

## Free Radical Scavenging Activity of *Solanum trilobatum* Linn. on Alloxan - Induced Diabetic Rats

A Doss\* and SP Anand

PG & Research Department of Botany, National College (Autonomous), Tiruchirappalli- 60001, Tamil Nadu, India

### Abstract

*Solanum trilobatum* is reported to have a wide range of biological activities such as antidiabetic, antibacterial, antifungal, antimutagenic, antioxidant, antitumours and is used for treatment of asthma, to arrest blood vomiting and several kinds of leprosy. In the present study, we report the effect of ethanol extracts of *S. trilobatum* leaf on free radical scavenging activity, in alloxan induced diabetic rats. The lipid peroxidation, superoxide dismutase and catalase were measured in liver homogenate, and Serum Glutamic Pyruvic Transaminase (SGPT), Serum Glutamic Oxaloacetic Transaminase (SGOT), cholesterol and glucose were measured in blood serum. Oral administration of single dose of alloxan monohydrate (1mg/kg), caused significant increases in Lipid Peroxidation (LPO), SGOT, SGPT and glucose levels, while Superoxide Dismutase (SOD) and Catalase (CAT) levels were significantly decreased. Further, the administration of ethanol extracts of *S. trilobatum* leaf to alloxan induced diabetic rats, at a dose of 100 and 200 mg/kg orally for 10 days, led to a significant decrease in lipid peroxidation, SGPT, SGOT and cholesterol levels. Consequently, superoxide dismutase and catalase levels were significantly increased. Glibenclamide was used as a positive control (10 mg/kg). It was observed that the effect of ethanol extracts of *S. trilobatum* on cholesterol, superoxide dismutase, SGPT, SGOT levels, were compared to that of those produced by the positive control. The activities of SOD and CAT were found to be increased in extract treated diabetic rats in selected tissues. The increased level of lipid peroxidation in diabetic rats also was found to be reverted back, to near normal state in extract treated groups. Thus, it may be concluded that the ethanol extract of *S. trilobatum*, may produce its hypoglycemic effect through antioxidant defense mechanism.

**Keywords:** Antioxidant; *Solanum trilobatum*; Ethanol; SGOT; SGPT; CAT; SOD

### Introduction

Diabetes Mellitus (DM) is a common metabolic disease, characterized by elevated blood glucose levels, resulting from absent or inadequate pancreatic insulin secretion, with or without impairment of insulin action. Such a deficiency results in increased concentrations of glucose in the blood, which in turn damages many of the body's systems, in particular the blood vessels and nerves [1]. It is the most prevalent disease in the world, affecting 25% of population and afflicts 150 million people, and is set to rise to 300 million by 2025 [2]. In India, it is estimated that presently 19.4 million individuals are affected by diabetes, and the figure is likely to go up to 57.2 million by the year 2025 [3]. Hyperglycemia alone does not cause diabetic complications. It is rather, the detrimental effect of glucose toxicity due to chronic hyperglycemia, mediated and complicated through augmented oxidative stress [4]. Hyperglycemia increases the production of reactive oxygen species (ROS) inside the aortic endothelial cells. ROS-induced activation of protein kinase-C isoforms, increased formation of glucose-derived advanced glycation end products, increased glucose flux through aldose reductase pathways and activation of cytokines are some of the known biochemical mechanisms of hyperglycemia-induced tissue and cell damage [2]. Many traditional medicinal plants that possess substantial quantity of antioxidant components have been found to be useful against diabetes and its related complications [5]. Hence, there is a huge prospect of development of potential hypoglycemic agent coupled with antioxidant activity, from traditional medicinal plants to combat diabetes and its complications. Apart from currently available therapeutic options, many herbal medicines have been recommended for the treatment of diabetes. Traditional plant medicines are used throughout the world, for a range of diabetic presentations. *Solanum trilobatum* (Solanaceae), a thorny creeper with bluish violet flowers, more commonly available in Southern

India has been used traditionally in Siddha system of medicines, to treat various diseases. Popularly called 'ththuvai' by the local tribes, villagers and herbalogists in Tamilnadu, this ethnobotanical herb is known to have unique medicinal properties [6]. It has been widely used to treat respiratory disorders, especially bronchial asthma [7]. It has hepatoprotective activity [8], antibacterial activity [9], antidiabetic activity [3] and antioxidant properties [10], and can treat several kinds of leprosy. Therefore, the present investigation was undertaken to evaluate, the role of ethanolic extract of *S. trilobatum* leaves (EEST), in effective management of diabetes through antioxidant defense mechanism.

### Materials and Methods

#### Plant material

Mature leaves of *S. trilobatum* were collected, in the month of December 2008 from the villages; Coimbatore District, Tamilnadu, India. The plant was authenticated by the Botanical Survey of India (Southern Circle), Coimbatore District, Tamilnadu, India. A voucher specimen was deposited at our laboratory for future reference.

\*Corresponding author: Doss A, PG & Research Department of Botany, National College (Autonomous), Tiruchirappalli- 60001, Tamil Nadu, India, E-mail: androdoss@gmail.com

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## Preparation of extract

The plant was dried under shade and powdered to coarse particles. The 2 kg powdered plant material was defatted, with petroleum ether (60-80°C) in a Soxhlet extraction apparatus and further, the same amount plant material extracted with ethanol. The ethanol extract, *S.trilobatum* (EEST) was selected for the present study.

## Animals

Wister albino rats (150-200gm) of either sex were procured from RVS Pharmaceutical College, Coimbatore, Tamilnadu. Before and during the experiment, rats were fed with standard diet (Lipton India Ltd). After randomization into various groups and before initiation of experiment, the rats were acclimatized for a period of 7 days at standard environmental conditions of temperature, relative humidity and dark/light cycle. (12 h light or 12 h dark cycle,  $25 \pm 3^\circ\text{C}$ , 35-60 % humidity). The experiments were conducted according to the ethical norms, approved by the Institutional Animal Ethical Committee guidelines of RVS Pharmaceutical College, Coimbatore (Approval No: IAEC1012/ C06/CPSEA- Coreres- 2008-2009).

## Induction of diabetes

The Group II, III, IV and V animals were then anesthetized with Alloxan monohydrate (120mg/kg), which was dissolved in saline immediately before use, and injected intraperitoneally. After 2 days, rats with moderate diabetes having glycosuria, indicated by Benedict's qualitative test and moderate hyperglycemia (180-200mg/dl) were used for the experiment.

## Experimental design

Animals were divided into five groups of six rats each. Group I: normal rats administered distilled water, 2.0 ml/kg, orally daily for 10 days. Group II: diabetic control rats, administered 0.2 ml of 2% aqueous gum acacia. Group III: diabetic rats administered EEST, 100 mg/kg, orally daily for 10 days. Group IV: diabetic rats administered EEST, 200 mg/kg, orally daily for 10 days. Group V: diabetic rats administered standard drug glibenclamide (10 mg/kg, orally) daily for 10 days. Body weights of experimental rats were measured on days 3, 5, 7 & 10. After 10 days of treatment, all the rats were anaesthetized and sacrificed by cervical dislocation; liver was excised and washed thoroughly to clear off blood. The tissues were immediately transferred to ice-cold saline and homogenized in 0.1N Tris-HCl buffer (pH 7.4).

## Preparation of liver homogenate

The liver was weighed and 10% liver homogenate was prepared with 0.1 M phosphate buffer (pH 7.0), after centrifugation at 1000 rpm for 15 min. The supernatant was used to measure protein, Lipid Peroxidation (LPO), SOD and CAT.

## Biochemical evaluations

The blood serum, glucose [11], SGPT & SGOT [12], cholesterol [13] and liver glycogen

## Protein content

The protein content was estimated using a previously reported method [14]. Liver homogenate was mixed (1.0 ml) with 2.25 ml of 90% alcohol, and centrifuged at 3000 rpm for 10 min. The supernatant was discarded and the precipitate was dissolved in 1ml of 0.1N NaOH, to which 1 ml of alkaline mixture was added; set aside for 10 min, 0.5 ml of folin reagent was added, and again set aside for 10 min for complete

color development. The absorbance was measured at 610 nm. Protein levels were calculated using standard bovine serum solution, 200 mg in 100 ml of distilled water.

## Lipid peroxidation

Lipid peroxidation was estimated by the method [15]. Liver homogenate mixed (1.0 ml) with 100  $\mu\text{l}$  of 8.1% sodium dodecyl sulfate (SDS), and 600 $\mu\text{l}$  of 20% acetic acid solution was kept for 2 min at room temperature, then 600 $\mu\text{l}$  of 0.8% solution of TBA, was added, heated at 95°C for 60 min in water bath and cooled with ice cold water at 4°C. The mixtures of n-butanol, and pyridine (15:1, v/v) were added, shaken vigorously and centrifuged at 10,000 rpm for 5 min. The absorbance of the organic layer was measured at 532 nm. Lipid peroxidation was expressed as nmoles of MDA/mg of protein.

## Superoxide dismutase

The SOD was estimated by the method [16,17], based on the reduction of Nitro Blue Tetrazolium (NBT) to water insoluble blue formazan. Liver homogenate (0.5 ml) was taken, and 1 ml of 50 mM sodium carbonate, 0.4 ml of 24  $\mu\text{M}$  NBT, and 0.2 ml of 0.1 mM EDTA was added. The reaction was initiated by adding 0.4 ml of 1 mM hydroxylamine hydrochloride. Zero time absorbance was taken at 560 nm, followed by 5 min at 25°C. The control was simultaneously run without liver homogenate. Units of SOD activity was expressed as, the amount of enzyme required to inhibit the reduction of NBT by 50%. The specific activity was expressed in terms of units/mg of protein.

## Catalase

Catalase was estimated using a previously reported method [18]. The reaction mixture contained 1.0 ml of 0.01 M Phosphate buffer (pH 7.0), 0.1 ml of liver homogenate, and 0.4 ml of 2 M  $\text{H}_2\text{O}_2$ . The reaction was stopped by the addition of 2.0 ml of dichromatic-acetic acid reagent. The control was carried out without addition of  $\text{H}_2\text{O}_2$ , and then absorbance was read at 620 nm. CAT activity was expressed as  $\mu\text{M}$  of  $\text{H}_2\text{O}_2$  consumed/min/mg protein.

## Statistical analysis

All the values of the present study were expressed as mean  $\pm$  standard error of mean (S.E.M.) and analyzed for ANOVA and post hoc Dunnet's T-test. Differences between groups were considered significant at  $p < 0.05$ ,  $p < 0.01$ ,  $p < 0.001$  levels.

## Results

Table 1 depicts the effect on rats with alloxan and treatment of ethanol extracts of *Solanum trilobatum* leaves (100 and 200 mg/kg) on the levels of LPO, SOD, CAT activity in liver homogenates. The values obtained clearly reveal that LPO, SGPT, SGOT, glucose and cholesterol were significantly ( $P < 0.001$ ) reversed, by administration of *Solanum trilobatum* extracts in alloxan induced diabetic rats for 12 days. Consequently, the levels of SOD and CAT were significantly ( $P < 0.001$ ) improved, after administration of *Solanum trilobatum* extracts in alloxan induced diabetic rats.

The blood serum of treated and control rats was used, to estimate the SGPT, SGOT, cholesterol and glucose (Table 2). A significant difference was observed between normal and diabetic rats in fasting blood glucose level. Ethanol extract of *S.trilobatum*, at the doses of 100 and 200 mg/kg body weight, significantly lowered fasting blood glucose level, and exhibited maximum reduction of 148.0 and 136.8 on Day 10, respectively. The results were compared with standard

Treatments	CAT (Unit/mg of protein)	SOD (Unit/mg of protein)	LPO MDA (nmol/mg of protein)	Liver glycogen (mg/gm of fresh Tissue)
Diabetic control	153.9 ± 0.1***	9.03 ± 0.05***	18.47 ± 0.05***	0.021 ± 0.04***
<sup>b</sup> Gliben (10 mg/kg)	327.9 ± 0.05***	15.8 ± 0.11***	9.06 ± 0.05**	0.062 ± 0.006***
Normal	334.8 ± 0.11	19.9 ± 0.1	7.96 ± 0.1	0.068 ± 0.01
<sup>a</sup> EEST (100 mg/kg)	246.7 ± 0.07**	12.9 ± 0.1**	14.93 ± 0.05*	0.05 ± 0.004**
<sup>a</sup> EEST (200 mg/kg)	277.9 ± 0.11**	14.6 ± 0.1***	11.9 ± 0.1**	0.061 ± 0.003**

<sup>a</sup>Ethanol extract of *Solanum trilobatum*

<sup>b</sup>Glibenclamide (Standard Drug)

Values are expressed as mean + SEM, n=6 in each group. \*\*\*P< 0.001, significant when compared to diabetic control

**Table 1:** Effect of *Solanum trilobatum* leaf extracts on CAT, SOD LPO and Liver glycogen levels in alloxan induced diabetic rats.

Treatments	Glucose (mg/dl)	Cholesterol (mg/dl)	SGOT (U/dl)	SGPT (U/dl)
Diabetic control	221.1 ± 1.5***	112 ± 1.8**	320.1 ± 0.1***	256.9 ± 0.15***
<sup>b</sup> Gliben (10 mg/kg)	114.5 ± 0.3***	92 ± 3.4**	118.9 ± 0.05***	69 ± 0.1***
Normal	108.4 ± 0.5	84 ± 2.01	62.06 ± 0.05	61.36 ± 3.0
<sup>a</sup> EEST (100 mg/kg)	126 ± 1.9**	104 ± 2.0**	199.1 ± 0.11**	152 ± 0.1**
<sup>a</sup> EEST 200 (mg/kg)	123.8 ± 0.5**	98.2 ± 2.8**	172.0 ± 0.05**	128 ± 0.11**

<sup>a</sup>Ethanol extract of *Solanum trilobatum*

<sup>b</sup>Glibenclamide (Standard Drug)

Values are expressed as mean + SEM, n=6 in each group. \*\*\*P< 0.001, significant when compared to diabetic control

**Table 2:** Effect of *Solanum trilobatum* leaf extracts on Serum, Glucose, Cholesterol, SGOT and SGPT levels in alloxan induced diabetic rats.

Treatments	Body weight profile (g)			
	3 <sup>rd</sup> Day	5 <sup>th</sup> Day	7 <sup>th</sup> Day	10 <sup>th</sup> Day
Diabetic Control	154.95 ± 2.1	151.5 ± 5.17	148.83 ± 1.7	144.71 ± 0.5
<sup>b</sup> Gliben (1 mg/kg)	145.76 ± 1.68	146.85 ± 5.54	149.57 ± 4.66	150.09 ± 5.09
Normal Control	142.48 ± 2.0	148.25 ± 0.25	155.14 ± 0.54	162.61 ± 3.14
<sup>a</sup> EEST (100 mg/kg)	144.75 ± 0.75	150.33 ± 3.42	154.8 ± 1.95	159.63 ± 0.92
<sup>a</sup> EEST (200 mg/kg)	149.61 ± 2.52	152.65 ± 6.45	156.55 ± 1.55	164.73 ± 4.17

<sup>a</sup>Ethanol extract of *Solanum trilobatum*

<sup>b</sup>Glibenclamide (Standard Drug)

Values are expressed as mean + SEM, n=6 in each group. \*\*\*P< 0.001, significant when compared to diabetic controls

**Table 3:** Effect of EEST leaves on body weight profile of normal and diabetic rats.

oral hypoglycemic agent, glibenclamide (10 mg/kg), which exhibited maximum reduction of 124.5 on Day 10 (Table 2). Glibenclamide is often used as an insulin stimulant in many studies, and also used as a standard antidiabetic drug in alloxan induced moderate diabetes, to compare the antidiabetic properties of a variety of hypoglycemic compounds.

Significant decrease in the level of liver glycogen was observed in diabetic rats, when compared with normal control groups. Oral administration of EEST at the selected doses, significantly increases liver glycogen level to its normal level and the result is comparable, to that of standard drug glibenclamide (Table 1). Ethanol extract of *S.trilobatum* treatment also, improved body weight profile with respect to diabetic control group (Table 3). The findings of our study shows that, protection provided by *Solanum trilobatum* extracts could maintain the levels of LPO, SOD, CAT, biomarker enzymes (SGOT, SGPT), cholesterol and glucose in alloxan induced diabetic rats.

## Discussion

Oxidative stress plays an important role in chronic complication of diabetes, and is postulated to be associated with increased lipid peroxidation. Alloxan is a toxic glucose analogue, which selectively destroys insulin-producing cells in the pancreas, when administered to rodents and many other animal species. This causes an insulin-dependent diabetes mellitus (called "Alloxan Diabetes") in these animals, with characteristics, similar to type1 diabetes in humans. Alloxan administration produced elevated level of lipid peroxidation,

hydroperoxides and conjugated dienes, that is a clear manifestation of excessive formation of free radicals and activation of lipid peroxidation system resulting in tissue damage [19]. In the present study, diabetes control rats, exhibited significantly elevated fasting blood glucose and cholesterol levels, as compared with normal control rats. Treatment with EEST, significantly reduced fasting blood glucose and cholesterol levels. The significant control of plasma lipid levels, suggests that the extract may produce its action, by improving insulin secretion [20]. Diabetic rats exhibited significantly lower level of liver glycogen level, which was significantly reverted back near to the normal status in EEST treated diabetic groups, may be due to the reactivation of the glycogen synthase system by improving insulin secretion. Diabetes is associated with weight loss. Imbalance between energy intake and expenditure results in a change in body weight. Alloxan induced diabetic rats showed decreased level of body weight. The decrease in body weight in diabetic rats clearly shows a loss (or) degradation of structural proteins. Weight loss, which is one of the clinical features of diabetes mellitus, may be due to the degeneration of the energy lost from the body due to frequent urination and over conversion glycogen to glucose. This shows that, the plant extract the degeneration of the adipose in adipose and muscle tissues which occurs during diabetic stress in order to make up for every loss from the body due to frequent urination, over conversion of glycogen urination and over conversion to glucose [21].

The SOD and CAT are the two major scavenging enzymes that remove free radicals in vivo. A decreased activity of these antioxidants, can lead to an excess availability of superoxide anion (O<sub>2</sub><sup>•-</sup>) and

hydrogen peroxide ( $H_2O_2$ ), which in turn, generate hydroxyl radicals (OH), resulting in initiation and propagation of LPO. The SOD can catalyze dismutation of  $O_2^{\cdot-}$  into  $H_2O_2$ , which is then deactivated to  $H_2O$  by catalase, or SOD works in parallel with selenium-dependent glutathione peroxidase, which plays an important role in the reduction of  $H_2O_2$ , in the presence of reduced glutathione forming oxidized glutathione, and it protects cell protein and cell membranes against oxidative stress. In our study, the SOD and CAT enzymes were significantly ( $P < 0.001$ ) decreased in alloxan induced diabetic control rats, may be due to inactivation caused by free radicals [22]. The SOD and CAT play a prominent role in scavenging free radical and restoring antioxidant activities in the tissue of diabetic animals [23]. The above observations may clearly suggest that, increased levels of SOD and CAT of *Solanum trilobatum* extracts has free radical scavenging activity, which may exert a beneficial effect against pathological alterations, caused by reactive oxygen species.

The elevation of biomarker enzymes, such as SGPT and SGOT was observed in diabetic control rats indicating the hepatic damage [24]. The diabetic complications such as increased gluconeogenesis and ketogenesis may be due to elevated transaminases activity [3]. There is significant decrease in serum cholesterol levels in *Solanum trilobatum* extract treated rats, as evident by reports exhibiting a potent hypocholesterolemic effect. However, the possible underlying mechanism is not elucidated at this stage of the study. The previous studies have reported that administration of *Momordica charantia* lead to decrease in cholesterol levels, probably by two mechanisms; one by decreasing absorption of cholesterol from intestine, by binding with bile acids within intestine and increasing the extraction of faecales bile acids, and the other by biosynthesis of cholesterol, especially by decreasing the activity of 3-hydroxyl-3-methyl-glutaryl coenzymes A reductase (HMG CoA reductase), an enzyme of cholesterol biosynthesis [25]. Same mechanism may be appropriate to explain the observed cholesterol lowering activity by *Solanum trilobatum* extracts.

Natural antioxidant strengthens the endogenous antioxidant defenses from reactive oxygen species and restores an optimal balance by neutralizing the reactive species. They are gaining immense importance, by virtue of their critical role in disease prevention. In the present study, it is concluded that *Solanum trilobatum* extract has free radical scavenging activity and improved antioxidant effect. The precise mechanism(s) and site(s) of action, as well as, constituents of *Solanum trilobatum* will be further determined including their toxicological effects.

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