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Original Research Article

Toxicological Studies of Leaf extract of *Stevia rebaudiana* Bertoni in Sprague-Dawley Rats

Md. A. Uddin[1], Ferdousi Akter[1], Imtiaj H. Chowdhury[1], Umma H. Asha[2], Shamia Z. Tanny[2], Tahmina A. Sony[1], Nuruzzaman Neon[1], Nilay Saha[1], Md. M. Sikder[3], Saquiba Yesmine[1]*

[1]*Department of Pharmacy, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh*
[2]*Bangabandhu Sheikh Mujibur Rahman Science & Technology University, Gopalganj, Bangladesh*
[3]*Department of Biochemistry, Kagawa University School of Medicine, 1750-1 Ikenobe, Miki, Kagawa 761-0793, Japan.*

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ABSTRACT

Stevia rebaudiana Bertoni is a perennial shrub native to South America. The study evaluated the safety profile of the leaf extract of stevia in Sprague-Dawley rats. Both acute and chronic toxicity studies were performed. Twenty-four (24) male Sprague-Dawley rats were divided into four groups of six animals in each group. Stevia was administered for 28 days via oral gavage to the rats at doses of 125, 500 and 2000 mg/kg body weight respectively and the control group was administered distilled water. Acute oral toxicity study was performed in Swiss albino mice of either sex for a 72h period and no death was recorded. In the chronic study, stevia extract demonstrated no adverse effects on body weights, absolute organ weights and relative organ weight ratio in the treated rats at any doses. Evaluation of the haematological and biochemical parameters demonstrated no toxic effect in the treated rats. Besides, stevia extract significantly decreased the serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), cholesterol, triglyceride and low-density lipoprotein (LDL) levels compared to the control animals. The results demonstrated a significant *($p< 0.05$) decrease in hemoglobin (Hb) level at 2000 mg/kg and red blood cell (RBC) count at 500 mg/kg doses of stevia respectively, though the changes did not follow dose dependent manner. Present study reported the non-toxic nature of stevia extracts on chronic oral administration. The LD50 values of the stevia extracts were considered to be more than 5000 mg/kg body weight (BW) as no toxicity was observed up to 5000 mg/kg BW in treated animals.

Keywords: *Stevia rebaudiana*, ALT, AST, Cholesterol, Triglyceride, LDL.

Introduction

Stevia is a noncaloric sweetener derived from the herb *Stevia rebaudiana* (Bertoni). Stevioside and rebaudioside A are the predominant steviol glycosides found in *S. rebaudiana.*[1] Although steviol glycosides cannot be patented, increasing concerns about managing appropriate caloric intake as well as consumer demand for more sugar-substitute options provided the commercial motivation to overcome the technical and regulatory hurdles for commercializing steviol glycosides as a food ingredient.[2] Stevia has the unique sweet taste, and it is 70 to 400 times sweeter than sucrose.[3] Stevioside is 300 times sweeter than sucrose in 0.4% solution[4] while rebaudioside A is 250 to 450 times sweeter than the same solution.[5] Stevia with its therapeutic properties has been proven to be safe and efficient for diabetic patients.[6] Stevia offers beneficial effects by reducing the risk of cardiovascular and metabolic diseases due to its non-caloric properties.[5,6]

Several clinical and experimental studies reported antidiabetic and antihypertensive effects of stevia in various publications.[7-9]

*Corresponding author. E mail: s.yesmine@juniv.edu
Tel: 8801715014867

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In the "Regulatory status" section of previously published review article reported that purified steviol glycosides (≥95%) found safer for consumption by population[10] but crude extracts of stevia may cause adverse effects on the fertility in animals.[11,12] The study evaluated the safety profile of the leaf extract of *Stevia rebaudiana* in Sprague-Dawley rats and acute oral toxicity in Swiss albino mice compared with high-purity steviol glycosides.

Materials and Methods

Collection and preparation of Stevia

Dried *S. rebaudiana* leaves were supplied by BRAC Herbarium at Gazipur in Dhaka during September 2019, identified and authenticated by the National Herbarium, Mirpur, Dhaka, Bangladesh. A voucher specimen was deposited there (Accession No. DACB 48392). The plant material was ground into fine powder using a Waring laboratory scale blender and sifted using a 30 MESH-sieve (590 µm, average). Powdered plant material was then packed in plastic bags and stored at −20°C. Stevia (3 g) was dissolved in 30 mL distilled water for daily intake of animal.

Experimental animals

Twenty-four male healthy Sprague-Dawley rats (150-170g) of eight weeks old were used. The rats were maintained at the animal house of the Department of Pharmacy, Jahangirnagar University. All the rats were kept in plastic cages having dimensions of 30 x 20 x 13 cm and soft wood shavings were employed as bedding in the cages. Feeding of animals was done ad libitum, along with drinking water and maintained at natural day night cycle. The animals were housed in a well-ventilated hygienic experimental animal house. Constant environmental parameters with adequate nutritional conditions were

maintained. The rats were fed with standard pellet diets (prepared according to the formula developed at BCSIR, Dhaka, Bangladesh). The animals were handled in accordance with the ethical guidelines and the study protocol was approved by the Biosafety, Biosecurity and Ethics Committee of Jahangirnagar University (Ethics approval no: BBEC, JU/ M 2018 (5)2), Savar, Dhaka, Bangladesh.

Acute toxicity test
Swiss albino mice of both sexes (25 - 30g) were used to perform acute toxicity study of the leaf extract of stevia following a method previously described by Islam *et al*.[13] Ten healthy mice of either sex were taken for each group and the mice were randomly divided into six groups. For conducting the acute toxicity study, the stevia leaf extract was administered at a dose of 100, 250, 500, 1000, 2000 and 5000 mg/kg to all the mice of each group a single dose after 24 h for three days via oral gavage. A close observation was made for 72 h in these mice and percent mortality was recorded as alive (A) and death (D) within seven days of stevia administration. Any changes in behavior, such as excitement, gait, weakness, diarrhea, sedation, piloerection, lacrimation, respiratory pattern, exploratory movements were carefully examined and recorded.

Chronic study: Experimental design
The male Sprague-Dawley rats were randomly divided into four groups; each group had six rats as follows: i) Control group (distilled water); ii) Stevia (Stevia 125 mg/kg); iii) Stevia (Stevia 500 mg/kg) and iv) Stevia (Stevia 2000 mg/kg). All the treatments were administered via oral gavage for a period of 28 days. For the toxicological experiment, the liquid was administered at a volume such that it would permit optimal dosage accuracy without contributing much to the total increase in the body fluid. Administration was carried out between the hours of 10 AM and noon each day. At the end of the 28-day treatment period, the animals were sacrificed after overnight fasting. The animals were euthanized using ketamine (500 mg/kg; intraperitoneal). Organs were excised and fixed in 10% formalin solution for further analysis.

Blood samples collection
Blood samples were collected from the post *vena cava* and transferred immediately into ethylene diamine tetra acetate (EDTA) tubes for hematological tests and tubes without EDTA to get serum for biochemical analysis. All analyses were completed within 24 h of sample collection.

Determination of the biochemical parameters
To obtain serum, blood was allowed to clot and centrifuged at 3000 rpm for 15 min using a bench top centrifuge (MSE minor, England). The supernatant serum samples were separated and were collected using dry Pasteur pipette and stored in the freezer (-20°C) for further analyses.

Serum biochemical parameters and lipid profiles:
The separated serum was further analyzed to investigate the biochemical parameters using Dimension® EXL 200 LM Integrated Chemistry System (Siemens Medical Solutions Inc., USA) automated chemistry analyzer according to the protocol established by the manufacturer. The biochemical parameters measured were: blood urea, serum creatinine, serum albumin, total protein, total serum bilirubin, serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), total cholesterol (TC), triglycerides (TG) and high-density lipoprotein cholesterol (HDL).[14, 15] The absorbances of all the tests were determined using spectrophotometer (UV-Visible Spectrophotometer Model No. UV-1601 PC.).

Serum low-density lipoprotein (LDL) level:
Serum low-density lipoprotein cholesterol (LDL) level was determined by differential subtraction of the sum of the cholesterol fractions from the total cholesterol according to the Friedewald equation.[14,15]

Haematological analysis
Blood samples were analyzed using automated Sysmex KX-21 hematology analyzer (Sysmex- Roche) by using the technology of electrical impedance method of direct current. [15] Following Blood parameters were estimated: red blood cell (RBC) count, hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width-coefficient of variation (RDW-CV), red blood cell dimension width-standard deviation (RDW-SD), white blood cells (WBC) count, Neutrophils, Lymphocyte, Monocyte, Eosinophils and platelets count, mean platelet volume (MPV), platelet dimensions width (PDW), platelet large cell ration (P-LCR), Erytheocyte Sedimentation Rate (ESR), Packed cell Volume (PCV), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC). Sysmex KX-21 used a non-cyanide hemoglobin method for the measurement of hemoglobin (Hb).

Histological study
All the animals were euthanized for gross pathological examinations of all major internal organs. Organs such as heart, lung, liver, kidney, spleen and testis were collected from all the animals to perform histology (only liver histological figure attached in this article). They were weighed and relative organ weights were calculated.
However, it was planned to perform histological examination for the control and high dose group initially, if any significant findings were observed with high dose group, the mid and low dose groups were to be studied. The selected organs were fixed in 10% neutral buffered formalin, trimmed and a 4-5µm thickness of tissue sections were stained with hematoxylin and eosin for histological investigation using established protocols.[16] The photomicrographs were taken using Olympus DP 72 (Japan) microscope at Wazed Miah Science Research Centre (WMSRC) at Jahangirnagar University, Dhaka, Bangladesh.

Statistical analysis
Data were expressed as Mean ± SEM (Standard Error of Mean). Statistical analysis was performed by one-way ANOVA followed by Dunnett'sand Bonferroni *post hoc* test. *P* values < 0.05 were considered significant and * < *0.05*, ** < *0.01*, *** < *0.001* were taken as the levels of significance. Statistical programs used were SPSS (version 16, IBM software Inc, USA).

Results and Discussion

Stevioside and Steviol glycoside were reported to have large LD_{50} value [9,15] and at a dose of 5000 mg/kg BW, stevia produced no mortality to the mice in acute toxicity study. In this study, we performed acute toxicity test and evaluation showed that leaf extract of stevia administered to the test mice was relatively safe as no death was recorded (Table 1). This result also suggests that stevia's LD_{50} could be more than 5000 mg/kg (Table 1). We assessed safety profile in rats after 28 days chronic administration with crude stevia leaf extract. The chronic administration of stevia leaf extract showed no significant change in body weight compared to the control rats (Table 2); even highest dose (2000 mg/kg) did not produce any significant effect on body weight (Table 2). The absolute liver weight (Table 3) was significantly decreased in high dose stevia leaf extract treated rats; however no significant changes were found in relative organ weights (Table 4) when compared with control group. Tissue hydration index determines the ratio of water in the organ. In this study, tissue hydration index of organs showed no significant changes except in spleen (Table 5). In spleen, tissue hydration index was found significantly increased ($P<0.05$) in the rats treated with highest dose of stevia leaf extract (Table 5).
Various biochemical parameters were analyzed and presented in the table 6. The ALT and AST which are important indicators of liver function were decreased significantly ($P<0.05$) compared with the control group.

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Table 1: Acute oral toxicity study of Stevia Leaf extract in *Swiss* mice

Name	100 mg/kg		250 mg/kg		500 mg/kg		1000 mg/kg		2000 mg/kg		5000 mg/kg	
	A	D	A	D	A	D	A	D	A	D	A	D
Stevia rebaudiana	10	0	10	0	10	0	10	0	10	0	10	0
% mortality	0		0		0		0		0		0	

Here, n = 10; A = alive; D = Death.

Table 2: Effect of Stevia Leaf extract on body weight of Male Sprague- Dawley rats

Weight (gm)	Control	Stevia 125 mg/kg	Stevia 500 mg/kg	Stevia 2000 mg/kg
Day 1	152.50 ± 10.55	155.83 ± 6.25	155.00 ± 5.35	156.33 ± 4.07
Day 7	166.17 ± 11.36	171.33 ± 5.57	169.00 ± 5.72	166.83 ± 3.35
Day 14	182.83 ± 12.02	186.00 ± 4.92	180.83 ± 7.12	177.33 ± 3.10
Day 21	186.33 ± 12.27	193.50 ± 4.42	190.83 ± 7.12	187.50 ± 4.54
Day 28	198.50 ± 12.69	206.00 ± 4.32	200.67 ± 7.06	199.17 ± 4.08

N.B: Data were analyzed by one-way ANOVA following Bonferroni post hoc test. Values are expressed as Mean ± SEM, n = 6; p.o.

Table 3: Absolute weight of all tissues of Male Sprague -Dawley rats

Organ weight (g)	Control	Stevia 125 mg/kg	Stevia 500 mg/kg	Stevia 2000 mg/kg
Heart	0.60 ± 0.03	0.61 ± 0.01	0.58 ± 0.01	0.58 ± 0.012
Lung	1.00 ± 0.04	0.96 ± 0.02	0.99 ± 0.05	1.00 ± 0.05
Liver	7.87 ± 0.13	7.56 ± 0.31	7.01 ± 0.30	6.79 ± 0.19*
Kidney	0.61 ± 0.03	0.68 ± 0.03	0.63 ± 0.03	0.61 ± 0.04
Spleen	0.50 ± 0.03	0.51 ± 0.03	0.49 ± 0.02	0.48 ± 0.02
Testis 1	1.24 ± 0.03	1.27 ± 0.04	1.21 ± 0.04	1.29 ± 0.03

N.B: Data were analyzed by one-way ANOVA following *Bonferroni post hoc* test. Values are expressed as Mean ± SEM, n = 6, p.o. *($p < 0.05$) = significant compared to control.

Table 4: Relative Organ weight profile of tissues of Male Sprague -Dawley rats

Organ weight (mg/kg body weight)	Control	Stevia 125 mg/kg	Stevia 500 mg/kg	Stevia 2000 mg/kg
Heart	0.30 ± 0.01	0.30 ± 0.01	0.29 ± 0.00	0.29 ± 0.01
Lung	0.51 ± 0.03	0.47 ± 0.02	0.46 ± 0.02	0.50 ± 0.03
Liver	3.81 ± 0.18	3.68 ± 0.18	3.49 ± 0.06	3.41 ± 0.08
Kidney	0.31 ± 0.01	0.33 ± 0.02	0.31 ± 0.01	0.32 ± 0.02
Spleen	0.26 ± 0.01	0.24 ± 0.02	0.25 ± 0.01	0.24 ± 0.01
Testis	0.63 ± 0.03	0.65 ± 0.01	0.61 ± 0.03	0.65 ± 0.02

N.B: Data were analyzed by one-way ANOVA following Bonferroni post hoc test. Values are expressed as Mean±SEM, n=6, p.o.

Table 5: Effect of Stevia Leaf extract on Tissue-hydration indices of Male Sprague -Dawley rats

Organ	Control	Stevia 125 mg/kg	Stevia 500 mg/kg	Stevia 2000 mg/kg
Heart	78.74 ± 0.38	78.56 ± 0.58	79.56 ± 1.40	82.11 ± 2.02
Lung	80.03 ± 1.01	78.59 ± 1.19	79.38 ± 0.59	81.84 ± 1.21
Liver	76.19 ± 1.58	75.21 ± 2.00	74.79 ± 0.51	79.16 ± 2.51
Kidney	79.02 ± 0.40	79.82 ± 0.85	80.1392 ± 1.12	81.87 ± 2.76
Spleen	71.17 ± 5.24	76.27 ± 1.12	76.49 ± 0.64	91.96 ± 10.66*

N.B: Data were analyzed by one-way ANOVA following Bonferroni post hoc test. Values are expressed as Mean ± SEM, n = 6, p.o. *($p < 0.05$) = significant compared to control.

The most interestingly data was found in lipid profile study where Cholesterol, Triglyceride and LDL levels significantly decreased (P<0.05) in the treated animal although other parameters were not changed significantly such as the HDL value was found unchanged (Table 6). After analyzing liver histological slides (H&E stain), we found that treated animals had less fat droplet compared with control group that could be caused by less TG level in treated group animals (Figure 1). Investigation of haematological parameters demonstrated normal ranges compared to the control group (Table 7) but there were some significant decreases (P<0.05) observed in haemoglobin, and RBC levels although these results did not follow any dose dependent manner (Table 7).

Stevia leaves contain steviol glycosides including stevioside and rebaudioside as active compounds which are 30-150 times sweeterness than sugar.[5,6] In this study, we found, stevia leaf extract is non-toxic and causes negligible increase in body weight after chronic administration compared to control group though the elevation in body weight was found not statistically significant, indicating normal phenomena of body weight gain in treated animals. If stevia leaf extract is safe and found no effect on vital organs, then biochemical parameters, haematological parameters, relative organ weight, tissue hydration index and histological data are needed to be confirmed safety against chronic toxicity study.[17]

Fluctuations in body weight are linked to responsible foreign agents for their toxic effect in body.[18] Previous study reported that fat accumulation or deposition is associated with body weight gain for plant extracts other than a toxic impression of drug or chemicals present in extract that results in lower calorie intake in animals and a physiological adjustment might occur.[18] Relative organ weights in animals is a highly sensitive indicator to observe in toxicity studies. So, toxicity can be imagined in those specific organs if there is significant change in weights of organs.[19] With chronic oral dosing with stevia leaf extract, vital body organs like heart, kidney and spleen showed no significant variation in weight but liver weight significantly decreased (Table 3). For chronic toxicity study, weights of the organs were recorded to calculate the responsiveness of specific organs to physiologic changes and enzyme induction producing histopathological changes.[20] As the decrease in liver weight might be due to increased fat oxidation which causes less lipid droplet in hepatocyte which may be beneficial to body because increasing lipid droplet in hepatocyte causes several diseases like NAFLD (Non-alcoholic fatty liver disease).[21,22] Nonalcoholic fatty liver disease (NAFLD) is common in mostly western countries associated with increased cardiovascular and liver related morbidity and mortality such as high level of free fatty acids.[23]

Metabolic alterations in lipid homeostasis which increase free fatty acids (FFA) and non-HDL cholesterol, and these two parameters have been reported to lead fatty liver onset resulting in several cardiac diseases. [24] Previous study showed that high levels of liver free fatty acid and cholesterol activated inflammation and fibrogenesis by switching on cellular signaling pathways in some patients, which in turn lead to non-alcoholic steatohepatitis (NASH). [25] Increased oxidative stress in the liver was reported to be a major pathway leading to fibrogenesis. [26] In fact, patients with fibrogenic NASH exhibit both an increase in radical oxygen and nitrogen species production [26] and a lack of endogenous antioxidant defenses. [27] Oxidative stress keeps a crucial role to develop deadly liver cirrhosis.[28,29] Different indicators of oxidative stress were determined to analyze the antioxidant capacity of stevia. Reduced glutathione (GSH) and lipid peroxidation are crucial indicators of oxidative stress at both lipophilic and hydrophilic levels, respectively. In cirrhotic rats, lipid peroxidation was increased and GSH level was decreased in the liver and treatment with stevia significantly prevented these alterations, demonstrating that stevia has strong antioxidant activity.[30]

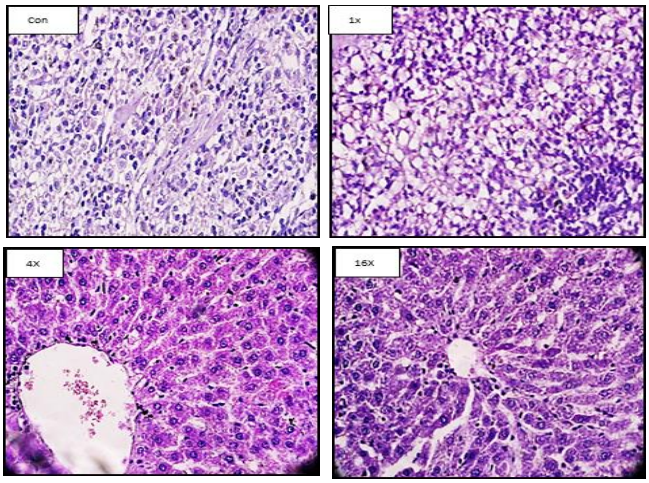

Figure 1: Photomicrographs of Liver sections of rats at the end of the 4th week (H&E stained paraffin-embedded sections). Control group and low dose (1x) contains more lipid droplets than higher dose (4x and 16x) of stevia treated animals (n=6).

Table 6: Effect of the Leaf extract of Stevia on Biochemical parameters of Male Sprague-Dawley rats

Parameters	Control	Stevia 125 mg/kg	Stevia 500 mg/kg	Stevia 2000 mg/kg
Blood Urea (mg/dL)	21.67 ± 1.02	22.17 ± 1.28	27.33 ± 0.71**	20.00 ± 1.69
Serum Creatinine (mg/dL)	0.71 ± 0.03	0.73 ± 0.03	0.81 ± 0.03	0.71 ± 0.04
Serum Bilirubin (mg/dL)	0.19 ± 0.01	0.19 ± 0.01	0.18 ± 0.012	0.22 ± 0.01
ALT (per Liter)	61.60 ± 2.94	47.67 ± 3.28*	41.83 ± 2.26***	45.33 ± 3.29**
AST (per Liter)	155.50 ± 4.72	113.00 ± 4.39***	89.33 ± 2.54***	105.00 ± 3.04***
Serum Cholesterol (mg/dL)	75.33 ± 1.87	67.00 ± 2.03*	63.50 ± 2.33*	64.83 ± 1.54*
Serum Triglyseride (mg/dL)	50.67 ± 1.40	43.50 ± 2.00*	40.00± 2.03*	42.33 ± 1.69***
HDL (mg/dL)	48.00 ± 1.73	50.17 ± 1.14	47.83 ± 1.74	46.33 ± 0.71
LDL (mg/dL)	17.33 ± 1.38	7.80 ± 0.58***	7.83 ± 0.95***	10.00 ± 01.18***
Serum Uric Acid (mg/mL)	1.92 ± 0.05	1.87 ± 0.10	1.82 ± 0.09	1.80 ± 0.7
Total Protein (g/dL)	7.12 ± 0.08	7.02 ± 0 .11	6.97 ± 0.13	6.78 ± 0.08
Serum Albumin (g/dL)	3.00 ± 0.09	2.90 ± 0.07	2.77 ± 0.13	2.92 ± 0.08

N.B: Data were analyzed by one-way ANOVA following Bonferroni post hoc test. Values are expressed as Mean ± SEM, n = 6., p.o. *(p< 0.05) = significant, ** (p< 0.01) = highly significant, *** (p< 0.001) = very highly significant compared to control.

Table 7: Effect of Leaf extract *S. rebaudiana* on the haematological parameters on whole blood of Male Sprague-Dawley rats

Parameters	Control	Stevia 125 mg/kg	Stevia 500 mg/kg	Stevia 2000 mg/kg
Hemoglobin (gm/dL)	13.32 ± 0.13	13.48 ± 0.12	13.37 ± 0.14	12.77 ± 0.12*
ESR	3.00 ± 0.26	2.50 ± 0.22	3.20 ± 0.37	3.50 ± 0.87
WBC (X 10^3)	5.70 ± 2.82	4.63 ±1 .86	7.07 ± 3.51*	5.12 ± 4.04
RBC (million/cumm)	7.58 ± 0.17	7.70 ± 0.20	6.65 ± 0.27*	7.23 ± 0.312
Platelet (X 10^3)	639.50 ± 18.87	692.17 ± 16.35	629.67 ± 28.22	500.50 ± 11.12***
DC (%)				
Neutrophil	30.12 ± 0.02	29.22 ± 0.01	30.21 ± 0.02	31.35 ± 0.02
Lymphocyte	65.22 ± 0.02	67.10 ± 0.01	65.31 ± 0.01	64.21 ± 0.02
Monocyte	3.16 ± 0.01	4.11 ± 0.10	3.14 ± 0.01	3.10 ± 0.12
Eosinophil	2.10 ± 0.01	1.14 ± 0.10	2.11 ± 0.01	2.13 ± 0.03
MCV (fL)	63.32 ± 2.51	58.47 ± 1.61	63.73 ± 1.53	57.72 ± 1.09
MCH (pg)	18.10 ± 0.43	15.94 ± 0.52	17.90 ± 0.46	17.50 ± 0.33
MCHC (gm/dL)	28.75 ± 0.69	26.33±0.72	26.60±0.43	29.05±0.92
RDW-SD (fL)	36.32 ± 1.59	30.02 ± 0.75	38.35 ± 2.73	31.93 ± 0.46
RDW-CV (%)	0.18 ± 0.01	0.18 ± 0.01	0.19 ± 0.00	0.18 ± 0.00
PDW (%)	9.18±0.21	9.67 ± 0.34	9.38 ± 0.21	9.17 ± 0.13
MPV (fL)	8.22 ± 0.23	8.53 ± 0.27	8.38 ± 0.15	7.83 ± 0.20
P-LCR (%)	12.14 ± 0.01	12.09 ± 0.01	11.31 ± 0.00	10.43 ± 0.00

N.B: Data were analyzed by one-way ANOVA following Bonferroni post hoc test. Values are expressed as Mean ± SEM, n=6, p.o. *($p < 0.05$) = significant, ** ($p < 0.01$) = highly significant, *** ($p < 0.001$) = very highly significant compared to control. Here, ESR = Erythrocyte sedimentation rate, WBC = White Blood Cell, RBC = Red Blood Cell, DC = Differential Count, MCV = Mean Corpuscular Volume, MCH = Mean Corpuscular Hemoglobin, MCHC = Mean Corpuscular Hemoglobin Concentration, RDW SD = Red Blood Cell Distribution Width SD, RDW-CV = Red Blood Cell Distribution Width-CV, MPV = Mean Platelet Volume, PDW: Platelet distribution width, P-LCR = Platelet-large cell ratio.

The leaves of stevia were found to contain flavonoids, phenolic acids, fatty acids, proteins and vitamins[31,32] which might contribute to its hepatoprotective effects. Published data reported that plants containing phenolic compounds demonstrate hepatoprotective property by increasing antioxidant balance.[33] Significant decrease of cholesterol, triglyceride, LDL level were observed, which cause less accumulation of fat droplets. Histological result also showed less lipid droplets in the rats treated with stevia leaf extract at 2000 mg/kg and reduction of liver weight compared to the control. Treatment with stevia leaf extract caused decrease in non-HDL cholesterol may act as a preventive to develop nonalcoholic fatty liver disease. Administration of stevia leaf extract orally at different doses decreased the levels of liver function enzymes (ALT, AST) in the rats. The lower levels of liver function enzymes were not considered to be adverse due to the small magnitude of difference from the control group value. The results revealed that stevioside in stevia leaves had no adverse effect on liver function enzymes due to its non-toxic nature. [33,34]

Tissues water content is beneficial to know in the development of physiologically based pharmacokinetic modeling[35] and in the interpretation of drug tissue distribution data.[36] Changes in tissue water content can affect tissue physiology and its metabolic functions which are associated with an increase in tissue weight, may cause tissue oedema.[37,38] The water content in spleen at the highest dose of stevia was increased significantly though the other organs' water contents were comparable to the control.

If there was any change in spleen function, this can cause poor absorption and delivery of nutrients and fluid which may develop fatigue and muscles weakness. There will also be poor water clearance from tissue and loss of ability to maintain blood circulation in the vessels. Investigation of haematological parameters is important to determine blood-related abnormalities after the ingestion of plant products.[39-41] Positive effects on haematological parameters were observed after chronic administration of stevia in different doses. However, a significant decrease in hemoglobin and RBC levels were observed at high doses (2000 mg/kg and 500 mg/kg respectively) of stevia extract treated groups though the values were within normal ranges and the changes were not dose dependent.

Conclusion

The current study demonstrated non-toxic nature of stevia crude extract on acute and chronic administration in Swiss albino mice and Sprague-Dawley rats at any of the dose levels. Regardless of the doses, the stevia extract was found to have no adverse effects on body weights, absolute organ weights and relative organ weight ratio in the treated animals. Various hematological and biochemical parameters measured in the treated rats showed no abnormal results and the values were comparable to those of the control rats. All animals survived till to the end of the study and no pathological alterations was observed in tissue histology after treatment with stevia. Besides, the study demonstrated a potential hepatoprotective effect of stevia extract as the results showed significant decrease in serum ALT, AST, LDL, Cholesterol and TG levels. However, long-term studies are needed to confirm the hepatoprotective effect of stevia after chronic administration.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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