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Antidiabetic activity of medium-polar extract from the leaves of *Stevia rebaudiana* Bert. (Bertoni) on alloxan-induced diabetic rats

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Abstract

Go to:

Objective:

Go to:

To investigate the medicative effects of medium-polar (benzene:acetone, 1:1, v/v) extract of leaves from *Stevia rebaudiana* (family Asteraceae) on alloxan-induced diabetic rats.

Materials and Methods:

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Diabetes was induced in adult albino Wistar rats by intraperitoneal (i.p.) injection of alloxan (180 mg/kg). Medium-polar extract was administered orally at daily dose of 200 and 400 mg/kg body wt. basis for 10 days. The control group received normal saline (0.9%) for the same duration. Glibenclamide was used as positive control reference drug against *Stevia* extract.

Results:

Go to:

Medium-polar leaf extract of *S. rebaudiana* (200 and 400 mg/kg) produced a delayed but significant ($P < 0.01$) decrease in the blood glucose level, without producing condition of hypoglycemia after treatment, together with lesser loss in the body weight as compared with standard positive control drug glibenclamide.

Conclusions:

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Treatment of diabetes with sulfonylurea drugs (glibenclamide) causes hypoglycemia followed by greater reduction in body weight, which are the most worrisome effects of these drugs. *Stevia* extract was found to antagonize the necrotic action of alloxan and thus had a re-vitalizing effect on β-cells of pancreas.

Keywords: Alloxan-induced diabetic rats, antidiabetic activity, benzene:acetone extract, Compositae, *Stevia rebaudiana*

As a devastating disease, diabetes is affecting approximately 3% of the population worldwide, 90% of which suffer from type 2 diabetes. [1] The World Health Organization (WHO) estimates that more than 180 million people worldwide have diabetes and this number is likely to more than double by 2030 and an estimated 1.1 million people died from diabetes in 2005. WHO estimates that over the next 10 years (2006–2015), China will lose $ 558 billion in foregone national income due to heart disease, stroke and diabetes alone. [2] India leads the world with the largest number of diabetic subjects, earning the dubious distinction of being termed the “diabetes capital of the world”. According to the Diabetes Atlas 2006 published by the International Diabetes Federation, the number of people with diabetes in India, currently around 40.9 million, is expected to rise to 69.9 million by 2025 unless urgent preventive steps are taken. [3]

Stevia rebaudiana (Bertoni) is one of the 950 genera of the Compositae (Asteraceae). The plant was rediscovered by Dr. Moises Santiago Bertoni in 1887. The plant was used extensively by Gaurani Indians for more than 1500 years. [4] *Stevia* has a long history of medicinal use in Paraguay and Brazil, and while many of the therapeutic applications of Stevia are anecdotal, they must be considered in that they have spanned generations. There are now known to be more than 150 *Stevia* species but this is the only one with significant sweetening properties; other species do contain other biochemicals of interest. Leaves contain approximately 4–15% of steviosides, which are intensely sweet compounds (150–300 times sweeter than sugar). The leaves have been traditionally used for hundreds of years in Paraguay and Brazil to sweeten local teas, medicines and as a “sweet treat”. [5]

S. rebaudiana possesses various activities like antimicrobial, [6] antifungal, [7] hepatoprotective, [8] hypoglycemic (water extract), [9] antitumor, [6] antirotavirus, [10] anti-HIV, [11] anti-hypertension, [12,13] antiviral activity, [14] etc. Other folk applications of *Stevia* and stevioside (primarily in Latin America and the Orient) include the following: stimulate alertness and counter fatigue; facilitate digestion and gastrointestinal functions; regulate blood glucose levels (BGLs); nourish the liver, pancreas and spleen; help the body sustain a feeling of vitality and well-being and external application for blemishes. Some *Stevia* and stevioside users report a decrease in desire for sweets and fatty foods. Additionally, some users have reported that drinking *Stevia* tea or *Stevia* enhanced teas helped to reduce their desire for tobacco and alcoholic beverages. [15] *Stevia* and stevioside have been shown in studies to inhibit the growth and reproduction of some bacteria that are responsible for tooth decay. [15,16]

Studies on the comparative effects of leaves and stevioside on glycemia and hepatic gluconeogenesis have already been reported. [17] Hypoglycemic effect [18] of stevioside has also been studied, together with protective effects of stevioside against the toxic actions of alloxan. [19] Chen *et al*.[18] suggested that stevioside was able to regulate BGLs by enhancing not only insulin secretion, but also insulin utilization in insulin-deficient rats; the latter was due to decreased PEPCK gene expression in rat liver by stevioside's action of slowing down gluconeogenesis. Further studies of this agent for the treatment of diabetes appear warranted. These studies on hypoglycemic actions were centralized on stevioside, a polar molecule, which can be extracted completely either with methanol or with water, [20] whereas non-polar and medium-polar solvents like *n*-hexane, benzene, methylene dichloride, ethyl acetate acetate and have lesser affinity toward extraction of stevioside. Polar (methanol and water) extracts containing stevioside are well studied for hypoglycemic action, whereas low-polar and medium-polar extracts are yet to be investigated. Therefore, our group decided to study the hypoglycemic effects of medium-polar extractive of its leaves. For this, we generated medium-polar solvent by mixing benzene:acetone in 1:1, v/v ratio. Benzene, being toxic in nature, was selected because it dissolves fatty

acids/esters, acetylenes and less to medium-polar plant components. Traces of benzene were removed before the use of extract on animals.

During our investigation of this sweet herb of Paraguay, we carried out extraction of its leaves with benzene:acetone, 1:1, v/v, after defatting the plant material with *n*-hexane. This medium-polar extract was taken for evaluation of *in vivo* antidiabetic effects to assess its hypoglycemic (antidiabetic) value.

Materials and Methods

Go to:

Plant material

Leaves of *S. rebaudiana* were purchased from Sun Fruits Ltd., Pune (India), which were pulverized manually through hands and filtered with a sieve of mesh size 14. A portion (1.0 kg) of these leaves was kept for extraction. *S. rebaudiana* was authenticated by Dr. Gyanendra Tiwari (Taxonomist, Department of Fruit Science, K. N. K. College of Horticulture, Mandsaur) and a voucher specimen (MIP/PD/HN/*Stevia*/S-01) of the plant was deposited in the herbarium of Department of Pharmacognosy, Mandsaur Institute of Pharmacy, Mandsaur (MP), India.

Extract preparation

Dried leaves of *S. rebaudiana* plant (1.0 kg) were first defatted with several extractions of *n*-hexane and then these leaves were again extracted using benzene:acetone in the ratio of 1:1, v/v. The medium-polar extract of the leaves thus obtained was distilled and simultaneously dried *in vacuo* using rotatory evaporator (Bóchi, Switzerland). Benzene was removed completely by distillation, making an azeotropic mixture with alcohol–water. [21] A portion (20 g) of this dried extract was stored under refrigeration at 4.0 ± 2°C until used for biological activity.

Thin-layer chromatographic profiles of *Stevia* extract

Few chromatographic signatures (plant metabolite profiling) of medium-polar (benzene:acetone) extract of *S. rebaudiana* were developed using thin-layer chromatography (TLC) coupled with densitometric detection. For this, we optimized three different mobile phases for three different categories of metabolites (non-polar, medium-polar and polar) present in benzene:acetone extract.

Non-polar, medium-polar and polar constituents of the extract were separated onto TLC plates [Figure 1a–c] using hexane:diethyl ether, chloroform:methanol, and chloroform:methanol:water in ratios of 1:1, 85:15, and 60:36:4, (all v/v), respectively. Pure stevioside was used as reference / marker component, which was a kind gift from Dr. Jaroslav Pól (University of Helsinki, Finland).

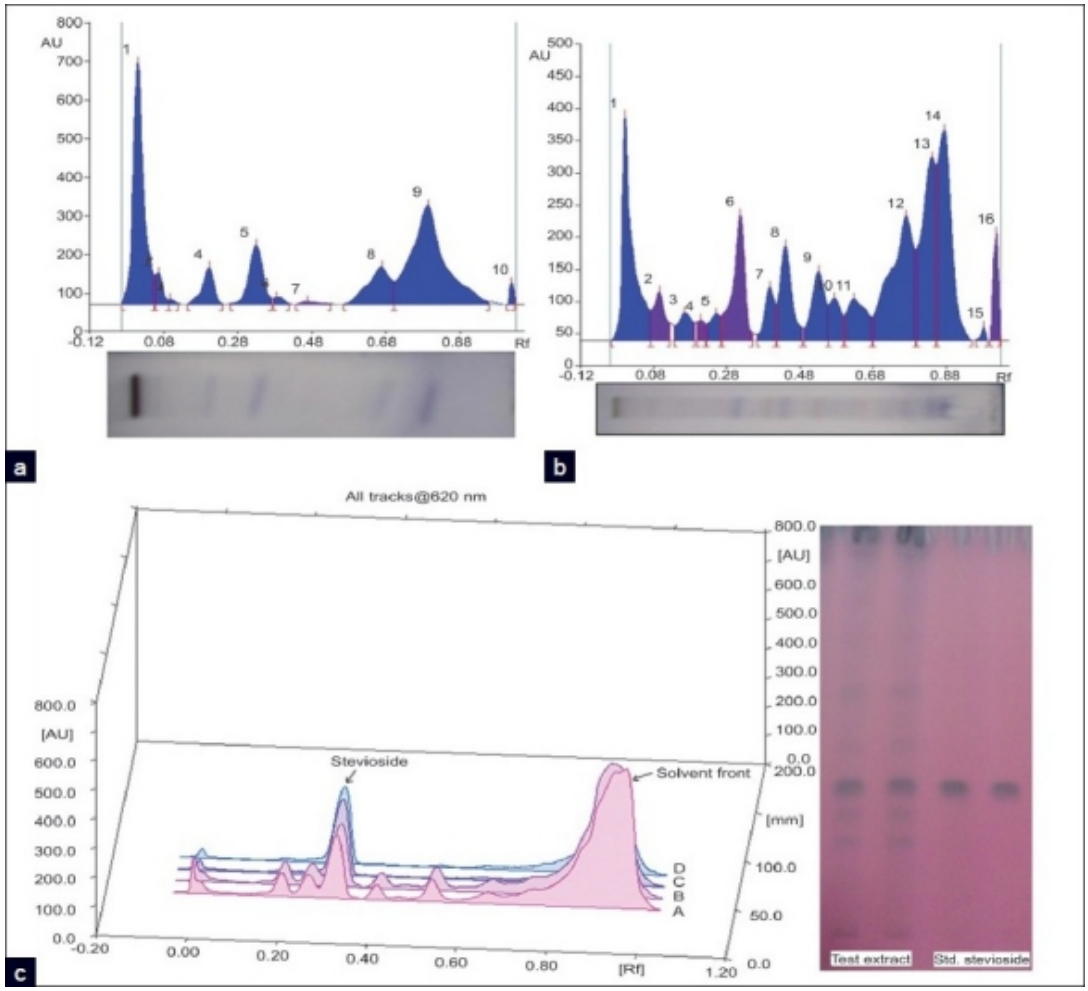

Figure 1

Non-polar (a), middle-polar (b) and polar (c) metabolite profiles of benzene:acetone extract [In (c), A and B = test benzene:acetone extract; C and D = standard stevioside tracks]

Extraction and determination of the extracted amount of stevioside

One gram sample of dried leaves was packed in an extraction thimble of Whatmann filter paper number 1 and extracted by hot soxhlet extraction over a water bath for 48 h. The extract thus obtained was dried *in vacuo* and redissolved in 10 mL methanol. Quantitative analysis was performed by spotting 5 μL of this test solution on pre-coated silica gel 60 F_{254} TLC plate (Item code: 1.05554.0007; Make: Merck, Darmstadt, Germany) against 5 μL of standard stevioside solution of concentration 1 mg/mL (methanol). TLC was developed in chloroform:methanol:water in a ratio of 60:36:4, v/v/v and allowed to stand in air for evaporation of the solvent completely. Dark green colored spots of well-resolved *Stevia* glycosides were visualized by heating derivatized plate on Camag TLC plate heater at at 110°C for 10–20 minutes. Post-chromatographic derivatization of TLC plate was performed with freshly prepared anisaldehyde–sulfuric acid reagent. [22] TLC was scanned via CAMAG TLC Scanner 3 in visible mode (tungsten lamp) at 620 nm. A calibration curve was plotted between increasing amounts of standard stevioside per spot and their peak area responses. A straight line was obtained between 1 and 10 μg/spot with a correlation coefficient (r) 0.99907 (r^2 = 0.998; sdv = 3.15). The linear regression equation ($y = mx + C$) was found to be $y = 268.2x + 52.07$, where y is the peak area, m is

slope of calibration curve, x is the concentration and C is the intercept. Stevioside content in benzene:acetone extract was found to be 0.45% dry leaves weight basis [Table 1].

Table 1

Stevioside content in benzene: Acetone extract

Extraction solvent	Stevioside content, % (dry leaves weight basis) (n = 3)	Mean stevioside content, % (dry leaves weight basis)	Standard deviation	Relative standard deviation, %
Benzene: acetone (1:1, v/v)	0.449 0.459 0.453	0.454	0.005	1.109

Chemicals and reference drugs

Alloxan monohydrate was procured from Loba Chemie, Mumbai, India, and other reagents used in the experiment were of analytical grade. Chemically, alloxan is 2,4,4,6-tetra oxo hexahydropyrimidine. Glibenclamide, a reference antidiabetic drug used in this study, was purchased from a local medical store (Aventis Pharma Ltd., Goa, India) and stored as per the instructions given on the pack (i.e., below 25°C).

Animals

Adult Wistar strain albino rats (120–140 g; n = 70) of either sex were obtained from B. R. N. C. P., Mandsaur animal house, after getting permission from Institution of Animal Ethics Committee (IAEC). The rats were maintained under standard laboratory conditions at 25 ± 2°C, relative humidity 50 ± 15% and normal photoperiod (12 h dark/12 h light) for the experiment. Standard pellet diet (Hindustan Lever Ltd., Mumbai, India) and water were provided *ad libitum*. The experimental protocol has been approved by the IAEC and by the Regulatory body of the government (Animal Ethical Committee number 1019/C/06/CPCSEA). Blood was collected by making a small cut at terminal tail vein for measuring BGL. Estimation of blood glucose was done by using Accu-Check Advantage blood glucose system (strip method).

Acute toxicity study

Acute toxicity study of extracts was carried out in albino rats of either sex by “up and down method” (OECD Guidelines 425). Animals were treated with extract (2000 mg/kg) and observed continuously for the first 4 hours for general behavioral, neurological, autonomic profiles and mortality within 24 h. One-fifth and one-tenth of safe dose was selected as the experimental dose. [23]

Evaluation of antidiabetic activity

Induction of diabetes

Hyperglycemia was induced in 18-h fasted adult Wistar rats (n = 50) weighing 120–140 g by a single intraperitoneal (i.p.) injection of freshly prepared alloxan monohydrate (180 mg/kg) [24] dissolved in normal saline; a 20% glucose solution was also injected intraperitoneally after 4–6 h. The rats were then kept for the next 24 h on 5% glucose solution bottles in their cages to prevent hypoglycemia. [25] Fasting BGLs were estimated by commercially available glucose kit based on glucose oxidase method. [26] The elevated glucose level in plasma, determined at 48 h after injection, confirmed hyperglycemia.

Rats with blood glucose more than 250 mg/dl were included in the study (n = 31). 1 unit of insulin i.p. was also given to prevent motility (due to triphasic response) after induction of diabetes. [23,27]

Experimental design

Animals were divided into five groups (five animals in each group). The first group (control/sham) received normal saline (0.9%) and the second group received alloxan monohydrate (180 mg/kg) and served as negative control. Groups from second to fifth were alloxan treated groups (diabetic animals). The third group received antidiabetic reference drug glibenclamide (10 mg/kg) as positive control. The remaining (fourth and fifth) groups received 200 mg/kg (body wt.) and 400 mg/kg (body wt.) of Stevia extract. The blood glucose concentrations of the animals were measured at the beginning of the study and the measurements were repeated on 3^{rd}, 7^{th} and 10^{th} days. [28] All the animals were regularly observed for their general behavior. Changes in the body weight were also measured.

Statistical analysis

All values were expressed as mean ± standard error mean (SEM). The differences were compared using one-way analysis of variance (ANOVA) followed by Dunnet's test. P values <0.01 were considered as significant.

Results and Discussion

Go to:

Alloxan, a β-cytotoxin, destroys β-cells of islets of Langerhans of pancreas, resulting in a decrease in endogenous insulin secretion and paves the way for the decreased utilization of glucose by the tissues. [29–31] *In vitro* studies have shown that alloxan is selectively toxic to pancreatic β-cells, leading to the induction of cell necrosis. [32,33] The cytotoxic action of alloxan is mediated by reactive oxygen species, with a simultaneous massive increase in cystolic calcium concentration, leading to a rapid destruction of β-cells. [34] Decreased utilization of glucose by the tissues results in the elevation of BGL.

Expression of elevated fasting BGL confirms induction of diabetes in alloxan-induced experimental rats. The experiment focused on exploring the competence of medium-polar (benzene:acetone, 1:1, v/v) extract from the leaves of *S. rebaudiana* for medication of diabetes against positive control reference drug glibenclamide. The difference in the initial and final fasting BGLs of different groups in long-term (10-day) studies exposed a significant elevation in BGL in diabetic controls as compared with that of normal control, extract treated and glibenclamide treated rats. Treatment of BGL with Stevia extract indicates the effectiveness of the extract in experimental diabetic animals.

Medium-polar extract from leaves of *S. rebaudiana*, when administered orally (200 and 400 mg/kg) for 10 days, produced a significant ($P < 0.01$) dose-dependent reduction in BGL [Table 2] as well as in the body weight [Table 3], although body weight was regained by rats treated with both glibenclamide and Stevia extract. *Stevia* extract exhibited a significant control of BGLs in diabetic rats, together with lowest decrease in the body weight, as compared with glibenclamide. Alternative exogenous treatments to diabetes include dosage of insulin and sulfonylurea drugs (e.g., glibenclamide), which cause hypoglycemia followed by greater reduction in body weight are the most worrisome effects. Treatment with Stevia extract did not cause hypoglycemia as well as significant decrease in body weight of diabetic rats. *Stevia* extract was found to revitalize β-cells of pancreas, antagonizing β-necrotic action of alloxan.

Table 2

Effects of *Stevia* extract on blood glucose level (mg/dL) of diabetic rats

Groups	Regimen	Blood glucose level (BGL) (mg/dL)			
		0 Day	3 Day	7 Day	10 Day
Group I	Normal control	82.40 ± 1.63	83.04 ± 2.30	85.20 ± 1.96	83.00 ± 1.30
Group II	Negative control	88.22 ± 3.05	374.82 ± 11.03	480.40 ± 12.32	460.40 ± 15.32
Group III	Positive control	85.42 ± 1.75	390.88 ± 16.96	80.40 ± 4.729***	80.20 ± 3.23***
Group IV	Drug treated L.E . 200	86.60 ± 1.75	424.00 ± 18.12	258.80 ± 19.46**	99.00 ± 7.98**
Group V	Drug treated L.E. 400	88.41 ± 2.54	404.65 ± 14.63	198.80 ± 16.09**	93.69 ± 9.33**

Values expressed as mean ± SEM ($n = 5$). $^{**}P < 0.01$, $^{***}P < 0.001$ vs. negative control (one-way ANOVA followed by Dunnet's test). LE: Leaves extract

Table 3

Effects of *Stevia* extract on body weight of diabetic rats

Groups	Treatment	Body weight (g)			
		0 Day	3 Day	7 Day	10 Day
Group I	Normal control	120.00 ± 4.89	120.00 ± 3.59	126.00 ± 3.74	132.00 ± 1.74
Group II	Negative control (alloxan)	142.04 ± 4.14	135.70 ± 5.87	96.00 ± 2.45	90.45 ± 3.51
Group III	Positive control (gilbenclamide)	129.10 ± 5.48	122.00 ± 7.38	118.50 ± 5.83**	120.39 ± 6.49**
Group IV	Drug treated L.E. 200	130.06 ± 5.48	116.00 ± 4.00	124.50 ± 6.78**	127.20 ± 8.94**
Group V	Drug treated L.E. 400	136.02 ± 7.48	113.08 ± 8.78	126.70 ± 12.41**	132.50 ± 7.37**

Values expressed as mean ± SEM ($n = 5$). $^{**}P < 0.01$ vs. negative control (one-way ANOVA followed by Dunnet's test). LE: Leaves extract

Excessive hepatic glycogenolysis and gluconeogenesis associated with decreased utilization of glucose by tissue is the fundamental mechanism underlying hyperglycemia in the diabetic state. [35] Aberration of liver glycogen synthesis or glycogenolysis in diabetes may be due to lack of or resistance to insulin, which is essential to activate glycogen synthase system. The significant increase of liver glycogen level in *Stevia* extract-treated groups may be due to reactivation of the glycogen synthase system by improving insulin secretion. Diabetes is associated with weight loss. [36] The reversal of weight loss in extract-treated diabetic group indicates that the restorative effect of the extract may be due to the reversal of gluconeogenesis and glycogenolysis.

Experimental results also reflect that the *Stevia* extract is capable of reducing the oxidative state associated with diabetes. Alloxan produces diabetes by liberating oxygen-free radicals which cause lipid peroxide-mediated pancreatic injury. [37] The extract may scavenge free radicals and facilitate reconstruction of pancreatic cells to release more insulin and ultimately produces an antidiabetic effect.

Effects on blood glucose level

Administration of benzene:acetone extract (200 and 400 mg/kg) produced a significant ($P < 0.01$) dose-dependant reduction in BGL of alloxan-induced diabetic rats. Alloxanized rats of group II (negative control) suffered from hyperglycemia as they did not receive any drug, whereas alloxanized rats of group III (positive control) treated with the reference antidiabetic drug glibenclamide showed significant reduction in BGL to the required standard blood glucose level on the 7th day and the levels were continuously maintained up to 10th day. Rats of group IV treated with *Stevia* extract (200 mg/kg) showed nearly normal BGL (99.00 ± 7.98 mg/dL) value on the 10 th day, whereas group V rats treated with *Stevia* extract (400 mg/kg) also showed decrease in blood glucose level to nearly normal (93.69 ± 9.33 mg/dL) value, which is very close to 0 day BGL of group V. Table 2 shows that positive control

glibenclamide treated rats attained normalized BGL on the 7th day of treatment, whereas *Stevia* extract treated rats attained nearby normal BGL on the 10th day.

Effects on body weight

Administration of benzene:acetone extract of *Stevia* (200 and 400 mg/kg) produced a significant ($P < 0.01$) dose-dependent reduction in body weight of alloxan-induced diabetic rats. Group II (alloxan-induced negative control) rats revealed 4.46, 32.41 and 36.32% decrease in the body weight on 3rd, 7th and 10th days, respectively, with respect to 0 day control value. Group III (positive control with glibenclamide) rats revealed 5.50, 8.21 and 6.71% decrease in the body weights monitored on 3rd, 7th and 10th days of treatment, respectively. Rats in group IV treated with *Stevia* extract (200 mg/kg) revealed 10.81, 4.27 and 2.20% decrease in the body weight, while group V (400 mg/kg) rats revealed 16.87, 6.85 and 2.59% decrease in body weight on 3rd, 7th and 10th days, respectively, as compared with 0 day value. Least decrease in body weight was observed in group IV rats (200 mg/kg), i.e., 2.20% on the 10th day [Table 3].

Glibenclamide versus *Stevia* extract treatment

The effects of oral administration of medium-polar (benzene:acetone, 1:1, v/v) extract of *S. rebaudiana* leaves are shown in Tables 2 and 3. Experimental studies clearly reveal that the medium-polar extract from *S. rebaudiana* leaves (200 and 400 mg/kg) orally administered for 10 days produced a delayed but significant decrease in the blood glucose level, together with lesser loss in the body weight, as compared with standard positive control drug in the model of alloxan-induced diabetes in rats.

Effects on liver, renal and pancreatic weights

Table 4 shows the effect of medium-polar extract of *S. rebaudiana* on renal, pancreatic and hepatic weights of normal, diabetic and diabetic treated rats. A significant intergroup difference ($P < 0.05$) was observed in glibenclamide treated group and diabetic control group. The liver weight of the normal rats was greater as compared to that of the diabetic control rats and treated diabetic rats. As shown in Table 4, administration of alloxan decreased the liver mass to 1.15 ± 0.2 g/100 g body weight, which showed significant difference ($P < 0.01$) with respect to non-diabetic rats. The liver mass was increased in diabetic treatment groups and glibenclamide treatment groups significantly ($P < 0.05$) with respect to diabetic control groups. Alloxan administration also caused a decrease in the pancreatic tissue weight. Treatment with the extract caused a significant increase in pancreatic tissue weight ($P < 0.05$) with respect to diabetic control. *S. rebaudiana* extract reduced the elevated kidney weight slightly as compared to untreated diabetic rats, although this did not reach statistically significant level.

Table 4

Effect on liver, pancreatic and kidney weights in diabetic rats

Groups	Treatment	Liver weight (g/100 g b.wt.)	Pancreatic weight (g/100 g b.wt.)	Kidney weight (g/100 g b.wt.)
Group I	Normal control	3.96 ± 0.75	0.6 ± 0.03	1.0 ± 0.14
Group II	Negative control (alloxan)	1.15 ± 0.2##	0.4 ± 0.05	0.9 ± 0.02
Group III	Positive control (gilbenclamide)	3.43 ± 0.032*	2.3 ± 0.36***	0.9 ± 0.02
Group IV	Drug treated L.E. 200	3.49 ± 0.15*	1.5 ± 0.09*	0.9 ± 0.0
Group V	Drug treated L.E. 400	3.52 ± 0.064*	1.58 ± 0.06*	0.9 ± 0.04

($n = 5$), Values expressed as mean ± SEM. *$P < 0.05$ values significant with respect to diabetic control, **$P < 0.01$ values significant with respect to non-diabetic control, ***$P < 0.001$ values significant with respect to diabetic control (one-way ANOVA followed by Bonferroni's Multiple Comparison Test)

Long-term pretreatment with sulfonylurea glyburide (GB) causes elevated basal insulin secretion (BIS) and decreased glucose-stimulated insulin secretion (GSIS). These characteristics may play an important role in the development of hypoglycemia and secondary failure. Results revealed that stevioside was able to counteract the desensitizing effects of GB and may be a putative new drug candidate for the treatment of type 2 diabetes mellitus. [38] Abudula *et al*. in 2004 [39] showed that rebaudioside A potentially stimulates insulin secretion from isolated mouse islets in a dose-, glucose- and Ca^{2+} -dependent manner. According to the study of Dyrskog *et al*., [40] rebaudioside A failed to show beneficial effects in diabetic animals. In continuation of the previous study, Abudula *et al*. in 2008 [41] reported the mechanism for the insulinotropic action of rebaudioside A.

According to the study of Gardana *et al*.[42] on the metabolism of stevioside and rebaudioside A from *S. rebaudiana* extracts by human microflora, both stevioside and rebaudioside A were completely hydrolyzed to their aglycon steviol in 10 and 24 h, respectively. Interestingly, the human intestinal microflora was not able to degrade steviol, which suggests that *Stevia* glycosides are zero calorie sweeteners and thus can be utilizable as a dietary supplement by diabetic patients or these sweeteners can also be used for preparing cough syrups.

Stevioside is not absorbed by the human gut; only bacteria of the colon degrade stevioside to steviol. Part of this steviol is absorbed by the colon and transported to the liver by portal blood. In the liver, steviol glucuronide is formed, which is released into the blood and filtered out by the kidneys into the urine. High levels of steviol glucuronide in the urine suggest that there is no accumulation of steviol derivatives in the human body. The steviol glucuronide still present is expected to be excreted in the urine during the next 24 h. Besides steviol glucuronide, no free steviol or any other possible steviol metabolite could be detected in blood or urine. Hepatic metabolism of steviol is extremely low, if existing at all, which is in agreement with the results of Koyama *et al*.,[43,44] who demonstrated by their *in vitro* experiments that the steviol metabolism by human microsomes was 4 times lower than that by rat microsomes, and the latter one was already very low. [44]

A recent *in vivo* study by Melis *et al*., in 2009, [45] carried out on 30 male rats, toward evaluation of the renal excretion of steviol suggested that steviol at all doses (0.5, 1.0 and 3.0 mg/kg/h) used, except the lowest (0.5 mg/kg/h), induced a statistically significant increase in glucose clearance when compared to control and exhibited a dose-dependent effect. In our medium-polar extract, the amount of stevioside was 0.45% (dry leaves weight basis) as determined by high-performance thin-layer chromatography (HPTLC) method. Thus, the antidiabetic (hypoglycemic) effects of this extract may be due to the presence of stevioside, rebaudioside A and other sweet glycosides, as was also shown in polar chromatographic signature/profile [Figure 1c] of benzene:acetone extract.

Conclusions

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In conclusion, the present data suggest that *Stevia* extract produced good antidiabetic effects together with lesser loss in body weight. Thus, purified *Stevia* sweeteners can also be used in the preparation of cough syrups and cold beverages for diabetes patients.

Acknowledgments

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Footnotes

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Conflict of Interest: None declared.

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