<u>Research Article</u>

ANTIHYPERGLYCEMIC AND ANTIOXIDATIVE ABILITY OF *STEVIA REBAUDIANA* (BERTONI) LEAVES IN DIABETES INDUCED MICE

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ABSTRACT

The main objective of the study was to evaluate the antihyperglycemic, antidyslipidymic and antioxidative properties of methanolic leaf extract of *Stevia rebaudiana* in alloxan induced diabetic mice. Four groups of mice were studied. Group NC consisted of control animals. Rest three were made diabetic with a single intraperitoneal injection of alloxan monohydrate. Group DR was treated for 21 days with leaf extract of *S.rebaudiana*. After completion of experimental duration mice were sacrificed and blood and organs were further used for detecting biochemical and histopathological changes. Alloxan administration resulted in higher blood glucose level as compared to normal animals. Further, serum lipid profile parameters such as total cholesterol (TC), triglyceride (TG), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) were also found to be significantly elevated, whereas high density lipoprotein (HDL) was reduced in diabetic animals. Oxidative damage in the tissues of diabetic animals shows a marked increase in the level of thiobarbituric acid reactive substances and reduced glutathione (GSH); and decrease in the activity of antioxidant enzymes such as Superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px). The daily treatment of methanolic extract of *Stevia rebaudiana* leaves at 300mg/kg for 21 days significantly lowered the blood glucose level and elevated hepatic glycogen content. Moreover, the levels of various lipid profile parameters also reversed towards normal. TBARS and GSH were also restored towards normal. Significant antihyperglycemic and antioxidant potential of the *Stevia rebaudiana* extract indicated that it may be used in the management of diabetes and resultant oxidative stress.

Keywords: *Stevia rebaudiana,* Antioxidant, Alloxan, Antidiabetic

INTRODUCTION

Stevia rebaudiana (Bertoni), commonly known as 'sweet leaf of Paraguay' is a herbaceous perennial plant of the Asteraceae family, native to Paraguay, where it grows wild in sandy soils [1]. It was used extensively by Gaurani Indians for more than 1500. This plant was rediscovered by Dr. Moises Santiago Bertoni in 1887. *S. rebaudiana* has been reported to posses sweetening property as well as have therapeutic values such as antihyperglycemic, anticancerous [2,3], and also act as antihypersensitive agent [3]. It helps in the prevention of dental cavities [4] and can also inhibit bacterial and fungal growth [5]. It has been found to be non toxic, non addictive, non mutagenic, non teratogenic and is devoid of genotoxic effect. Sweetness of this plant is attributed to the presence of natural active components present in the leaves that is stevioside, rebaudiosides A,B,C,D and E, dulcoside A and steviolbioside. Leaves contain approximately 4-15% of steviosides, which are intensely sweet compounds (150-300 times sweeter than sugar). Moreover, it has been reported that *S. rebaudiana* shows ability to maintain blood glucose level with glucose tolerance enhancement in diabetic patients [6].

Diabetes mellitus (DM) being a chronic metabolic disorder, is characterized by hyperglycemia and disturbances in the metabolism of carbohydrates, lipids and proteins. Apart from hyperglycemia [7] increased oxidative stress is also reported to play a major role in the parthenogenesis of this disease. Oxidative stress occurs due to the glucose autoxidation and glycation of proteins [8], which thereby depletes the antioxidant defence system [9] and thus promotes free radical generation [10].

In modern medicine, the beneficial effects of drugs on glycemic levels are well documented but their preventive activity against progressive nature of diabetes and its micro and macrovascular complications are not always effective [11]. Moreover, synthetic antioxidants are suspected to be carcinogenic and hence are in no more use [12]. Before the discovery of insulin, the only option for the treatment of diabetes and related consequences were the traditional practices. The only ethically approved drug for the treatment of diabetes till today is metformin, which is derived from a medicinal plant *Galega officinalis* [13]. Therefore, the search for antidiabetic and antioxidative agents from natural origin has been greatly felt in recent years [14]. The major merits of herbal medicine seem to be their efficacy, low incidence of side effects and low cost [15].

The present study is an attempt to investigate the antidiabetic, antidyslipidemic and antioxidative properties present in S.*rebaudiana*, which help in managing diabetes in alloxan induced mice.

MATERIAL AND METHODS

Chemicals

Alloxan monohydrate was purchased from CDH (India). All other chemicals used in the study were of analytical grade and obtained from HIMEDIA (India), SRL (India) and Qualigens (India). ERBA Mannheim (Transasia Bio medicals Ltd. Daman, India) kits were used for the estimation of Total cholesterol, Triglycerides and HDL cholesterol.

Animal care and diabetes induction

Healthy swiss albino mice (4-5 months old, weighing 20 -30 gms) were procured from C.C.S Agricultural University, Hissar. Prior to the commencement of the experiment, all the mice were acclimatized to the new environmental conditions for one week. They were housed under standard laboratory conditions of 12 hours light and 12 hours dark cycle, fed with standard pellet diet. Maintenance and treatment of all the animals was done in accordance with the principles of Institutional Animal Ethics Committee constituted as per the directions of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India.

All experimental mice were divided into 4 groups each containing 7 mice. Group 1-Normal control, Group 2- Alloxan-induced diabetic control, Group 3- Standard drug treatment (glibenclamide) and Group 4- Crude methanolic extract of leaf of plant *Stevia rebaudiana* (Bertoni). Experimental mice of group 2, 3 and 4 were made diabetic by a single intraperitoneal injection of alloxan monohydrate with a dose of 150 mg/kg body weight in overnight fasted mice. Subsequent to diabetes induction, the mice had free access to food and water, and were provided with 50% glucose solution to drink overnight to counter drug induced hypoglycemic shock. One week after alloxan injection, the fasting blood glucose (FBG) concentration was determined by means of one touch ultra glucometer (Johnson & Johnson Company, USA) and compatible blood glucose strips [16]. Mice showing fasting blood level greater than 140 mg/dl were considered diabetic [17] and were selected for treatment with drug (10 mg/kg body wt.) or leaf extract (300 mg/kg body wt.).

Preparation of crude extract

Taxonomically identified leaves were procured from Vallabh Bhai Patel University of Agriculture and Technology, Meerut. Dried leaves were subjected to size reduction to a coarse powder, which was then soxhlet extracted with methanol and consumption to dryness under reduced pressure in vacuum rotator evaporator. The extract was air dried till solid to semisolid mass was obtained. The suspension of leaf extract was prepared in 20% tween 20 in normal saline.

Biochemical assays

For estimating lipid profile, serum was isolated from the blood collected from reterorbital plexus of overnight fasted mice on day 21st of all experimental groups, and serum total cholesterol (TC), triglyceride (TG) and HDL-cholesterol were estimated using diagnostic kits. VLDL and LDL cholesterol were calculated as per Friedevald's equation:

$$VLDL\text{-}cholesterol = \frac{Serum\ triglyceride}{5}$$

LDL-cholesterol = Serum total-cholesterol – VLDL-cholesterol – HDL-cholesterol.

Results were expressed in mg/dl.

Liver, pancreas and kidney were removed, freed from adhering tissues and washed with ice-cold normal saline solution (0.9%). Weight of all the organs was taken only after drying the tissue.

1g tissue was homogenized in 10 ml of 0.2 M tris-HCl with the help of homogenizer. The homogenate was filtered and then centrifuged at 10,000 rpm for 20 minutes at 4°C. The supernatant obtained was used for estimation of superoxide dismutase [SOD] [18], glutathione peroxidase [GSH-Px] [19], reduced glutathione [GSH] [20] and thiobarbituric acid reactive substances [TBARS] [21].

Statistical Analysis

Results are expressed as mean ± Standard Error of Mean [SEM]. Statistical analysis was performed using one-way Analysis of Variance [ANOVA] followed by Tukey's post-hoc multiple comparison test using SPSS [version 16.0] and student's 't'-test using SigmaPlot [version 8.0]. The values of *P<0.05 were considered as statistically significant.

RESULTS

The present study has been designed to elucidate the role of methanolic leaf extract of *S.rebaudiana* in managing the sugar level and oxidative stress caused by alloxan induction.

Effect on body weight and blood sugar level

Alloxan administration resulted in reduction in the body weight of groups (Table 1). Further, blood sugar level was significantly increased as compared before and after alloxan induction. 21 day treatment with leaf extract causes a significant reduction of 39.84% (193.0 ± 63.57to 116.1± 41.03) in the sugar level of DR group.

Table 1: Effect of methanolic leaf extract of *S.rebaudiana* on body weight and fasting blood sugar level of experimental mice.

Paremeter	Days	NC	DC	SD	leaf
Body weight[gms]	BD	22.4 ± 2.3	22.2 ± 1.25	21 ± 0.81	23.8 ± 1.9
	0	22.6 ± 2.1[a]	21.7 ± 0.5[a]	20.2 ± 1.8[a]	22.1 ± 1.6[a]
	21	24.2 ± 1.9[b]	20 ± 1.15[b]	21 ± 3.4[b]	27.2 ± 4.7[b*]
Blood sugar level[mg/dl]	BD	96.6 ± 25.4	104 ± 29.2	112.8 ± 19.9	84.5 ± 17.28
	0	96.6 ± 25.4[a]	175.2 ± 26.3[a*]	151.8 ± 5.6[a*]	193.0±63.57[a*]
	21	101.8 ± 17.7[b]	197.6 ± 14.6[b]	135.6 ± 17.2[b]	116.1±41.03[b*]

Values are mean ± SEM of 7 observations. Before diabetes [Basal values]; Student's 't'- test is significant at *P<0.05. a*significant [*P*<0.05] difference, ainsignificant difference [*P*>0.05] compared to basal values; b* significant [*P*<0.05], b insignificant [*P*<0.05] difference compared to values obtained after alloxan injection.to basal values; c* significant [*P*<0.05] difference compared to values obtained after alloxan injection
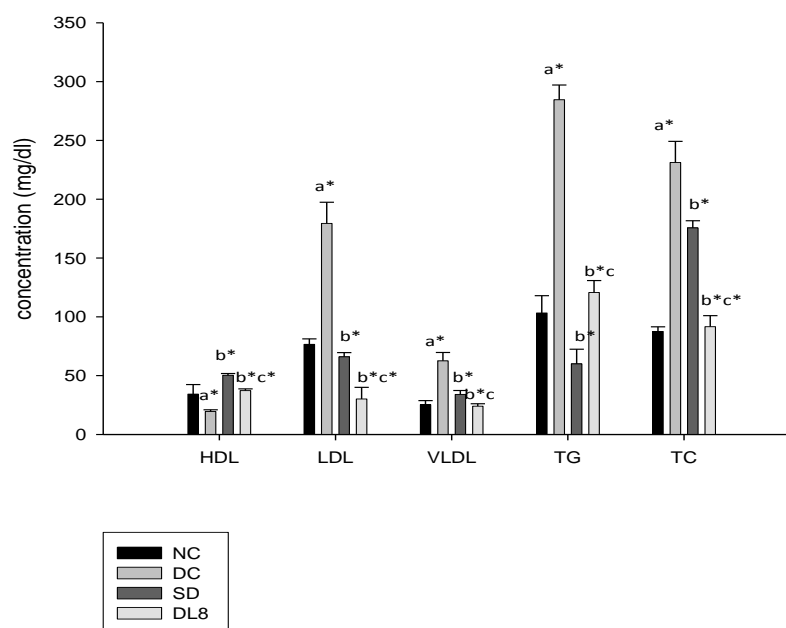
Fig. 1: Graphical representation of effect of methanolic leaf extract of *S.rebaudiana* on Total lipid profile of experimental mice.

Values are mean ± SEM of 7 observations. Student's 't'-test is significant at *P*<0.05. a*significant [*P*<0.05] difference compared to NC; b*significant [*P*<0.05] difference compared to DC; c*significant [*P*<0.05] difference and cinsignificant [*P*>0.05] difference compared to SD.

ANOVA between groups: TC – F = 86.410, *P*<0.05; TG – F =55.529 , *P*<0.05; HDL – F 22.448= , *P*<0.05; LDL – F = 115.288 , *P*<0.05; VLDL – F = 36.803 , *P*<0.05

Effect on serum lipid profile

Alloxan administration at the dose of 150mg/kg body weight resulted in significant increase in the level of serum Total cholesterol [TC], triglyceride [TG], low density lipoprotein [LDL] and Very low density lipoprotein [VLDL] in DC i.e. from 87.6 ± 3.9 to 231.2 ± 18; 103.3 ± 14.7 to 284.6 ± 12.5; 76.6 ± 4.7 to 179.4 ± 18 and 25.3 ± 3.5 to 62.6 ± 7.09 respectively compared to NC, whereas the level of high density lipoprotein significantly reduced from 34.3 ± 8.1 to 19.6 ± 1.5 in DC. However the methanolic leaf extract reduce significantly the level of Total cholesterol [TC], triglyceride [TG], low density lipoprotein [LDL] and very low density lipoprotein [VLDL] from 231.2 ± 18 to 91.6 ± 9.4; 284.6 ± 12.5 to 120.7 ± 10.1; 179.4 ± 18 to 30.2 ± 9.9 and 62.6 ± 7.09 to 24.1 ± 2 respectively as compared to DC, whereas the level of high density lipoprotein significantly increased from 19.6 ± 1.5 to 37.3 ± 1.5. Glibenclamide treatment reduced the level of TC, TG, LDL and VLDL by 231.2 ± 18 to 175.6 ±

6.11; 284.6 ± 12.5 to 60 ± 12.5; 179.4 ± 18 to 66 ± 3.6 and 62.6 ± 7.09 to 34 ± 3.4 respectively and enhances the level of HDL from 19.6 ± 1.5 to 50.3 ± 1.5 (Fig 1).

Biochemical parameters

Data of the present study revealed that the alloxan administration significantly reduced the activity of GSH (Fig 2) and GPx (Fig 3) in liver, pancreas and kidney. Treatment with leaf extract significantly alleviated the level of GSH and GPx in all selected tissues. However, the glibenclamide administration also proved to be sufficient in significantly increasing the level of GSH.

Induction of alloxan significantly increased the level of TBARS in hepatic, renal and pancreatic tissues (Fig 4). TBARS concentrations were significantly [p<0.05] increased after the treatment with leaf extract and were found to be convincingly higher than the glibenclamide treated group.
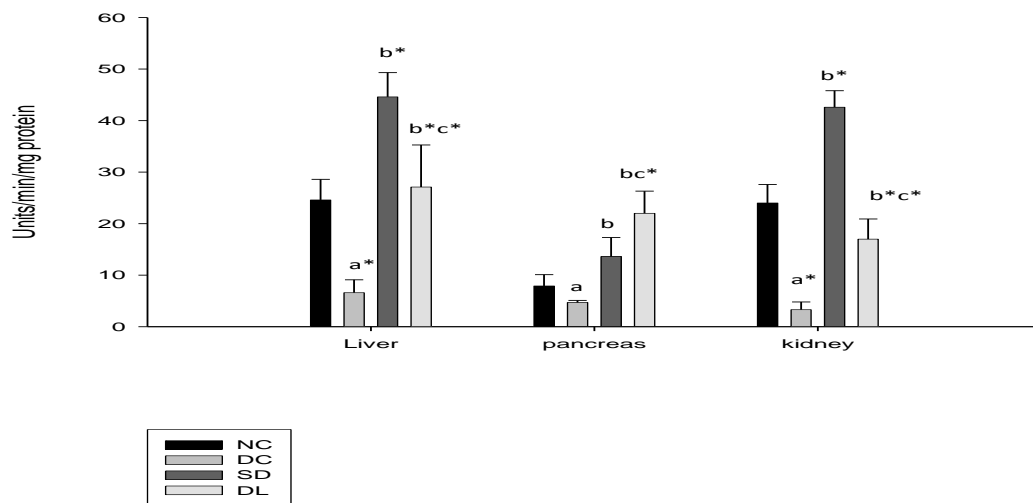
Fig. 2: Graphical representation of effect of methanolic leaf extract of *S.rebaudiana* on hepatic, renal and pancreatic GSH of experimental mice.
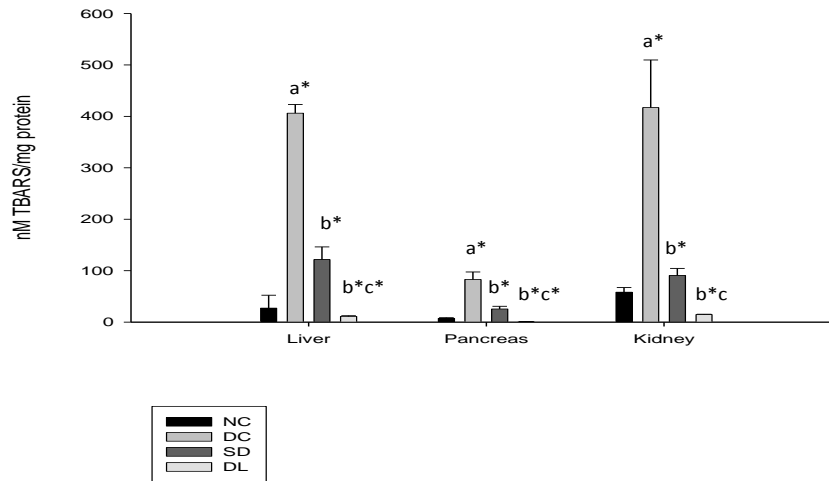
Values are mean ± SEM of 7 observations. Student's 't'-test is significant at *P*<0.05. [a*]significant [*P*<0.05] difference, [a]insignificant [*P*>0.05] difference compared to NC; [b*]significant [*P*<0.05] difference, [b]insignificant [*P*>0.05] difference compared to DC; [c*]significant [*P*<0.05] difference, [c]insignificant [*P*>0.05] difference compared to SD.

ANOVA between groups: Hepatic GSH – F = 159.1, *P*<0.05; Pancreatic GSH – F = 294.6, *P*<0.05; Renal GSH – F = 344.1, *P*<tissues0.05.
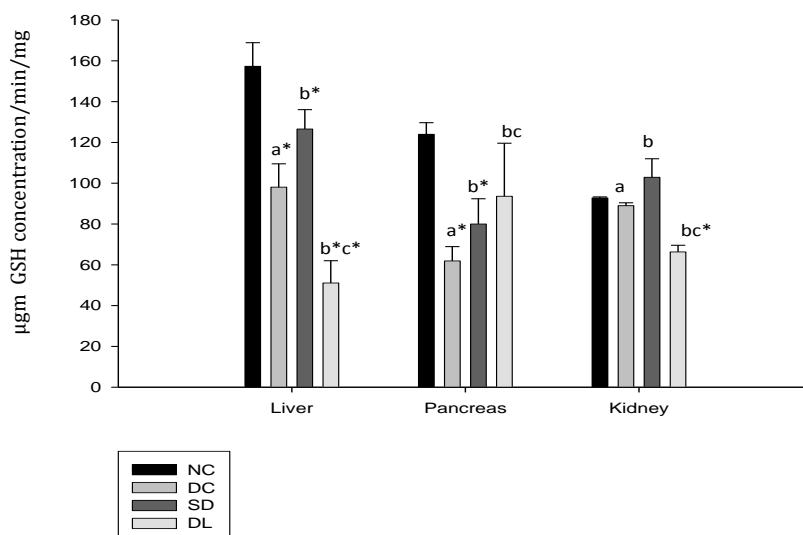
Fig. 3: Graphical representation of effect of methanolic leaf extract of *S.rebaudiana* on GPx of hepatic, renal and pancreatic tissues of experimental mice.

Values are mean ± SEM of 7 observations. Student's 't'-test is significant at *P*<0.05. [a*]significant [*P*<0.05] difference, [a]insignificant [*P*>0.05] difference compared to NC; [b*]significant [*P*<0.05] difference, [b]insignificant [*P*>0.05] difference compared to DC; [c*]significant [*P*<0.05] difference, [c]insignificant [*P*>0.05] difference compared to SD. Anova between the groups: Hepatic GPx – F =17.64, *P*<0.05; Pancreatic GPx – F =55.12, *P*<0.05; Renal GPx – F =26.93, *P*<0.05
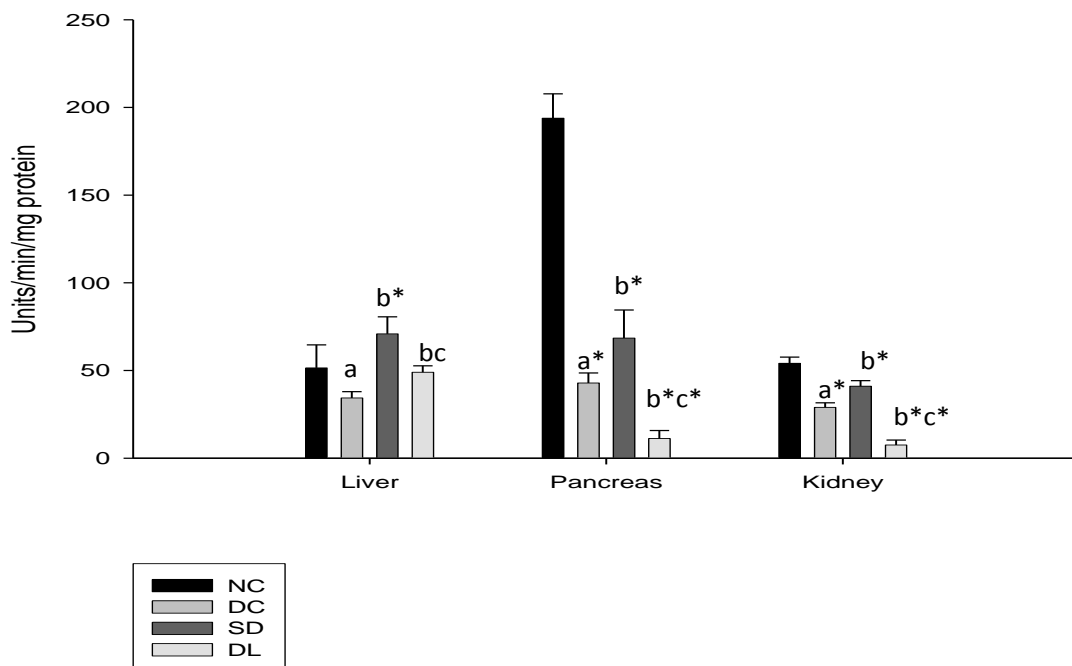
Fig. 4: Graphical representation of effect of methanolic leaf extract of *S.rebaudiana* on TBARS production in hepatic, pancreatic and renal tissues of experimental mice.

Values are mean ± SEM of 7 observations. Student's 't'-test is significant at $P<0.05$. [a*]significant [$P<0.05$] difference, [a]insignificant [$P>0.05$] difference compared to NC; [b*]significant [$P<0.05$] difference, [b]insignificant [$P>0.05$] difference compared to DC; [c*]significant [$P<0.05$] difference, [c]insignificant [$P>0.05$] difference compared to SD. ANOVA between the group: Hepatic TBARS – F =500.5, $P<0.05$; Pancreatic TBARS – F =77.06, $P<0.05$; Renal TBARS – F =51.90, $P<0.05$

Alloxan induction significantly [p<0.05] reduced the activity of SOD in kidney and pancreas but the decrease in the liver was not observed to be significant. The 21 day treatment with leaf extract did not significantly improve the activity in liver and pancreas but was found to be significant in kidney. But the glibenclamide treatment proved to be significantly capable in alleviating the activity of SOD in all organs (Fig 5).

Fig. 5: Graphical representation of effect of methanolic leaf extract of *S.rebaudiana* on SOD of hepatic, renal and pancreatic tissues of experimental mice.

Values are mean ± SEM of 7 observations. Student's 't'-test is significant at $P<0.05$. [a*]significant [$P<0.05$] difference, [a]insignificant [$P>0.05$] difference compared to NC; [b*]significant [$P<0.05$] difference, [b]insignificant [$P>0.05$] difference compared to DC; [c*]significant [$P<0.05$] difference, [c]insignificant [$P>0.05$] difference compared to SD. ANOVA between the groups: Hepatic SOD – F = 27.23, $P<0.05$; Pancreatic SOD – F =131.3, $P>0.05$; Renal SOD – F =85.06, $P>0.05$.

DISCUSSION

The present study is directed to investigate the role of leaf extract of *S.rebaudiana* in managing the diabetic complications in alloxan induced mice. For such an assessment body weight and FBG level along with the oxidative stress condition and lipid peroxidation level in hepatic, renal and pancreatic tissues of animals were studied.

Alloxan is a specific toxin that causes massive destruction of the pancreatic beta cells, provoking a state of primary deficiency of insulin without affecting other islet types and thus creating a hyperglycaemic condition [22]. In our study also, the blood sugar level was increased subsequent to alloxan administration. Diabetogenic effect of alloxan is due to excess production of reactive oxygen species [ROS] leading to cytotoxicity in pancreatic β-cells

which reduces the synthesis and the release of insulin, [23], while effecting organs such as liver, kidney, pancreas and haemopoitic system [24]. However, continuous treatment of diabetic mice with *S.rebaudiana* for 21 days considerably lowered the blood sugar level. This could be due to the presence of some biomolecules present in the plant extract 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 [25].

The characteristic features of diabetic dyslipidemia are a high plasma triglyceride concentration, low HDL cholesterol concentration and increased concentration of small dense LDL-cholesterol particles [26]. Hyperlipidemia is mostly coupled with hyperglycemia. High levels of triglycerides and LDL cholesterol are associated with high risk of coronary dysfunction, whereas increase in HDL cholesterol is associated with decrease in coronary risk [27]. The main cause of the lipid changes associated with diabetic dyslipidemia is the increased free fatty-acid release from insulin-resistant fat cells [28, 29]. The increased flux of free fatty acids into the liver in the presence of adequate glycogen stores promotes triglyceride production, which in turn stimulates the secretion of apolipoprotein B [ApoB] and VLDL cholesterol. The increased number of VLDL cholesterol particles and increased plasma triglyceride levels decrease the level of HDL cholesterol and increase the concentration of small dense LDL- cholesterol particles. In our study, the increase in the level of all parameters except the HDL in alloxan induced diabetic mice is in accordance with the previous findings of Howard [30]. However, with the treatment of leaf extract of *S.rebaudiana* for a period of 21 days normalize all the parameters attributing the antidyslipidemic ability of plant [31, 32, 33].

Complications in diabetes are attributed to various factors such as glucose auto oxidation leading to free radical generation, cellular oxidation/reduction imbalances and reduction of antioxidative potential. SOD protects cell wall by catalyzing superoxide ions into H_2O and H_2O_2 . Catalase works at high concentration of H_2O_2, and it readily detoxify it into H_2O and O_2. Peroxidases catalyze the degradation by oxidizing glutathione with the formation of its conjugates [34]. Results depicted in the present study revealed a decline in the antioxidant enzymes viz., SOD, GSH-px in the diabetic animals [35].

Treatment with leaf extract of *S. rebaudiana* does not show a considerable improvement in the SOD level of pancreatic and renal tissues. However, hepatic level was normalised in case of SOD. Moreover, treatment with leaf extract causes a significant increase in the level of GPx, suggesting its compensatory role in reducing H_2O_2 produced thus diminishing the toxic effects of free radicals produced by it in various secondary reactions.

GSH is a major non protein thiol in living organisms which plays a central role in coordinating the antioxidant defence process in the body. GSH plays a vital role in many cellular functions.GSH has reported in destroying reactive oxygen intermediates and free radicals formed during metabolism.It also protects membrane lipids and helps in transport of proteins across membrane [36]. In the present study the level of selected tissues GSH level was significantly decreased after the alloxan induction [37]. But after the extract treatment for 21 days, a significant increase in the GSH level was observed [38], which indicates that the leaf extract of *S .rebaudiana* have the potential to increase the biosynthesis of GSH which thereby reduces the oxidative stress. Lipid peroxidation is one of the characteristic features of chronic diabetes, and lipid peroxidation mediated tissue damage has been observed in diabetic conditions [39]. Hyperglycemia generates reactive oxygen species [ROS], which in turn cause lipid peroxidation and thus membrane damage [40]. In our study also, alloxan induction resulted in increase in the level of TBARS concentrations in diabetic mice. However, the treatment of methanolic leaf extract significantly reduced the level of TBARS.

CONCLUSION

It can thus be concluded from the results obtained that *S. rebaudiana* possess significant antihyperglycemic, antidyslipidemic and antioxidative properties. The bioactive component[s] responsible for

the observed activities are not precisely known but it may be one or more of the phytochemical constituents established to be present in the plant. However, further studies are needed to investigate and elucidate the possible mechanism of action of the active ingredients, establish complete safety profiles and evaluate the potential value of *S.rebaudiana* extract for the management of diabetes and hyperlipidemia in the clinic.

ACKNOWLEDGMENT

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