned into 5 µm pieces. They were then hydrated with alcohol, dewaxed with xylene and stained with Hematoxylin and Eosin (H&E). A fluorescent orthochromatic microscope (Haier, Qingdao, China) was used to take the pictures. For every location, ten microscopical fields were selected at random. The Panoramic Viewer v1.15.3 program (3DHISTECH Ltd., Budapest, Hungary) was used to calculate the villus height and crypt depth.

Villus height was calculated as the distance between the tip of the villus and the villus-crypt junction; crypt depth was calculated as the depth of the invagination between neighboring villi.

Statistical analysis: The experimental data were analysed using the IBM SPSS Statistics V25.0 program (IBM Corp., Armonk, New York, United States of America). Intestinal measures and digestive enzyme activity were assessed for significance using a One-way Analysis of Variance (ANOVA). At $p \leq 0.05$, a difference was considered significant.

RESULTS

Serum and intestinal antioxidant capacity: Table 1 displays the serum antioxidant capacity indicators after the four-week trial. The MDA levels in the rat serum were considerably lower ($p \leq 0.05$) when compared to the control values, whereas SOD and GPx activities increased ($p \leq 0.05$) and CAT activity remained unchanged ($p \leq 0.05$). The same trend was observed in the intestinal samples: The MDA concentration in treated rats was significantly lower ($p \leq 0.05$) compared to the control samples while the activities of SOD and GPx were significantly higher ($p \leq 0.05$). The CAT activity did not change compared to the control rats ($p \leq 0.05$).

Digestive enzyme activity of serum, pancreatic tissue and intestinal contents: Figure 1-3 depict the activity of digestive enzymes (α-amylase, lipase and protease, respectively) in blood, pancreatic tissue and intestine contents. The data show a significant increase ($p \leq 0.05$) in serum α-amylase, lipase and protease activities in treated rats compared to control values. Similarly, the activities of these enzymes in both pancreatic tissue homogenates and intestinal contents were significantly higher ($p \leq 0.05$) compared to control animals.

Intestinal histomorphometry: The results of the intestinal histomorphometry were shown in Table 2 and were graphically depicted in Fig. 4. They show the villus length (µm) and crypt depth (µm) of various intestinal segments in rats

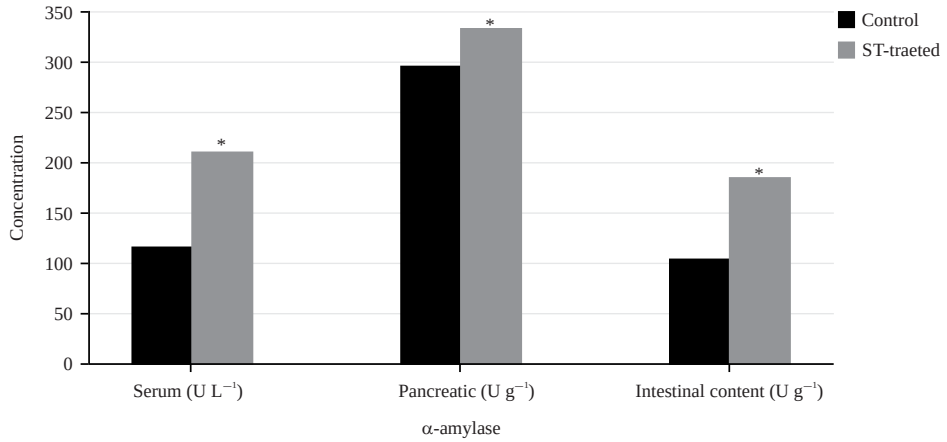

Fig. 1: α-Amylase activity in serum, pancreatic tissue and intestinal contents of rats treated with Steviana (ST) sweetener
*Significant difference from its control at $p \leq 0.05$

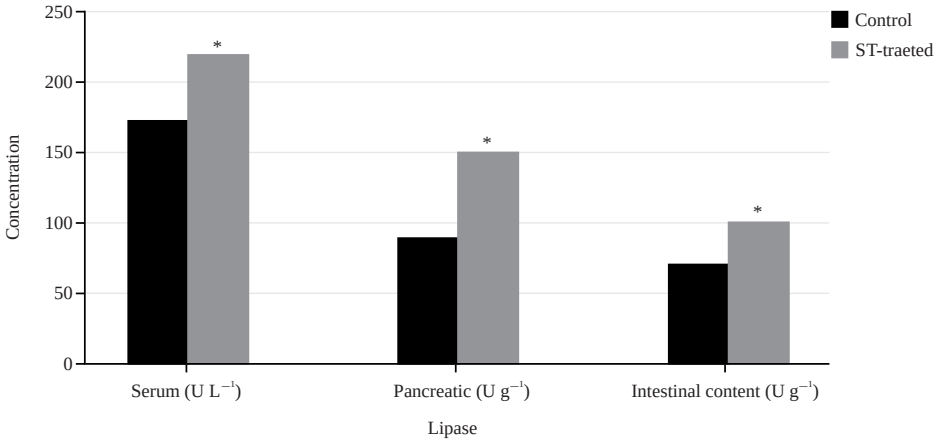

Fig. 2: Lipase activity in serum, pancreatic tissue and intestinal contents of rats treated with Steviana (ST) sweetener
*Significant difference from its control at $p \leq 0.05$

Table 1: Effects of Steviana administration on the serum and intestinal segment antioxidant capacity of rats after four weeks of treatment

	Enzyme							
	MDA (nM mL^{-1})		SOD (U mL^{-1})		CAT (U mL^{-1})		GSH-PX (U mL^{-1})	
Element	C	ST	C	ST	C	ST	C	ST
Serum	2.70±0.08^{a}	1.05±0.02^{a}	80.06±5.53^{b}	100.20±5.55^{b}	2.50±0.11	2.35±0.11	280.20±12.47^{c}	305.20±13.70^{c}
	MDA (nM mg^{-1} protein)		SOD (U mg^{-1} protein)		CAT (U mg^{-1} protein)		GPx (U mg^{-1} protein)	
	C	ST	C	ST	C	ST	C	ST
Duodenum	0.40±0.02^{a}	0.20±0.01^{a}	92.40±2.09^{b}	119.23±3.04^{b}	325.64±7.08	320.036±14.01	110.10±6.66^{c}	141.00±4.70^{c}
Jejunum	0.45±0.01^{a}	0.22±0.02^{a}	98.40±2.11^{b}	130.23±3.04^{b}	305.64±7.08	311.036±14.01	118.10±6.66^{c}	154.00±5.00^{c}
Ileum	0.42±0.02^{a}	0.18±0.02^{a}	97.40±2.01^{b}	122.23±2.05^{b}	322.64±7.08	321.036±14.01	109.10±6.66^{c}	138.00±3.50^{c}

Values are Means±SE, n = 5, within a row, means with the same superscript letter differ at $p \leq 0.05$, ST: Steviana sweetener, C: Control group, MDA: Malondialdehyde, SOD: Superoxide dismutase, CAT: Catalase and GPx: Glutathione peroxidase

given Steviana for four weeks. When compared to control animals, Steviana-supplemented rats showed a statistically significant increase in crypt depth measurements ($p \leq 0.05$). No significant change was recorded in villus length.

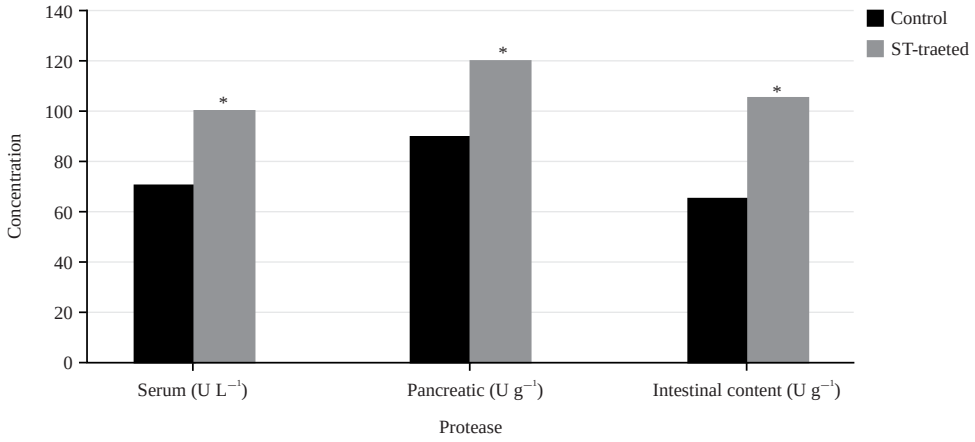

Fig. 3: Protease activity in serum, pancreatic tissue and intestinal contents of rats treated with Steviana (ST) sweetener
*Significant difference from its control at $p \leq 0.05$

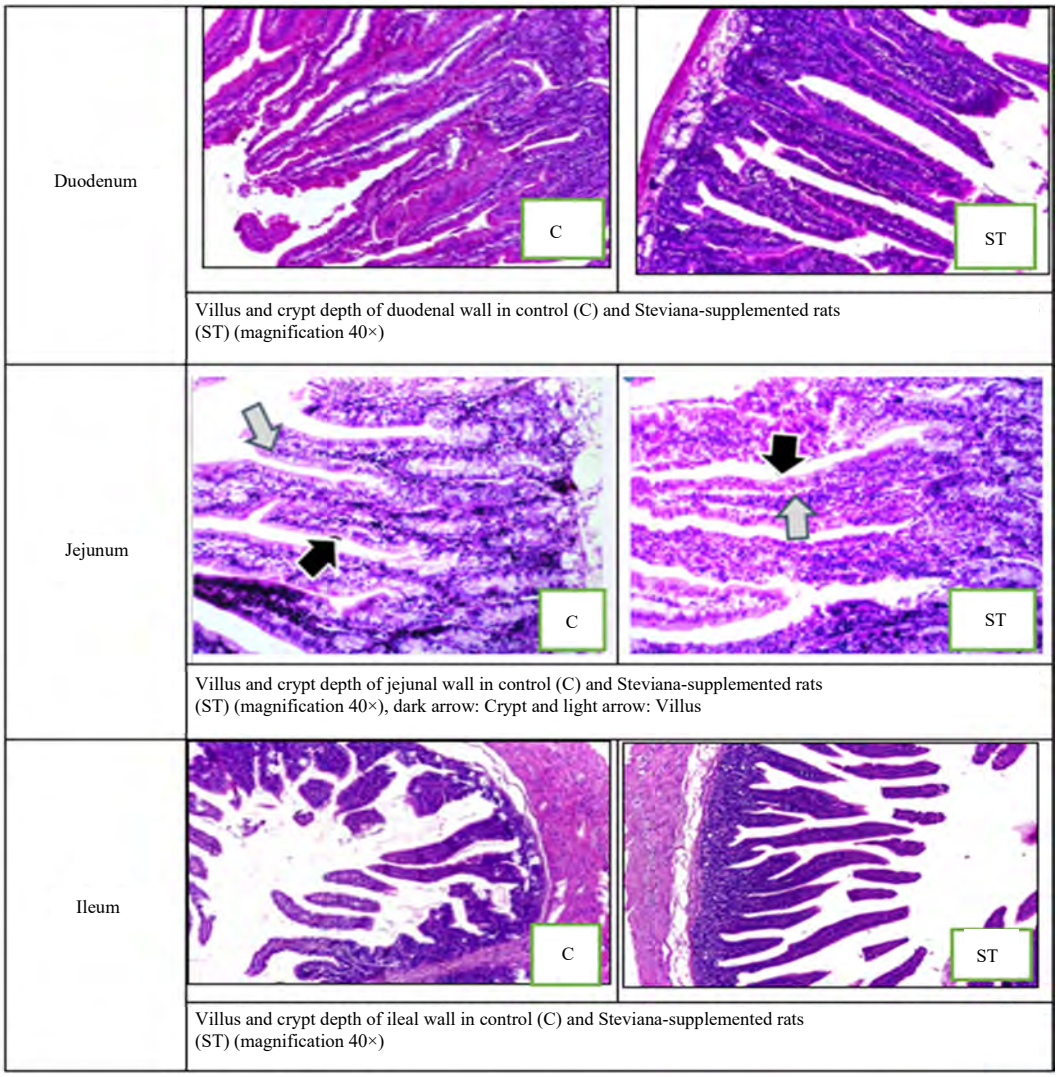

Fig. 4: Villus and crypt depth of intestinal segments of rats treated with Steviana sweetener for 4 weeks

Table 2: Villus length (μm) and crypt depth (μm) of small intestinal segments in rats supplemented with Steviana for four weeks

	Groups			
	Control		Steviana (5 mg kg^{-1} b.wt.)	
Segment	Villus length	Crypt depth	Villus length	Crypt depth
Duodenum	64.00±3.70	18.60±1.20	79.00±14.60	38.25±2.08*
Jejunum	115.00±14.10	39.25±4.56	118.75±15.30	60.00±8.20*
Ileum	136.87±12.80	31.60±2.01	142.19±14.40	48.91±2.40*

Values are Means±SE, (n: 10 rats/group), *Values are significantly different from the corresponding control values at $p \leq 0.05$

DISCUSSION

The present study showed that Steviana sweetener has a strong antioxidative potential at the level of blood and intestinal tissue, which both showed a decrease in the levels of MDA (a marker of oxidative stress) and an increase in SOD and GPx antioxidant enzyme activities compared to untreated controls. Lemus-Mondaca *et al.*[23] demonstrated that stevia and its extracts contain antioxidant, anti-inflammatory and antimicrobial factors and exhibit properties that control glucose and lipid metabolism. Formigoni *et al.*[24] suggested that the bioactive components of stevia, such as chlorogenic acid, flavonoids, quercetin and protocatechuic acid, may be connected to its physiological effects. According to some research, chlorogenic acid, which has antioxidant activity, is the primary polyphenol in stevia residues[25].

The majority of investigations on natural sweeteners have focused on *in vitro* or *in vivo* trials in mice. By stimulating the Akt/Nrf2/HO-1 pathway, Zhao *et al.*[26] discovered that stevia scavenged different free radicals and greatly raised the serum total antioxidant (T-AOC) content and SOD activity while decreasing MDA levels in oxidatively challenged mice. Stevia has been shown by Zhao *et al.*[27] to enhance serum antioxidant status in mice. According to the results obtained in this study, the administration of Steviana significantly improved the antioxidant capacity of blood and intestinal tissues in rats. Other studies have proved that treatment with stevia increased blood GPx, duodenal GPx and CAT activity and decreased ileal MDA levels in mice[28].

The current investigation indicated that the activities of α-amylase, lipase and protease were considerably elevated in the blood, pancreatic homogenate and intestinal contents of rats fed with Steviana for four weeks. The enhanced enzymatic activities in the intestinal content might be attributed to an increased rate of enzyme synthesis and/or release from pancreatic cells, or to increased enzymatic activities mediated by a more favorable pH value of the GIT[29]. Increased activity and secretion of these digestive enzymes from glandular mother cells (exocrine pancreatic cells) occurs due to a positive feedback response. When this is primarily driven by neural factors rather than gut hormones, it is most likely the result of increased availability of nutrients (substrates) within the GIT[30]. A significant amount of the digestive enzymes released by the pancreas are absorbed into the blood and recycled in enteropancreatic circulation[31,32]. It is possible that the antioxidant properties of Steviana could be a stimulatory factor for the enhanced release of digestive enzymes. The removal of toxic molecules and the breakdown of large dietary proteins may contribute to increased crypt depth seen in all intestinal segment mucosa of Steviana-treated rats. Alternatively, this histological change may be a complementary process to accommodate the increased rates of digestion and absorption mediated by the increased activities of digestive enzymes. According to histological analyses of mouse pancreatic tissue following treatment with stevia, the islet of Langerhans and its surrounding regions, the connective tissue capsule and the cellular distribution were all normal[33]. Based on the present findings, it can be said that using the natural sweetener Steviana is safe because of its benefits for the process of digestion and absorption as well as its oxidation resistance, particularly for those who have diabetes. Other in-depth studies must be conducted in the future to confirm the long-term safety of using the natural sweetener Steviana on other vital functions in the body.

CONCLUSION

In conclusion, giving rats 5 mg kg^{-1} b.wt. of Steviana sweetener a commercial form of stevia leaf powder for four successive weeks led to improvements in redox balance, the quantities and activities of digestive enzymes and the histomorphology of the small intestine. In each case, the crypt depth was also noticeably greater in the Steviana-treated rats. As a result, the processes of digestion and absorption might be enhanced. Steviana's activity appeared to be mediated by increasing the health of the local GIT via its antioxidant capacity, thereby stimulating the rate of endogenous digestive enzyme production and secretion. More research is necessary to determine the impact of Steviana on the colonization of GIT bacteria.

SIGNIFICANCE STATEMENT

Since the sixteenth century, stevia, a plant with a strong sweet flavor, has been used to create tea and sweeten other drinks. It is a natural supplement and non-nutritive sweetener. In 1987, the Food and Drug Administration (FDA) of the United States outlawed the sale of stevia as a food ingredient. One may categorize stevia as "zero-calorie". The sugary substances that were separated and refined from stevia leaves, such as glycosides, stevioside, rebaudiosides and steviolbioside. The most abundant of them are stevioside and Rebaudioside A (reb A). It has demonstrated potential health advantages as a sugar substitute that is healthy for diabetics. The purpose of the study is to elucidate how Steviana affects the oxidative state, intestinal histology and digestive enzyme activity in rats.

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