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Hypoglycemic, Hypolipidemic and Antioxidant Potency of the Aqueous Extract of *Stevia rebaudiana* (Bert.) Leaves

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ABSTRACT

Eight sweet diterpene glycosides were quantitatively identified in the lyophilized aqueous extract of *Stevia rebaudiana* leaves using HPLC analysis zorbax NH_2 column and internal standard. The hypoglycemic effect was evaluated in alloxan-induced diabetic rats by orally treatment with *Stevia* extract (2.5g/kg b. wt) for a period of 21 days. Treatment with aqueous extract caused a significant decrease in blood glucose levels of diabetic rats (43.12%) as compared to the diabetic control. Blood glycated hemoglobin (HbA_1), creatinine, total cholesterol and total lipids were significantly decreased by 60.68, 30.10, 39.05 and 42.07% respectively. Liver function enzymes; aspartate and alanine aminotransferases (AST & ALT) were reduced by 38.16 and 69.19%, respectively. On the other hand, the aqueous extract caused non significant decrease in both sodium and urea levels, while potassium was significantly increased. The antioxidant effect of the aqueous extract was evaluated in the liver tissues of diabetic rats. A significant increase in both vitamins C & E (90.06 & 61.42%) and a significant decrease in lipid peroxides (-14.7%) were observed. A significant increase in glutathione (42.58%), glutathione peroxidase (46.79%) and glutathione reductase (28.84%) were also noticed as compared to diabetic controls. In conclusion, *Stevia rebaudiana* leaves could be considered not only as a safe natural alternative sweetener but also its aqueous extract have antidiabetic, hypolipidemic and antioxidant activities.

INTRODUCTION

Stevia rebaudiana Bertoni, belonging to the Asteraceae family, is a sweet herb native to Brazil and Paraguay. *Stevia rebaudiana* is a non-caloric natural source alternative to artificially produced sugar substitutes [1, 2]. *Stevia* sweeteners extracted from the leaves are commercially available in Japan, Korea, China, South-East Asia and South America, where they have been used for some decades to sweeten a variety of foods including beverages, confectionery and pickled vegetables. *Stevia* extracts have been extensively used as the dietary supplements in USA [3]. Many researchers have demonstrated that *Stevia* may use as hypoglycemic [4,5], anti-hypertensive [6] and anti-inflammatory [7] agents. In addition it exerts antibacterial and antimicrobial effects [8].

Diabetes mellitus is one of the most prevalent chronic diseases in the world. The mortality rate in diabetes mellitus patients is generally not due to classical diabetes-related complications (micro and/or macro vascular complications), but rather to an increased risk of hepatocellular failure [9]. Diabetes mellitus and familial combined hyperlipidaemia had been observed by many investigators [10]. Abnormal lipid metabolism is a main cause of dyslipidaemia, which is a major risk factor for cardiovascular disease, obesity, cholestasis and overall mortality [11]. It had been reported that high levels of fat increase fat-mediated oxidative stress and attenuate antioxidants [12]. Reactive oxygen species (ROSs) have detrimental effects on hepatocytes by damaging DNA, lipids and proteins, leading to a disruption in cellular homeostasis and aggravating metabolic syndrome features [13].

Medicinal plants are in general known to exert their beneficial effect (s) on diabetes via various modes and mechanisms depending on the phytochemicals and bioactive agents endowed in such plants or a collection of plants [14]. In the present study, we reported a more rapid and better resolved HPLC separation of sweet glycosides in the aqueous extract. In addition, different biological studies were carried out for evaluation of this extract as hypoglycemic, hypolipidemic and antioxidant agent.

MATERIALS AND METHODS

Plant Materials:

Stevia rebaudiana seeds were kindly secured by Prof. Dr Jose Walter Pedroza Carneiro, Maringa University, Brazil. As *Stevia* seeds were poorly germinated and establishment of the seedlings was slow, so tissue culture technique was conducted in order to obtain the plants which consequently give up the cuttings used for cultivation of appropriate area to provide the leaves materials [15]. The leaves were collected, air dried and powdered.

HPLC of Sweet Glycosides:

Eight sweet diterpene glycosides were quantitavely and qualitatively identified in the aqueous lyophilized extract (100 mg from which 25µl was dissolved in MeOH HPLC grad using the following conditions; Shimadzu system Controller Scl-10AVP with UV-Visible detector, and Shimadzu liquid chromatography pump, column: zorbax NH_2 Du pont (4.6m x 25 cm). Eluting solvent: 84-70% v/v acetonitrile - water (pH: 5) changed over a period of 15 min. Flow rate: 2ml/min. UV detector: 210 nm [16]. The results were recorded in Table 1.

Macro and micro elements of *Stevia rebaudiana* Leaves:

Dried leaves and its dried aqueous extract (0.5g) were separately digested using a mixture of conc. sulfuric acid (3ml) and conc. nitric acid (1ml) [17]. In acid digested solutions, iron, zinc, manganese, copper, magnesium, selenium and chromium were determined using atomic absorption (Table 2).

Alloxan-induced diabetes

Diabetes was induced in 12 hours fasted male Wistar albino rats by intraperitoneal administration of aqueous alloxan monohydrate (Sigma-Aldrich Co.) at a single dose of 150 mg/kg body weight dissolved in 0.9% NaCl [18]. Vehicle group intraperitoneally injected with 0.9% NaCl. After alloxan application, the pancreas secretes insulin at high levels. As a consequence, fatal hypoglycemia can occur. To prevent this adverse effect, 5 ml 20% glucose solution were injected intraperitoneally 4–6 h after alloxan injection.

Experimental design:

Rats were divided into three groups (6 rats each). Group I: Normal control rats. Group II: Control non-treated diabetic rats Group III: Diabetic rats treated orally with aqueous extract (2.5 g/kg b.

wt**)** over a period of 21 successive days. The blood samples were withdrawn from the retro-orbital venous plexus at zero, 10 and 21 day.

Samples preparations:

Serum sample: Animals were sacrificed under anesthesia and blood samples were withdrawn from the retro-orbital venous plexus in clean and dry test tubes. Blood left 10 min to clot and centrifuged at 3000 rpm for 20 minutes at 4ºC. The supernatant serum was collected and stored at –80ºC for further determination of glucose and glycohemoglobin levels, liver and kidney function tests, lipid profile and serum electrolytes.

Liver homogenate: A portion of liver was weighed and homogenized with saline (0.9% NaCl) (1:9 w/v) using a glass homogenizer at 4ºC. The homogenates were centrifuged at 3000 rpm for 10 minutes at 4ºC and the clear supernatant was used for further determination of the antioxidant parameters.

Biochemical determinations:

The following biochemical parameters were determined including, glycated hemoglobin (HbA_1), fasting glucose, urea, creatinine, sodium, potassium**,** cholesterol and total lipids (Biodiagnostic kits, Cairo, Egypt). Liver function enzymes; ALT and AST were estimated by Gella et al. [19]. The oxidative stress biomarkers; vitamin C [20], vitamin E [21], lipid peroxides [22], glutathione [23], glutathione peroxidase [24] and glutathione reductase [25] were estimated.

Statistical analysis:

Student t-test and analysis of variance (ANOVA) tests were done to compare between means of all groups at the level of significance $p<0.05$.

RESULTS AND DISCUSSION

Many analytical methods have been applied for the separation and quantification of sweet glycosides from the leaves of *Stevia rebaudiana.* Mizukami et al. [26] quantified only stevioside levels enzymatically and Sakaguchi and Kan [27] quantified total glycosides content by gas chromatography after acid hydrolysis. Fullas et al. [28] applied TLC to identify four more

abundant glycosides. Makapugay et al. [29] quantified the diterpene glycosides by HPLC using Soxhlet extraction. Gasmalla et al. [8] and Mauri et al. [30] employed a capillary electrophoresis method to analyze *Stevia rebaudiana* glycosides where they obtained rebaudioside A and steviolbioside using a semi preparative HPLC.

In the present study, eight sweet diterpene glycosides were quantitavely identified using internal standard and HPLC analysis of the aqueous extract of the leaves. The two most abundant and important compounds were rebaudioside A (22.78%), and stevioside (13.64%). The rest of constituents were in minor concentration (Table 1).

In our study also, the blood sugar level was increased subsequent to alloxan administration (Table 3). Singh et al. [5] stated that the diabetogenic effect of alloxan is due to excess production of reactive oxygen species leading to cytotoxicity in pancreatic β-cells which reduces the synthesis and the release of insulin, while affecting organs such as liver, kidney, pancreas and haemopoitic system. The continuous treatment with aqueous extract (2.5g/kg b.wt) caused a significant decrease in the blood glucose levels (43.12%) as compared to the diabetic control rats. The potency of aqueous extract of *Stevia* leaves as hypoglycemic agent was due to presence of sweet glycosides. Certain inorganic mineral elements (potassium, zinc, calcium, traces of chromium) are known to play an important role in the maintenance of normal glucose tolerance and in the release of insulin from beta cells of islets of langerhans [31]. Chromium is essential for the regulation of insulin action, and its supplementation has been studied as a potential therapy of insulin resistance and lipid and protein abnormalities [32]. Diabetic patients should probably take zinc supplements, as it plays a key role in the regulation of insulin production by pancreatic tissue and glucose utilization by muscles and fat cells. Also zinc is used as cofactor of many enzymes like lactate dehydrogenase and alkaline phosphatase beside it is the main constituent of many enzyme systems [33]. In the present work, using the atomic absorption, it has been found that *Stevia rebaudiana* dry leaves and its aqueous extract contain different levels of nutritionally important microelements (Table 2) which are zinc (50, 35 mg/l), magnesium (675, 1270 mg/L), selenium (279, 55 mg/L) and chromium (4, 4 mg/L). These results indicate the richness of *Stevia* leaves with some biomolecules that may have stimulate the beta cells of langerhans to release insulin, leading to the improvement in the carbohydrate metabolizing enzymes and thus establishing normal blood glucose level.

Measurement of glycated hemoglobin (HbA_1) percentage is unique in reflecting blood glucose levels in a period of about eight weeks. Unlike blood glucose conc., HbA_1 percentage does not fluctuate from hour to hour, so it is useful in the long term assessment of control diabetes [34]. The present work revealed an increase in HbA_1 percentage (19.66%) in diabetic control rats reflected as an increase in mean serum fasting glucose levels (160 mg/dl) over a period of 21 days. Group III treated with aqueous extract restored the normal level of HbA_1 percentages (7.73%) as compared to diabetic control rats (Table 4).

The important role of the kidney in physiological glucose homeostasis is often overlooked. It helps to regulate blood glucose by gluconeogenesis, by utilization of glucose from the circulation, and by re-absorption of glucose from the glomerular filtrate.

These homeostatic mechanisms are altered in type 2 diabetes which may contribute to hyperglycaemia [35]. Serum creatinine levels are frequently used as screening test for renal dysfunction [36]. The level of serum creatinine was significantly decreased by 30.10 % in the treated groups in comparison to the diabetic control rats (Table 3). These results gave an additional support that the plant extract improved the renal function and support the glomeruli.

One primary problem with diabetes is that the amount of glucose in the blood can offset the proportion of serum electrolytes. With hyperglycemia, the body tries to rid itself of the excess blood glucose by increasing urinary output. Increased urination produces water and electrolyte loss, which then upsets the body's balance of electrolytes. The balance is especially disturbed between sodium and potassium [37]. Concerning to the serum electrolytes; sodium was significantly elevated in diabetic group as compared to the control, while potassium was significantly decreased. These results were in parallel with the finding of Bukonla et al. [37]. Treatment with plant extract improved the serum electrolytes; sodium and potassium as well as the urea level (Table 5).

Treatment of diabetic rats with aqueous extract not only caused blood glucose homeostasis but also reversed changes in lipid metabolism. In the alloxan-induced control diabetic rats, the rise in blood glucose is accompanied by an increase in serum total cholesterol (87.5 mg/dl) and total lipids (393 mg/dl) levels after 21 days as compared to the normal control. The treatment of the diabetic rats with aqueous extract (Table 4) of *Stevia* leaves for 21 days resulted in decrease in

serum total cholesterol and total lipids levels (39.05, 42.07%, respectively), as compared to the diabetic control rats. These findings were in accordance with the results of Ali et al. [38]. It has been suggested that the increase in the total cholesterol and triglycerides levels in diabetes mellitus was due to the resistance to insulin that depending on glucose uptake which consequently increase the serum glucose levels, leading to increase the synthesis and secretion of hepatic total cholesterol and triglycerides [39].

The aminotransferases constitute a group of enzymes that catalyze the interconversion of amino acids and α-oxo-acids by transfer of amino groups. In liver diseases, serum ALT and AST levels are elevated, where ALT level is characteristically higher than AST level. Elevated serum AST activity with no ALT elevation indicates muscle necrosis or myocardial infarction [40]. In diabetic control rats, serum ALT and AST levels (30.5 U/ml and 66 U/ml, respectively) were significantly higher than normal rats (19.33 U/ml and 16.16 U/ml, respectively). Treatment with the aqueous extract led to significant decreased in ALT and AST activities (38.16 and 69.19%, respectively) as compared to diabetic control rats (Table 6).

Hyperglycaemia is thought to be associated with increased oxidative stress via glucose autoxidation which produces superoxide radicals and free radicals generated from glycosylated proteins [41]. In present study it was found that lipid peroxidation level was increased in diabetic rats. This observation was in accordance with the results of Ali et al. [38]. On the other hand, diabetic rats treated with aqueous extract showed significantly decreased of lipid peroxidation by 14.7%.

Raza and Sohn [42] reported that injection of rats with alloxan can induce production of reactive oxygen species which can damage the cellular elements. In diabetes, it was found that the final pathway of pancreatic beta cell destruction is referred to the hypothesis that beta cytotoxic events involve the macromolecular damage resulting from an increase in the levels of free radicals (superoxide and H_2O_2) relative to antioxidants (superoxide dismutase, catalase, glutathione peroxidase) and vitamins A, C and E. The basis for this idea is the fact that antioxidants are capable of preventing beta cell damage resulting from the administration of alloxan in animals [43]. Singh et al. [5] suggesting the compensatory role of the extract in reducing H_2O_2 produced thus diminishing the toxic effects of free radicals produced by it in various secondary reactions.

Vitamin C is a strong reducing agent, it is a naturally occurring suppressor of free radicals and also it enhances vitamin E efficiency in reducing lipid peroxidation. Vitamin E the major chain breaking antioxidants in plasma, red cells and tissues. It represents first line of defense against peroxidation of polyunsaturated fatty acids in cellular and subcellular membrane phospholipids [44]. Palsamy et al. [45] observed significant reduction in Vitamins C and E in diabetic patients and rats, respectively. In accordance of our study, Frei et al. [46] reported that peroxyl radicals are trapped by ascorbate thus, the level of the enzyme and vitamin C decreased during the free radical scavenging process. Also, the reduction of vitamin E in diabetes occurs since the vitamin adds as a soluble antioxidant to protect biological membranes against oxidative stress which leads to distribution of cell function. Also, Sokal et al. [47] reported that vitamin E protect hepatocytes against lipid peroxidation and toxic injury.

In this study, *Stevia* aqueous extract was used to control diabetes and scavenging agents for control free radicals associated with diabetes. Therefore, *Stevia* aqueous extract was considered as antioxidant agent. The present results showed that diabetic rats treated with aqueous extract resulted in a significant increase in the levels of both vitamins C and E by 90.06 and 61.42%, respectively, while lipid peroxide was significantly decrease by 14.70%.

In this respect, Singh et al. [5] and Muralikrishnan and Shyamaladevi [48] reported that glutathione concentration is an important defense mechanism against certain toxic substances such as some drugs and carcinogens. If the level of glutathione is lowered in tissue, then the tissue is more susceptible to injury by various chemicals that would normally be conjugated to glutathione. Also it participates in the decomposition of potentially toxic hydrogen peroxide in the reaction catalyzed by glutathione peroxidase. In this study, a significant decrease in glutathione level in diabetic group which in parallel with the results of with Parveen et al. [49] was observed. A significant increase in glutathione level was found in diabetic rats treated with aqueous extract (42.58%). Also, glutathione peroxidase and reductase activities were significantly increased (46.79, 45.81%, respectively) as compared to diabetic control rats (Table 7) which indicates that the extract had the potential to increase the biosynthesis of GSH which thereby reduces the oxidative stress [5].

Table 1: HPLC of the aqueous extract of *Stevia rebaudiana* leaves

Peak No	Rt (min)	Conc. %	Identified Compounds
1	1.31	3.13	Unidentified
2	2.18	13.92	Unidentified
3	2.79	7.31	Unidentified
4	3.42	2.64	Steviolbioside
5	3.87	1.35	Unidentified
6	4.22	3.56	Unidentified
7	5.27	6.52	Dulcoside A
8	6.22	2.03	RebaudiosideB
9	6.92	13.64	Stevioside
10	7.70	1.50	RebaudiosideC
11	8.49	7.76	Unidentified
12	8.94	22.78	RebaudiosideA
13	11.93	11.15	Rebaudioside E
14	12.28	2.72	Rebaudioside D

Table 2: Concentration of macro and micro elements in the dried leaves and aqueous extract of *Stevia rebaudiana*

Elements	Dried leaves	Dried aqueous extract
Nitrogen (N)	1720	480
Phosphorus (P)	2532	1953
Potassium (K)	2240	2632
Sodium (Na)	150	130
Magnesium (Mg)	675	1270
Iron (Fe)	299	54
Zinc (Zn)	50	35
Manganese (Mn)	225	104
Copper (Cu)	16	9
Selenium (Se)	279	55
Chromium (Cr)	4	4

Table 3: Effect of the aqueous extract of *Stevia rebaudiana* leaves on serum fasting glucose and creatinine levels in diabetic rats

Time	Glucose level (mg/dl)			Creatinine (mg/dl)		
	GI	GII	GIII	GI	GII	GIII
Zero						
Mean±S.E	86.66±2.47^{Aa}	160±3.89^{Ba}	163.5±3.64^{Ba}	0.65±0.05^{Aa}	0.86±0.06^{Ba}	0.89±0.05^{Ba}
Range	74-89	150-177	150-176	0.47-0.70	0.6-1.04	0.88-1.03
10th day						
Mean±S.E	88.33±3.32^{Aa}	156.50±2.18^{B}	108.33±5.74^{C}	0.68±0.08^{Aa}	0.83±0.04^{Aa}	0.72±0.04^{Aab}
Range	78-100	148-164	85-122	0.44-0.95	0.64-1.0	0.56-0.88
% Change			**-30.77**			**-13.25**
21st day						
Mean±S.E	89.00±3.32^{Aa}	143.00±3.49^{Bb}	81.33±.88^{Cc}	0.61±0.04^{Aa}	0.93±0.05^{Ba}	0.68±0.06^{Ab}
Range	78-93	133-152	**80-85**	0.45-0.80	0.77-1.09	0.42-0.80
% Change			**- 43.12**			**-26.88**

Number of rats / group = 6 Means with different capital letters superscripts A, B... for rows and small letters a, b… for columns with a significant difference (P value) at ≤ 0.05. Percentage of change compared to diabetic rats. GI: normal control rats. GII: diabetic control rats. GIII: diabetic rats treated with aqueous extract.

Table 4: Effect of the aqueous extract of *Stevia rebaudiana* leaves on serum glycohemoglobin, total lipids and total cholesterol levels in diabetic rats

Time	Glycohemoglobin (HbA_1 %)			Total lipids (mg/dl)			Total cholesterol (mg/dl)		
	G1	GII	GIII	GI	GII	GIII	GI	GII	GIII
Zero Mean ±S.E	6.63±0. 10 Aa	11.00±0.36 Ba	11.00±0.61 Ba	229.33±7.64 Aa	250.5±4.28 Ba	258.33±4.05 Ba	45.33±1.33 Aa	54.66±1.77 Ba	56.83±1.89 Ba
range	6.30-7.0	10-12	10 -13.1	200-246	240-268	250-270	39-47	47-58	55-60
21st days Mean ±S.E range	6.50±0. 25 Aa	19.66±0.60 Ba	7.73±0. 72 Ba	234.83±8.78 A	393±9.13 Ba	227.66±5.57 Ba	48.33±1.77 A	87.50±2.67 Ba	53.33±2.48 Ba
% change	5.4 -7.2	17.7-22	6- 9.5 **-60.68**	200-257	351-418	201 -239 **-42.07**	44-56	76-94	45- 60 **-39.05**

Number of rats / group = 6 Means with different capital letters superscripts A, B... for rows and small letters a, b… for columns with a significant difference (P value) at ≤ 0.05. Percentage of change compared to diabetic rats. GI: normal control rats. GII: diabetic control rats. GIII: diabetic rats treated with aqueous extract.

Table 5: Effect of the aqueous extract of *Stevia rebaudiana* leaves on serum sodium, potassium and urea levels in diabetic rats

Time	Sodium (mEq/L)			Potassium (mEq/L)			Urea (mg/dl)		
	G1	GII	GIII	GI	GII	GIII	GI	GII	GIII
Zero Mean ±S.E range	152.66±4.43 Aa 143-165	157.83±2.45 Aa 146-163	150.66±4.71 Aa 136 -170	5.06±0.13 Aa 4.7-5.5	5.73±0.31 Aa 4.4-5.9	5.80±0.26 Aa 4.8-6.4	19.68±2.42 Aa 11-20	27.66±0.06 Ba 22-30	28.±1.06 Ba 23-30
10th day Mean ±S.E range % change	145.33±1.66 Aa 140-150	172.16±5.20 Bb 156-190	159.83±4.55 Cab 143-177 - 7.16	4.93±0.25 Aa 4-5.8	3.98±0.23 Bb 2.9-4.6	6.11±0.28 Ca 5.3 -7.2 **53.51**	21.4±1.51 Aa 16-26	28.66±0.91 Ba 27-33	26.66±1.22 Ba 24-32 **-6.97**
21st days Mean ±S.E range % change	148.85±4.59 Aa 136-164	176.00±5.10 Bb 163-190	166.16±3.82 Bb 155-177 -5.59	4.71±0.21 Aa 3.7-5.2	3.43±0.18 Bb 2.8-4	5.33±0.18 Ca 5 -5.9 **55.39**	21.71±1.48 Aa 15-26	30±1.46 Ba 25-35	27.33±1.35 Ba 22-32 **-8.9**

Number of rats / group = 6 Means with different capital letters superscripts A, B... for rows and small letters a, b… for columns with a significant difference (P value) at ≤ 0.05. Percentage of change compared to diabetic rats. GI: normal control rats. GII: diabetic control rats. GIII: diabetic rats treated with of the aqueous extract.

Table 6: Effect of the aqueous extract of *Stevia rebaudiana* leaves on serum AST and ALT levels in diabetic rats .

Time	AST (u/ml)			ALT (u/ml)		
	G1	GII	GIII	GI	GII	GIII
Zero Mean±S.E Range	15.50±1.60^{Aa} 11-21	34.00±3.67^{Ba} 27-47	36.66±2.43^{Ba} 30-45	21.33±2.17^{Aa} 15-22	20.83±2.30^{Aa} 15-28	20.83±2.02 ^{Aa} 14 -27
10th day Mean±S.E Range % Change	15.83±1.30^{Aa} 11-19	69.66±6.50^{Cb} 41-88	28.83±2.42^{Bb} 23-36 **-58.61**	15.66±3.29^{Aa} 10-20	23.33±2.26^{Ba} 13-28	23.16±0.48^{Ba} 21 -25 **-0.72**
21st day Mean±S.E Range % Change	16.16±2.79^{Aa} 11-27	66.00±6.00^{Bb} 52-85	20.33±1.94^{Ac} 14-28 **-69.19**	19.33±2.37^{Aa} 11-29	30.5±1.82^{Bb} 22-35	18.86±1.14^{Ab} 16 -23 **-38.16**

Number of rats / group = 6 Means with different capital letters superscripts A, B... for rows and small letters a, b… for columns with a significant difference (P value) at ≤ 0.05. Percentage of change compared to diabetic rats. GI: normal control rats. GII: diabetic control rats. GIII: diabetic rats treated with of the aqueous extract.

Table 7: Effect of the aqueous extract of *Stevia rebaudiana* leaves on liver glutathione, glutathione peroxides, glutathione reducatase, Vitamins C & E and lipid peroxides levels in diabetic rats

Groups	Glutathione mg/g tissue	Glutathione peroxidase µmole/g tissue	Glutathione reductase µmole/g tissue	Vitamin C mg/g tissue	Vitamin E mg/g tissue	Lipid peroxides µmole/g tissue
Group I Mean±S.E	31.69±0.022^{A}	2.98±0.07^{A}	14.05±0.64^{A}	6.31±0.06^{A}	7.27±0.08^{A}	0.28±0.02^{A}
Group II Mean±S.E	20.62±0.39^{B}	2.03±0.04^{B}	9.43±0.29^{B}	4.63±0.04^{B}	4.77±0.04^{B}	0.34±0.01^{B}
Groups III Mean±S.E	29.40±0.52^{C}	2.98±0.03^{A}	12.15±0.28^{C}	8.8±0.11^{C}	7.7±0.08^{C}	0.29±0.01^{A}
% of change	**42.58**	**46.79**	**28.84**	**90.06**	**61.42**	**- 14.7**

Number of rats / group = 6 Means with different capital letters superscripts A, B... for rows with a significant difference (P value) at ≤ 0.05. Percentage of change compared to diabetic rats. GI: normal control rats. GII: diabetic control rats. GIII: diabetic rats treated with of the aqueous extract.

CONCLUSION

In conclusion, the aqueous extract of *Stevia rebaudiana* leaves may have potential use as antidiabetic and hypolipidemic agents as well as an alternative natural sweetener. Also they could be used as antioxidant agents for controlling free radicals in diabetes.

Competing interests:

The authors declare no conflict of interest.

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