Ameliorative potential of *Solanum trilobatum* leaf extract and fractions on lipid profile and oxidative stress in experimental diabetes

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Abstract: *Solanum trilobatum* Linn is a medicinal plants used in India from many centuries to cure various diseases. The purpose of the study is to evaluate the ameliorative potential of the ethanolic leaf extract and fractions of *Solanum trilobatum* (*St*) against alloxan induced diabetic rats. *Solanum trilobatum* leaf extract and fractions were administered orally at two different concentration (100-200mg/kg body weight) to alloxan induced diabetic rats. The antidiabetic efficacy was validated through various biochemical parameters, enzyme assays, renal markers and antioxidant properties were also determined. The phytochemical analyses of *St* leaves were done by estimating their Chlorophyll, carotenoids, total sugar, protein, amino acid and minerals contents. The results revealed that the leaf extracts and fractions of *St* were efficient anti hyperglycemic agents and posses potent nephro-protective activity. However, the extracts of *St* leaves at a concentration of 200mg/kg bw exhibit higher efficacy in all tested concentrations. From the result it can be concluded that the leaf extracts of *St* can be a potential candidate in treating the hyperglycemic conditions and justifies its use in ethano medicine and can be exploits in the management of diabetes

Keywords: *Solanum trilobatum,* Alloxan, oxidative stress, antioxidant, hyperglycemia, hypolipidemic.

INTRODUCTION

Diabetes mellitus (DM) is a major health problem worldwide and among Asians the disease is feared to increase 2–3 folds in recent time (Jasemine Shabeer *et al*., 2009). The focus on plant research has improved throughout the world and the use of medicinal plants in various traditional systems has been established scientifically. Many plant products are used widely in folklore medicine because of their therapeutic potential. A multitude of herbs species and other plant materials have been described for the treatment of diabetes throughout the world (Kesari *et al*., 2006).

Solanum trilobatum Linn is one of the common Indian medicinal plants and it has been used in traditional medicine for many centuries (Mohan *et al*., 1998). The constitutents of this plant include sobatum, β solamarine, solaine, solasodine, glycoalkaloid, diosogenin and tomatidine. *St* has a broad spectrum of antibiotic, anticancer and antibacterial (Mohan *et al*., 1996) and also has hepatoprotective and antioxidant activities (Shahjahan *et al*., 2004, Doss A and Anand SP 2012). The leaves are used to cure cough, ear aches, lung diseases increases male fertility, acts against snake poisioning for curing cough, gastrics and ear aches (Govindhan *et al*., 2004). The paste prepared from the plant is used to cure tuberculosis (Subramni *et al*., 1989). Based on the above perception an attempt was made to determine the possible role of *St* against experimental diabetes and associated complications.

MATERIALS AND METHODS

Collection and preparation of plant material

The leaves of *St* were collected from Vellore District, TN, India. The plant materials were cleaned with distilled water, shade dried at room temperature and authenticated from the Department of Botany, C. Abdul Hakeem College, Melvisharam, Vellore Dt, Tamil Nadu and voucher specimens (CAHC-9c/2009) was stored. The dried leaves were coarsely powdered by using electric blender and stored separately in an air-tight container for further use.

The powdered leaf sample was first macerated in ethanol for 24 h for proper extraction, similarly the leaf sample was successively macerated for 24hr with ethyl acetate and methanol. The residue was removed by filtration. The filtrate was concentrated under reduced pressure in a rotary evaporator at 60±10^{o}C to yield required quantity of crude extract and the resultant extract was used for further studies.

Chemicals and solvents

Alloxan was purchased from SD Fine Chem. Limited, Mumbai, India and all other chemicals and solvents were of analytical grade and obtained from Fischer Inorganic and Aromatic Limited, Chennai, India.

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Proximal composition and mineral analysis

Dried leaves of *St* were used to evaluate their proximal composition. Chlorophyll and carotenoids (Arnon, 1949), total sugar (Dubois *et al.,* 1956), total protein (Lowry *et al*., 1951), total amino acid (Moore and Stein, 1948), total lipid (Folch *et al.,* 1957). Mineral content like Zinc, Phosphorus, Magnesium, Potasium, Manganese, Calcium and Sulphur was determined using atomic absorption spectrophotometer as per the method suggested by the Association of Official Analytical Chemists (AOAC, 1990) and express in per cent of dry matter.

Experimental animal

Experiments were performed using colony bred, sexually mature male albino rats weighing around 180-200g. Rats were provided with standard laboratory chow (Hindustan Lever Ltd., Bangalore. India). They had free access to water. The experiments were designed and conducted in accordance with the institutional guidelines (Reg. No: 1011/c/06/CPCSEA).

Acute toxicity studies

The acute oral toxicity study was carried out according to the guidelines set by OECD, 2001. 50mg/kg bw to 5000 mg/kg bw was evaluated for toxicity, the animals were maintained individually in the polypropylene cages and monitored continuously for two days for any changes like behavioral, neurological and autonomic profiles and for any lethality.

Experimental induction of diabetes

Diabetes was induced in male wister albino rats by intraperitoneal injection of alloxan monohydrate in normal saline by the method described by Nagappa *et al*., 2003. Two days after alloxan injection blood was taken from the tip of the tail vein and measured using Gluco Chek glucose estimation kit (Aspen diagnostic (P) Ltd. Delhi, India), animals with blood glucose level of 300-400mg/dl were taken for the study. During the study period animals were allowed free access to water and pellet diet and maintained at room temperature in polypropylene cages.

Experimental design

Assessment of antihyperglycemic effect of St leaves extract and fractions in normal and alloxan induced diabetic rats

Rats were divided in to seven groups of six rats in each group. (i) Normal control rats, (ii) alloxan induced untreated control rats (iii) alloxan induced rats treated with *St* extract (100mg/kg bw), (iv) alloxan induced rats treated with *St* extract (200mg/kg bw), (v) alloxan induced rats treated with with *St* (EA) fraction extract (200mg/kg bw), (vi) alloxan induced rats treated with with *St* (M) fraction extract (200mg/kg bw), (vii) alloxan induced rats treated with drug control glibenclamide (600 µg/kg bw) (Pari and Uma Maheswari, 2001).

Normal control and diabetic control rats were fed distilled water alone. The leaf extract and the drug glibenclamide were given in aqueous solution daily using an intragastric tube for 25 days. Fasting blood glucose was monitored for every week throughout the experiment.

Sacrifice study

At the end of the experimental period, the animals were deprived of food overnight and then sacrificed by decapitation. Blood was taken from the jugular vein and collected in two tubes, one with potassium oxalate and sodium fluoride solution for plasma and another without anticoagulant for serum separation. The kidney was immediately dissected out, washed in ice-cold saline, patted dry and weighed to measure their antioxidant status.

Estimation of blood glucose and body weight

The fasting blood glucose level was determined after 25 days of treatment with leaves extracts and drug control. The blood was collected from the tip of the tail vein from the overnight fasted rats and the blood glucose was measured using Gluco Chek glucose estimation kit (Aspen diagnostic (P) Ltd. Delhi, India). The results were expressed in terms of milligrams per deciliter (mg/dl) of blood. Body weight of all experimental animals was recorded using a digital weighing scale.

Estimation of insulin

Insulin was estimated using Radio immuno assay (RIA) kit supplied by Linco research Inc, Stat diagnostic, Mumbai, India.

Estimation of lipid profile

Total cholesterol (TC), high density lipoprotein (HDL-C) cholesterol and triglycerides (TG) were estimated using standard kits purchased from Transasia Bio Medical Limited, Mumbai, India. For the determination of very low-density lipoprotein (VLDL-C) and low density lipoprotein (LDL- C) cholesterol Friedewald's formula was used which states: VLDL cholesterol= Triglycerides/5 and LDL cholesterol = Total cholesterol-(VLDL-C + HDL cholesterol) (Friedwald *et al*., 1972).

Estimation of urea and creatinine

Urea, Uric acid and creatinine were estimated using standard reagent kits purchased from Coral clinical systems, Goa, India.

Estimation of antioxidant enzymes

Thiobarbituric acid reactive substances (TBARS) in kidney were estimated by the method of Fraga *et al*., 1998. The Hydro peroxide was estimated by the method of Jiang *et al*., 1992. The activity of SOD was assayed by the method of Kakkar *et al*., 1984, Catalase activity was estimated according to Abei's 1984, Glutathione per oxidase was assayed by the method of Paglia and Valentine's 1967.

STATISTICAL ANALYSIS

Values are presented as means ± SEM. The statistical significance was evaluated by one-way using the statistical software SPSS Version 17 (Origin Lab Corporation, USA). The data were analyzed by one way analysis of variance (ANOVA) followed by Tukey's test.

RESULTS

Proximal composition and mineral analysis of St leaf

Phytochemical screening of *St* leaves were analyzed on dry weight basis. The result of proximal composition shows higher values (fig 1). Mineral analysis also showed that the presence of high amount of phosphorus, nitrogen, potassium, magnesium, manganese, zinc, calcium and sulphur (fig 2).

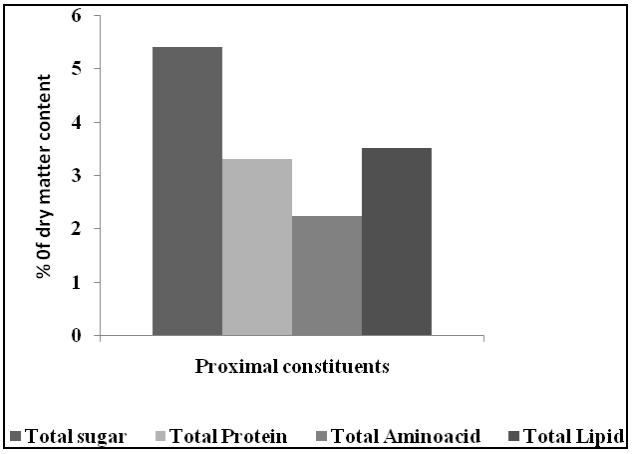

Fig. 1: Proximal composition of *St* leaf

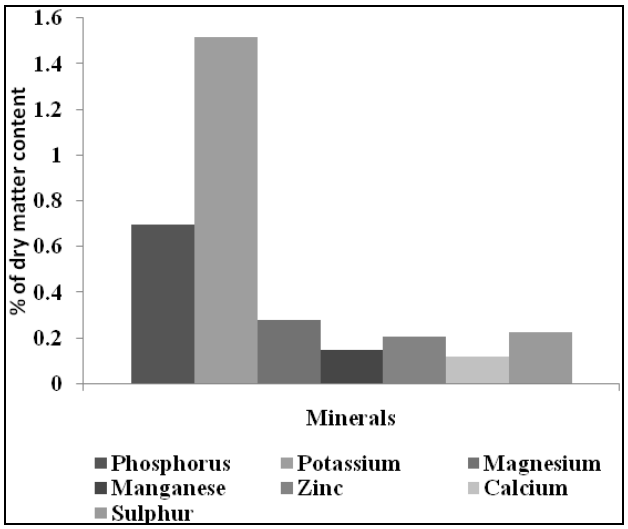

Fig. 2: Mineral content of *St* leaf

Acute toxicity studies

Acute toxicity study revealed the non-toxic nature of the extracts. There was no lethality or any toxic reactions found at any of the doses selected until the end of the study period.

Effects of St leaf extract and fractions on body weight, blood glucose and insulin in normal and experimental rats

Table 1 explicates the body weight, blood glucose and plasma insulin levels in normal and experimental animal groups. In alloxan induced diabetic animals, the body weight decreased significantly ($p<0.001$) when compared to the levels in normal animals. After administration of the leaves extract and fractions of *St* at a gradient dose of 100-200mg/kg bw the animals regained their body weight to near normal level. Similarly the levels of blood glucose and insulin levels was found to be altered in experimental rats, but after treatment the altered levels were brought back to near normal level. On prolong treatment for 25 days showed a significant ($p<0.001$) effect when compared to diabetic control groups. *St* M fraction treated group showed much significant changes having comparable antidiabetic effect.

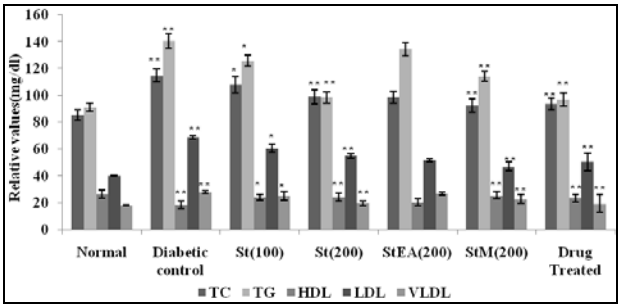

Fig. 3: Effect of *St* leaf extract and fractions on lipid profile in control and experimental group of animals.

The data were expressed as mean ± SEM and each value represents six individual observations, evaluated by one- way ANOVA followed by Tukey's test. 'P' denotes the statistical significance, *P<0.01, **P<0.001, ***P<0.0001. Diabetic control was compared with normal control and treated groups were compared with diabetic control.

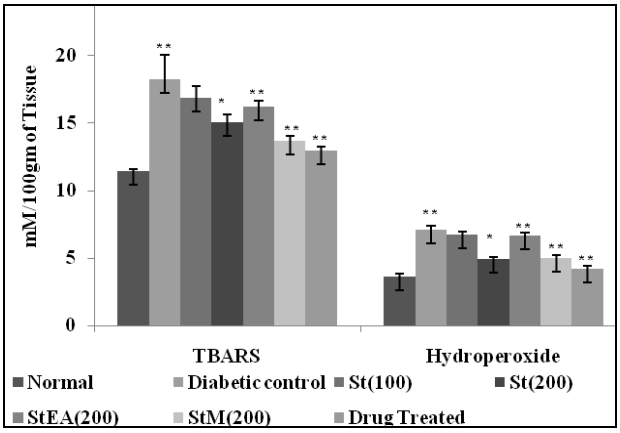

Fig. 4: Influences of methanol leaf extract of *St* on lipid peroxidation and hydroperoxide in kidney of control and experimental group of animals.

The data were expressed as mean ± S.E.M and each value represents six individual observations, evaluated by one- way ANOVA followed by Tukey's test. 'P' denotes the statistical

significance, * P<0.01, ** P<0.001, ** *P<0.0001, Diabetic control was compared with normal control and treated groups were compared with diabetic control.

Effects of St leaf extract and fractions on lipid levels in normal and experimental rats

Alloxan induction showed Significant increase in TC, TG, LDL-C and VLDL-C, where as HDL-C levels was significantly (p<0.001) reduced. Treatment with ethanolic leaf extract and fractions of *St* caused decrease in TC, TG, LDL-C and VLDL-C, similarly an increase in HDL-C levels were also observed. The observed changes were statistically significant (p<0.01- 0.001) (fig 3).

Effect of St leaf extract and fractions on hexokinase, Glucose -6-phosphatase, Fructose-1.6-bisphospatase and glycogen in normal and alloxan-induced experimental diabetes in rats

The levels of hexokinase, glucose-6- phosphatase, fructose-6-phosphatase and glycogen in normal and experimental animals in each group were summarized in table 2. A significant decrease (P<0.001) in hepatic hexokinase and glycogen levels was noted, simultaneous increase (P<0.001) in glucose-6- phosphatase and fructose-6-phosphatase level was observed in alloxan induced diabetic rats. Administration of *St* leaf extract and fractions normalized the altered levels. The observed changes were statistically significant (p<0.01- 0.001).

Effects of St leaf extract and fractions on urea, uric acid and creatinine levels in normal and experimental rats

Table 3 explains the levels of urea, uric acid, and creatinine in normal and experimental animals in each group. In diabetic rats, the all three tested renal markers urea, uric acids and creatinine were significantly increased (p<0.001) than the normal control Group. Administration of ethanolic leaf extract and fractions of *St* at dosage of 100 and 200mg/kg. b.w, reversed the altered changes to near normal levels.

Effects of St leaf extract and fractions on TBARS and hydro peroxides levels in kidney of normal and experimental rats

Significant increase (p<0.001) in TBARS and hydro peroxides was observed in alloxan induced experimental rats (fig. 3). Administration of *St* leaf extract and fractions leads to significant decrease in the levels of TBARS and hydro peroxides. Similarly, *St*(M) leaf extract administered Group showed much pronounce decrease in the levels of TBARS and hydro peroxides. This decrement was comparably equal to that of drug treated groups.

Effects of St leaf extract and fractions on SOD, CAT, GPx in kidney of normal and experimental rats

To validate the antioxidant property of the leaf extract of *St*, the activity of super oxide dismutase (SOD), catalase (CAT) and glutathione per oxidase (GPx) of kidney were measured (table 4). Reduced levels of antioxidants were observed in diabetic animals. Treatment with *St* leaf extract and fractions to experimental groups significantly increased (p<0.001) the levels when compared with diabetic control rats. The observed results were comparable with that of drug treated groups.

DISCUSSION

The present study was undertaken to evaluate the antidiabetic, hypolipidimic, reno protective and antioxidant status of *St* leaf extract and fractions on alloxan induced diabetes.

The proximal analysis shows that *St* leaves has a good source of carotenoids, carbohydrates, protein, lipids and minerals. It is proven that the minerals like Potassium, Sodium, manganese and iron are playing a vital role in various metabolic activities and responsible for normal growth and function of various organ systems. Potassium is necessary for muscular weakness, which is associated with malaria, and also slows down sclerosis of the vascular system. It contributes to fight against bacteria and cleanses the digestive system. Sodium takes part in the metabolism of water, promotes digestion, assimilation, osmosis, cleanses the digestive system, combats stomach acidity and alkalize the blood. Lack of magnesium can be responsible for tetany, tuberculosis, diabetes, cancer and all nervous diseases (Claude and Paule, 1979).

Manganese, according to Claude and Paule, 1979 is necessary for the functioning of the pituitary gland, the pineal gland and the brain. It promotes hepato-renal function, combat anaemia and it is also essential for growth. Iron is an energizer but excess can cause fatigue but we hardly have excess if taken from natural source (Gbolahan, 2001). The result of the mineral composition clearly suggests that *St* leaves contain rich source of minerals indicating the usefulness of the leaves to complement the cure of various ailments during treatment with this plant product (fig. 1).

Alloxan-induced diabetes is characterized by severe loss of body weight (Odetola *et al*., 2006), caused by the loss or degradation of structural proteins (Rajkumar *et al*., 1991). Diabetes affects both glucose and lipid metabolism (Bhagavan, 2002). In the post prandial state elevated serum insulin increases lipoprotein lipase activity in adipose tissue and promotes fuel storage as triglycerides in normal metabolism (Joy and Kuttan, 1999). Administration of extracts to diabetic rats showed a significant decrease in the blood glucose and an increase in the body weight, serum insulin levels (table 1). Hence, the possible mechanism by which *St* brings about its hypoglycemic action may be by potentiating the insulin

Table 1: Effect of *St* leaf extract and fractions on body weight, blood glucose and serum insulin levels in control and experimental group of animals.

Groups	Bodyweight (g)	Blood Glucose (mg%)	Plasma insulin (µU/ml)
I Normal control	189.43±3.64	119.61±3.46	13.83±0.51
II Diabetic Control (DC)	131.60±6.17**	298.63±19.63**	6.04±0.42**
% of Change (N Vs DC)	-30.52	+119.66	-56.32
III *St* Treated (100mg/Kg bw)	142.17±5.35	240.70±17.28*	8.63±0.44*
% of Change (Group III Vs DC)	+8.03	-19.39	+42.88
IV St Treated (200mg/Kg bw)	159.83±6.13*	198.34±15.62**	10.14±0.51**
% of Change (Group IV Vs DC)	+21.45	-33.58	+68.37
V *St* (Ea) Treated (200mg/Kg bw)	162.76±6.07*	239.46±17.18**	8.71±0.45*
% of Change (Group V Vs DC)	+23.67	-19.81	+44.20
VI *St* (M) Treated (200mg/Kg bw)	168.21±6.72*	163.56±12.47**	11.92±0.49*
% of Change (Group V Vs DC)	+27.81	-45.22	+97.35
VII Drug Treated	174.58±7.63*	142.17±10.16**	12.18±0.53*
% of Change (Group VII Vs DC)	+32.64	-52.39	+101.65

Table 2: Effect of *St* Leaf extract and fractions on Hexokinase, Glucose -6-phosphatase, Fructose-1.6-bisphospatase and Glycogen in control and experimental group of animals.

Groups	Hexokinase µmoles of glucose phosphorylated/ min/g protein	Glucose- 6-phosphatase (µmol of Pi liberated/ min/mg protein)	Fructose-1,6-bisphospatase (µmol of Pi liberated/h/mg protein)	Glycogen (mg/100 g tissue)
I Normal control	12.16±0.39	0.18±0.2	0.31±0.06	10.95±0.4
II Diabetic Control (DC)	8.39±0.32**	0.3±0.05**	0.51±0.03**	6.21±0.89
% of Change (N Vs DC)	-31	+66.66	+64.51	-43.28
III St Treated (100mg/Kg bw)	10.01±0.36	0.26±0.04	0.46±0.04	7.86±0.56
% of Change (Group III Vs DC)	+19.30	-13.33	-9.80	+26.24
IV St Treated (200mg/Kg bw)	10.64±0.32**	0.23±0.05**	0.41±0.03**	9.62±0.42**
% of Change (Group IV Vs DC)	+26.81	-23.33	-19.60	+54.91
V St (Ea) Treated (200mg/Kg bw)	9.78±0.37	0.25±0.03	0.44±0.04	8.44±0.3
% of Change (Group V Vs DC)	+16.56	-16.66	-13.72	+35.90
VI St (M) Treated (200mg/Kg bw)	10.95±0.34**	0.21±0.02**	0.39±0.02**	9.78±0.4**
% of Change (Group V Vs DC)	+30.51	-30	-23.52	+57.48
VII Drug Treated	11.02±0.33**	0.20±0.02**	0.38±0.03**	10.12±0.38**
% of Change (Group VII Vs DC)	+31.34	-33.33	-25.49	+62.96

The data were expressed as mean ± SEM and each value represents six individual observations, evaluated by one- way ANOVA followed by Tukey's test. 'P' denotes the statistical significance, * P<0.01, ** P<0.001, ***P<0.0001. Diabetic control was compared with normal control and treated groups were compared with diabetic control. + & - indicates the percentage of change over the diabetic control and treated groups.

effects of plasma by increasing either the pancreatic secretion of insulin from the existing beta cells or by its release from the bound form.

Hexokinase plays an important role in the maintenance of glucose homeostasis (Bopanna *et al*., 1997). In the diabetic state, several workers have observed increased activities of glucose-6-phosphatase (Venkateswaran and Pari, 2002). Activation of glucose-6-phosphatase is due to state of insulin deficiency since under normal condition insulin function as a suppressor of glucose-6- phosphatase enzymes. Administration of St and fractions showed a significant reversal on the enzyme activity (table 2). The reduction in enzyme activity corresponded to the decrease in serum glucose as less glucose was being produced and released into the blood stream. Glycogen is the primary intracellular storable form of glucose and its levels in various tissues especially skeletal muscle are direct reflection of insulin activity (Garvey, 1992). Treatment of *St* to diabetic rats significantly stimulates the secretion of insulin there by improved glycogen content of liver in experimental rats.

Table 3: Effect of *St* leaf extract and fractions on urea uric acid and creatinine levels in control and experimental group of animals.

Groups	Urea (mg%)	Uric acid (mg%)	Creatinine (mg%)
I Normal control	36.83±2.25	1.55±0.22	0.80±0.15
II Diabetic Control (DC)	67.5±3.81**	2.25±0.38**	2.01±0.29**
% of Change (N Vs DC)	83.27	+45.16	+151.25
III *St* Treated (100 mg/Kg bw)	57.50±3.05	2.0±0.30*	1.85±0.25*
% of Change (Group III Vs DC)	-14.81	-11.11	-7.96
IV St Treated (200 mg/Kg bw)	50.0±3.05*	1.90±0.27**	1.47±0.22**
% of Change (Group IV Vs DC)	-25.92	-15.55	-26.86
V *St* (Ea) Treated (200 mg/Kg bw)	55.33±2.71*	2.03±0.30**	1.83±0.26*
% of Change (Group V Vs DC)	-18.02	-9.77	-8.95
VI *St* (M) Treated (200 mg/Kg bw)	47.0±3.05*	1.85±0.26**	1.45±0.22*
% of Change (Group V Vs DC)	-30.37	-17.77	-27.86
VII Drug Treated	37.0±2.09*	1.6±0.22**	0.83±0.21*
% of Change (Group VII Vs DC)	-45.18	-28.88	-58.70

The data were expressed as mean ± SEM and each value represents six individual observations, evaluated by one- way ANOVA followed by Tukey's test. 'P' denotes the statistical significance, * P<0.01, ** P<0.001, ***P<0.0001. Diabetic control was compared with normal control and treated groups were compared with diabetic control. + & - indicates the percentage of change over the diabetic control and treated groups

Table 4: Effect of *St* leaf extract and fractions on SOD, Catalase, GPx in control and experimental group of animals

Groups	SOD[a]	CAT[b]	(GPx)[c]
I Normal control	13.81 ±1.84	36.13±1.61	5.6±0.91
II Diabetic Control (DC)	8.7±1.44**	20.41±1.48**	2.90±0.16**
% of Change (N Vs DC)	-37	-43.5	-48.21
III St Treated (100 mg/Kg bw)	10.1±1.8	21.2±1.58	2.98±0.15
% of Change (Group III Vs DC)	+16.09	+3.87	+2.75
IV St Treated (200 mg/Kg bw)	10.9±1.4*	28.7±1.32**	3.8±0.1**
% of Change (Group IV Vs DC)	+25.28	+40.61	+31.03
V St (Ea) Treated (200 mg/Kg bw)	10.6±1.32*	27.76±1.42*	2.96±0.2
% of Change (Group V Vs DC)	+21.83	+36.01	+2.06
VI St (M) Treated (200 mg/Kg bw)	11.4±1.22*	31.6±1.46**	4.2±0.1**
% of Change (Group V Vs DC)	+31.03	+54.82	+44.82
VII Drug Treated	13.2±1.42**	32.8±1.36**	4.9±0.13**
% of Change (Group VII Vs DC)	+51.72	+60.70	+68.96

The data were expressed as mean ± S.E.M and each value represents six individual observations, evaluated by one- way ANOVA followed by Tukey's test. 'P' denotes the statistical significance, *P<0.01, **P<0.001, ***P<0.0001, Diabetic control was compared with normal control and treated groups were compared with diabetic control. [a]50% inhibition of epinephrine auto oxidation / min [b]µmolH_2O_2/min/mg protein, [c]µmol glutathione/min/mg protein.

In diabetes, hyperglycemia is accompanied with dislipidemia (Garber, 2000) i.e., characterized by increase in TC, TG, LDL-C, VLDL-C and fall in HDL-C. An increase in these lipid profile was observed in our study and this altered lipid profile was reversed towards normal after treatment with the *St* extract and fractions. The elevated atherogenic index, i.e. TC/HDL ratio, which is a useful determinant of cardiovascular risk (Grover *et al*., 1999), was also shifted towards normal after treatment with *St* (fig. 3).

Hypercholesterolemia, hypertriglyceridemia and hyper urea have been reported to occur in alloxan diabetic rats (Resmi, 2001). Similarly, a significant rise in creatinine, a marker of renal function was found in the diabetic animals. Creatinuria occurs in any condition associated with extensive muscle breakdown as in starvation and poorly controlled diabetes mellitus (Ganong, 1995). The oral administration of *St* to diabetic rats decreased the altered levels to near normal.

Hyperglycemia results in the generation of free radicals, which can exhaust antioxidant defenses thus leading to the disruption of cellular functions, oxidative damage to membranes and enhanced susceptibility to lipid per oxidation (Giugliano *et al*., 1996; Van Dam *et al*., 1995).

The alloxan induced diabetes caused marked alterations in lipid per oxidation (fig. 4) and antioxidant enzyme (table 4) levels in kidney. Administration of plant extract reversed these levels to near normal levels, but higher reduction of enzymes was found in *St* M treated groups. Thus the antioxidant property of the extract is evident and synergistically it helps in treating diabetes mellitus.

In this study, the *St* extract and fractions reversed the complication associated diabetes to near normalcy, hence *St* exhibits remarkable potential to be an ideal candidate to pursue further exploration in developing the choice of an antidiabetic agent.

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