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ORIGNAL ARTICLE

Therapeutic Attributes of *Stevia rebaudiana* Leaves in Diabetic Animal Model

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ABSTRACT

Background: Medicinal plants contain organic chemicals with different properties. As synthetic medications can create deleterious effects, therefore, use of safe natural medicinal adjuncts like *Stevia rebaudiana* is endorsed.

Objectives: The purpose of this study was to assess the antidiabetic, antioxidant, antihyperlipidemic, hepatoprotective and renoprotective attributes of *Stevia rebaudiana* leaves in the diabetic rat model.

Methodology: Single dose of alloxan monohydrate was given to induce *Diabetes mellitus* in the rats. Plant extract treatment along with synthetic drug glibenclamide was given to rats for about 28 days to check their efficacies (antidiabetic, antioxidant, antihyperlipidemic, hepatoprotective and reno-protective) by using commercially available kits.

Results: Treatment showed a significant decrease in blood glucose, glycated hemoglobin HbA1c, and a rise in insulin, although it could not normalize these biomarkers after 28 days of treatment. Catalase (CAT) activity was restored yet it was not significantly improved in the case of Superoxide Dismutase (SOD) and Reduced Glutathione (GSH). Changes in lipid peroxidation products were trivial. Ingestion of *Stevia rebaudiana* significantly reduced Alanine Transaminase (ALT) and Aspartate Transaminase (AST) levels, however, changes in Gamma-Glutamyl Transpeptidase (GGT), and Total Protein (TP) were not significant. Similarly, treatment with *Stevia rebaudiana* reduced serum urea, creatinine and urinary albumin in diabetic animals.

Conclusion: It is established that *Stevia rebaudiana* leaves have multiple benefits and can be an exceptional nutraceutical.

Keywords	*Address of Correspondence	Article info.
Antidiabetic, Antioxidant, Hepatoprotective, Renoprotective, Stevia rebaudiana.	*fatmauaf@yahoo.com*	*Received: October 28, 2020* *Accepted: April 02, 2021*

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INTRODUCTION

Stevia rebaudiana Bertoni (Asteraceae family) is taken as a sugar substitute or artificial sweetener. Different types of glycosides present in Stevia leaves are stevioside, rebaudioside (A to E), steviolbioside, and isosteviols[1-4]. Stevioside with about 300 times more sweetness than sucrose has commercial value worldwide as an alternative sugar. Numerous studies have suggested that steviosides possess anti-infertility, hypotensive, antiseptic, diuretic, anti-fertility, cardiotonic, antimicrobial, anticancer, antidiabetic and antioxidant potentials[5-7]. Phytoconstituents contribute prominent insulinotropic, glucagonostatic, and antiplatelet effects[8]. *Stevia rebaudiana* leaves contain various antioxidant compounds such as ascorbic acid and phenolic compounds including flavonoids and tannins[9]. It is found that Stevia powder enhances the reduced level of glutathione[10]. One of the major therapeutic interventions to cure diseases is the use of medicinal plants, an option recently focused by researchers and the pharmaceutical sector. As synthetic medications can create deleterious effects, therefore, use

of safe natural medicinal adjuncts like *Stevia rebaudiana* is endorsed. Henceforth, current project was planned to assess the selected therapeutic effects of *Stevia rebaudiana* leaves in diabetic animals.

MATERIAL AND METHODS

Plant Sample Collection and Extract Preparation

S. rebaudiana leaves powder was extracted in methanol at ambient temperature. After solvent removal, it was dried on rotary evaporator. Same procedure was performed thrice after 3 days and final plant sample was reconstructed in dimethyl sulfoxide[11].

Experimental Design

Healthy albino male rats (weight 200-250g) were kept in stainless steel cages at adequate environmental conditions (25 ± 2˚C temperature, 60 ± 5% humidity, 12hours light-dark cycle). Ethical approval for trial was granted by institutional biosafety committee.

Induction of *Diabetes mellitus* was done by alloxan monohydrate (60mg/kg body weight) freshly prepared in normal saline as 5% solution. Rats with blood glucose levels more than 200mg/dL at fasting were assumed as diabetics. Animals were divided into four groups of seven animals each. First (Dc) and second (Nc) groups were diabetic control and normal control (non-diabetic), respectively. These groups were given standard diet and water *ad libitum*. Diabetic rats that received *S. rebaudiana* extract (12-15mg) and synthetic drug glibenclamide were included in third (Dse) and fourth (Dgd) groups, respectively. *S. rebaudiana* extract dose of 500ppm/kg body weight/day was given orally for 28 days. All the rats were slaughtered, blood sample and tissue homogenates were collected[12].

Biochemical Analysis

Antidiabetic (fasting glucose, glycated hemoglobin HbA1c, insulin), antioxidant (superoxide dismutase, lipid peroxidation product, glutathione, catalase), antihyperlipidemic (total cholesterol, HDL-C, LDL-C, triglycerides), hepatoprotective (AST, ALT, GGT), and reno-protective (serum urea, creatinine, urinary albumin) profiles were assessed by commercially available kits.

Statistical Analysis

The data were presented as mean ± SEM. A comparison among variables was assessed by analysis of variance technique with P-value < 0.05 as significant. SPSS software was used for data analysis.

RESULTS

Antidiabetic Potential

A momentous decrease in blood glucose, glycated hemoglobin HbA1c, and a rise in insulin were estimated after treatment with Stevia (Table. **1**). The diabetic control group had higher blood glucose levels as compared to normal control. In diabetic group treated with Stevia extract (Dse), significant decline (151.8 ± 29.49mg/dL) in elevated glucose concentrations was observed as compared to Dc group (265.6 ± 30.26mg/dL). However, synthetic drugs exhibited the highest hypoglycemic effect (120.1 ± 39.47mg/dL). Diabetic animals (Dc) had reduced insulin concentration (4 ± 0.3U/ml) and treatment with Stevia extract improved these levels in Dse group (7.5 ± 1.8U/ml). Whereas, Diabetic Glibenclamide Drug Group (Dgd) showed highly significant effect as drug treatment increased insulin levels (9.3 ± 1.7U/ml). Regarding glycated hemoglobin levels, significant decline was noted in the Dse group (6.9 ± 1.6%) as compared to Dc group (10.6 ± 0.7%). Glibenclamide treatment showed highly significant reduction (4.9 ± 0.7%) as compared to Dc group. Although treatment with Stevia extract exhibited significant changes in blood glucose, HbA1c and insulin levels, it could not normalize these biomarkers after 28 days of treatment.

Antioxidant Potential

Alloxan administration reduced hepatic antioxidant enzyme activities significantly by 59% for SOD, 31.7% for GSH, and 50.57% for CAT in diabetic animals (Table. **1**). Decreased concentrations of antioxidant enzymes in diabetes are associated with increased generation of Reactive Oxygen Species (ROS). When rats were fed with Stevia leaves extract, although CAT activity was restored yet it was not significantly improved in the case of SOD and GSH. CAT activity was enhanced by up to 37.7%. Changes in lipid peroxidation products were trivial in the present study.

Antihyperlipidemic Activity

Prominent antihyperlipidemic activity of plant extract was observed as the administration of *S. rebaudiana* extract reversed hyperglycemic condition by significantly reducing almost all lipid parameters, except High Density Lipoprotein-Cholesterol (HDL-C) that remained almost the same after treatment (Table. **1**).

Table 1. Antidiabetic, Antioxidant and Antihyperlipidemic Effects of *Stevia rebaudiana*.

Parameters / Potentials	Study Groups			
	Dc	Nc	Dse	Dgd
Antidiabetic profile				
Serum glucose (mg/dL)	265.6 ± 30.26	99.5 ± 13.12	151.8 ± 29.49*	120.1 ± 39.47**
Serum insulin (U/ml)	4 ± 0.3	12 ± 1.2	7.5 ± 1.8*	9.3 ± 1.7**
HbA1c (%)	10.6 ± 0.7	5.5 ± 0.6	6.9 ± 1.6*	4.9 ± 0.7**
Antioxidant profile				
Superoxide dismutase (Units/mg protein)	3.69 ± 0.18	9.20 ± 0.42	5.07 ± 0.31^{NS}	6.43 ± 0.42**
Glutathione peroxidase (Red) (Units/mg protein)	6.44 ± 0.29	9.43 ± 0.43	7.10 ± 0.30^{NS}	8.52 ± 0.43**
Catalase (Units/mg protein)	41.20 ± 3.76	83.33 ± 2.93	66.22 ± 3.16*	71.30 ± 3.60**
Lipid peroxidation (mM/100g of tissue)	1.77 ± 0.08	0.85 ± 0.03	1.51 ± 0.44^{NS}	0.96 ± 0.07**
Antihyperlipidemic potential				
Triglycerides (mg/dL)	166.81 ± 4.3	89.15 ± 6.37	136.33 ± 11.4*	78.32 ± 5.7**
Total cholesterol (mg/dL)	210.2 ± 4.77	54.67 ± 7.64	179.17 ± 3.49*	123.75 ± 2.80**
HDL-C (mg/dL)	20.86 ± 3.65	27.99 ± 7.21	23.892 ± 2.5	27.54 ± 1.67
LDL-C (mg/dL)	132.98 ± 0.9	8.85 ± 5.61	109.19 ± 1.81*	56.55 ± 2.97**

Data expressed as mean or percentage ± SEM of triplicate measurements for groups of seven animals each. Dc: Diabetic control group, Nc: Normal Non-diabetic control group, Dse: Diabetic stevia extract group, Dgd: Diabetic glibenclamide drug group.

* Significant P < 0.05 as compared to diabetic control.

** Highly significant P < 0.05 as compared to diabetic control.

NS: Non-Significant.

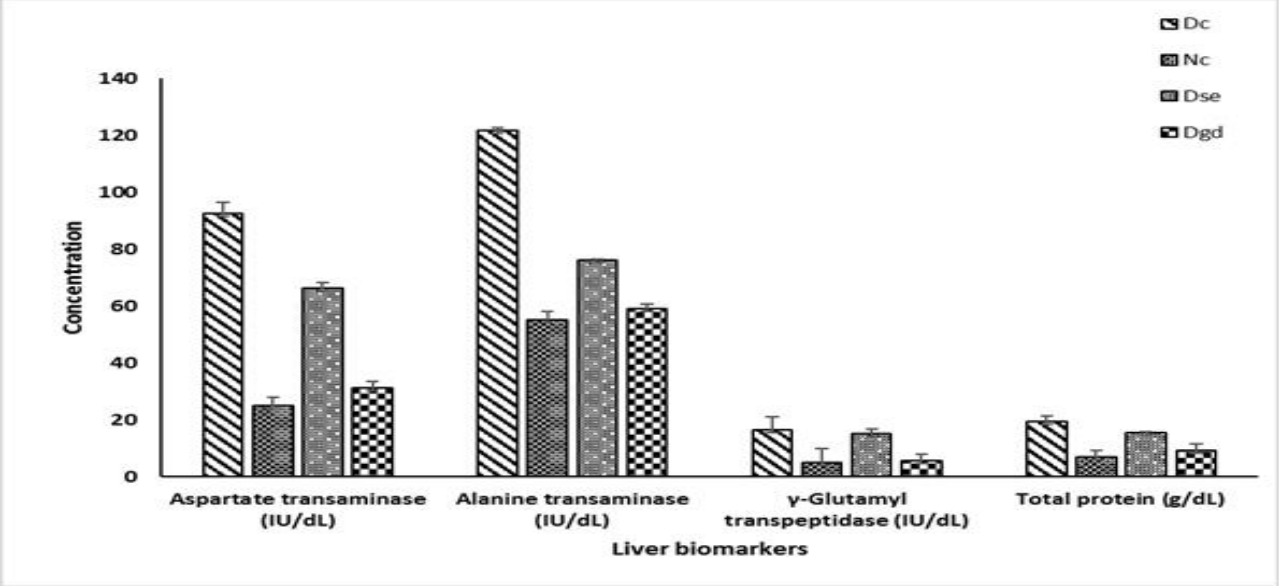

Figure 1. Hepatoprotective effect of *Stevia rebaudiana.*

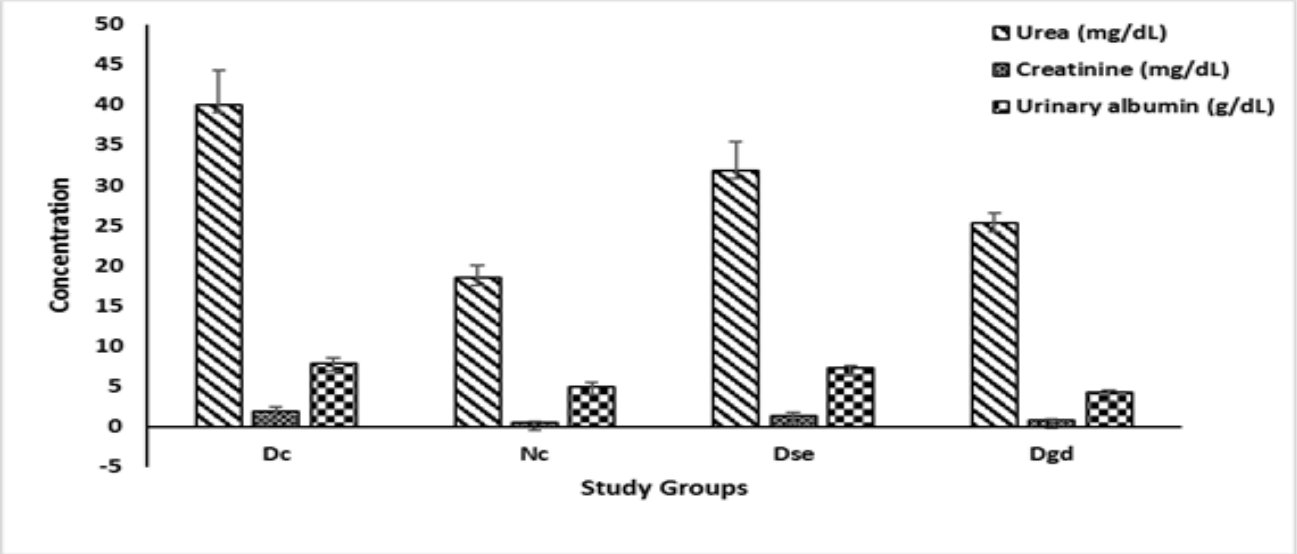

Figure 2. Renoprotective effect of *Stevia rebaudiana*.

Data expressed as mean or percentage ± SEM of triplicate measurements for groups of seven animals each. Dc: Diabetic control group, Nc: Normal Non-diabetic control group, Dse: Diabetic stevia extract group, Dgd: Diabetic glibenclamide drug group.

Hepatoprotective Activity

Ingestion of *Stevia rebaudiana* significantly reduced AST and ALT levels, however, changes in GGT and total protein were not significant (Fig. **1**). The diabetic group (Dgd) that received synthetic drug showed maximum reduction in hyperlipidemia, as drug ameliorated alloxan-induced hepatic damage normalizing the lipid profile.

Renoprotective Activity

Stevia rebaudiana had a protecting role as it declined renal function parameters (Fig. **2**). Treatment with *Stevia rebaudiana* reduced serum urea, creatinine and urinary albumin in diabetic treated group.

Data expressed as mean or percentage ± SEM of triplicate measurements for groups of seven animals each. Dc: Diabetic control group, Nc: Normal Non-diabetic control group, Dse: Diabetic stevia extract group, Dgd: Diabetic glibenclamide drug group.

DISCUSSION

Bioactive compounds like steviosides exert antihyperglycemic potential by affecting the pancreas to enhance insulin secretion[3-4, 13]. In current study, although Dse group exhibited decline in blood glucose, HbA1c levels, and increase in insulin concentration, but it was unable to normalize these parameters. Current results are in accordance with previous studies[13-16]. Insulin, PPARγ and mRNA expression levels are increased by treatment with Stevia. Steviosides and other bioactive ameliorate alloxan-induced hepatic damage, leading to an increase in insulin concentrations and hypoglycemic effect[13, 14]. Earlier, Shivanna *et al*.[17] stated that *Stevia rebaudiana* leaf extracts significantly reduced glucose and HbA1c. Steviosides may sensitize insulin receptors and stimulate pancreatic beta cells to release insulin in diabetic animals. The overall effect is improved carbohydrate metabolism[15]. Glycated hemoglobin levels were reduced by Stevia treatment (Dse group) as compared to Diabetic control group (Dc) but Stevia treatment was not effective in normalizing this biomarker. Phytoconstituents present in *S. rebaudiana* leaves may initiate glycogenesis[15] thereby, reducing HbA1c levels in Dse group and exhibiting an antidiabetic attribute.

Regarding antioxidant effects of stevia in present study, current results are not consistent with the previous reports that recognized the achievement of either normal or increased levels of antioxidant enzymes along with reduced lipid peroxidation after administration of stevia extracts[10, 17]. It has been reported that a new polyphenol family known as chlorogenic acid along with

hydroxycinnamic acid and quinic acid have exceptional antioxidant potential along with stevioside[18, 19]. Our results can be justified by the fact that stevia has the potential to reduce free radicals directly instead of moderating mitochondrial antioxidant enzymes system[20].

Reduction in Triglycerides (TG), Total Cholestrol (TC) and Low Density Lipoprotein-Cholestrol (LDL-C) might be due to activation of lipase activity or receptor up-regulation by saponin glycosides of *Stevia rebaudiana* leaves[21]. However, HDL-C level was increased by 14%. It is noteworthy that none of the lipid profile parameters was restored to the normal level either by plant extract or by the synthetic drug. The results of the present research are confirmed by earlier findings of Assaei *et al.,*[13]; Singh *et al.,*[14]; and Ritu and Nadini[16] that Stevia has the potential to ameliorate hyperlipidemia.

Assaei *et al.,*[13] and Najafi *et al.,*[22] stated that Stevia caused a decline in aminotransferases activities in treated rats and prevented hepatic damage. Previously, Ibrahim *et al*.,[9] reported that in diabetic control rats, serum ALT and AST levels were significantly higher than normal rats. In another study, Kuntal and Kathirity[23] observed that the levels of liver enzymes (AST, ALT, GGT) were significantly decreased with the administration of *Stevia* extract. Probably *Stevia rebaudiana* exhibits hepatoprotective potential due to its stimulation of nuclear factor-E2-related factor 2 Nrf2 expression, repression of nuclear factor kappa-B and obstruction of various profibrogenic signaling paths[24].

Renoprotective role of Stevia evaluated in the current study is supported by previous research works[17, 25-28]. It is suggested that Stevia attenuate diabetes-induced renal impairment by opposing oxidation, inflammation, and apoptosis through bio-signaling pathways[26].

CONCLUSION

It is concluded Stevia leaves have strong therapeutic potentials especially with reference to diabetic manifestations and it can be incorporated in daily diet as a value-added healthy component. Further studies on underlying mechanisms by which *S. rebaudiana* defends against different ailments should be planned.

ETHICAL APPROVAL

Institutional Biosafety/ Bioethics Committee gave ethical approval (D. No. 5497/ ORIC; dated: 11-10-2018) for *in vivo* animal trial under the guidelines of National Biosafety Committee (NBC) and Punjab Biosafety Rules 2014.

CONFLICT OF INTEREST

None.

FUNDING SOURCE

None.

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LIST OF ABBREVIATIONS

AST	Aspartate transaminase
ALT	Alanine transaminase
CAT	Catalase
Dc	Diabetic control
Nn	Normal non-diabetic control
Dse	Diabetic stevia extract
Dgd	Diabetic glibenclamide drug
GGT	Gamma-glutamyl transpeptidase
GSH	Glutathione (reduced)
HDL-C	High density lipoprotein cholesterol
LDL-C	Low density lipoprotein cholesterol
Nn	Normal non-diabetic control
PPARγ	Peroxisome proliferator-activated receptor-γ
ROS	Reactive oxygen species
SEM	Standard error mean
SOD	Superoxide dismutase
SPSS	Statistical package for social sciences
TC	Total cholesterol
TG	Triglyceride

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