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Antidiabetic Evaluation of *Dalbergia Sissoo* against alloxan induced diabetes mellitus in wistar albino rats

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ABSTRACT

In Indian traditional system of medicine, *Dalbergia sissoo* (DS) Roxb.(Family Fabaceae) is prescribed for the treatment of diabetes mellitus. In the present study, the antidiabetic effect of ethanolic extract of DS bark was investigated in alloxan (AL) induced diabetic rats. Oral administration of DS at the doses of 250 and 500 mg/kg was studied in AL-diabetic rats. The two doses caused significant reduction in blood glucose levels in all the models. The effect was more pronounced in 500mg/kg than 250 mg/kg. DS also showed significant increase in body weight and glycogen content in liver of AL-induced diabetic rats while there was significant reduction in the levels of serum triglyceride and total cholesterol. DS also showed significant improvement in the pancreas of AL-induced diabetic rats. The antidiabetic effect of DS was compared with glibenclamide, a well-known hypoglycemic drug. The results indicate that ethanolic extract of *Dalbergia sissoo* bark possesses significant antidiabetic activity.

Keywords: Type-II Diabetes, *Dalbergia sissoo*, Antioxidant Enzymes.

INTRODUCTION

Diabetes mellitus (DM) consists of a group of syndromes characterized by hyperglycemia; altered metabolism of lipids, carbohydrates, and proteins; and an increased risk of complications from vascular disease [1,2] Apart from currently available therapeutic options for diabetes like oral hypoglycemic agents and insulin, which have limitations of their own, many herbal medicines have been recommended for the treatment of diabetes [3] The hypoglycemic effect of many herbs has already been reviewed [4,5]. Antioxidants are used as supportive therapy in the treatment of DM [6] and hypoglycemic plants have been shown to regulate the oxidative complications of DM [7]. Hence the present study was carried out to evaluate the antidiabetic activity of *Dalbergia sissoo*.

Traditionally, *Dalbergia sissoo* Roxb.(Fabaceae) has been used in folk medicine as an aphrodisiac, abortifacient, expectorant, antihelminthic, antipyretic, and in the treatment of various digestive disorders and skin diseases [8,9]. The alternative wood is used in India for doils, eruptions, leprosy and [10]. Its barks reported as antioxidant activity, anti-Inflammatory activity, anti-spermatogenic activity [11, 12,13]. Taking into consideration the traditional claims and reported activities, the present study was planned to investigate the effect of *Dalbergia sissoo* Roxb. on Alloxan induced diabetes mellitus in rats.

Abbreviations: DS, *Dalbergia sissoo*; AL, Alloxan; DM, Diabetic mellitus; TC, Total cholesterol TG, Triglyceride; CAT, Catatase; SOD, Superoxide dismutase; MDA; Malonaldehyde, GSH, Reduced glutathione

MATERIALS AND METHODS

2.1. Plant extract

The barks of *Dalbergia sissoo* (DS) Syn. Shisav (Family: Fabaceae) were collected in the month of November-December from local areas near Pune. The DS sample was authenticated and certified by Dr. P. G. Diwakar from Botanical Survey of India, Pune. Voucher specimen number (BSI/2011/KIVPDAS1) was deposited. The shade dried barks of DS were subjected for size reduction to coarse powder. The powder was defatted with petroleum ether (60-80°C) and then extracted with 90% ethanol using solvent in soxhlet apparatus at 80 °C under vacuum. The ethanolic extract was concentrated to dryness under reduced pressure and controlled temperature (48°C–50°C) with a rota vapour. The extract was dried in order to produce a dark brown solid extract. The ethanolic extract of DS was then subjected to phytochemical screening.

2.2. Animals

Male and female Swiss albino mice (25–30 g was used. They were maintained at 25± 2°C and relative humidity of 45 to 55% and under standard environmental conditions (12 hour light: 12 hour dark cycle). Animals were allowed to take specified amount of standard laboratory feed (VRK Nutrition, Pune) and water ad libitum. All the experimental procedures and protocols used in this study were reviewed and approved by the Institutional Animal Ethical Committee (IAEC) of AISSMS College of Pharmacy, Pune which is constituted under Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA), approval no. CPCSEA/IAEC/PC-06/05-2K11. Ethical guidelines were strictly followed during all the experiments.

2.2 Chemicals

Alloxan (Ozone Interanational, Mumbai) and glibenclamide were purchased from local market. Trichloroacetic acid (TCA), Ethylene diamine tetra acetic acid (EDTA), Ferrous sulphate (FeSO₄) and other chemicals were purchased from local chemical supplier. Biochemical kits for estimation of serum glucose, total cholesterol and triglyceride were purchased from Biolab diagnostic (I) Pvt. Ltd., Tarapur Boisar, India.

2.3. Experimental design

2.3.1. Acute oral toxicity study

Acute oral toxicity assay was performed in healthy nulliparous and non pregnant adult female albino Swiss mice (20–35 g) divided into different groups as per the OECD guidelines-425 (OECD, 2001). Two groups of three albino Swiss mice were treated with *D. sissoo* 5000 mg/kg, orally. The control group received saline at the same volume. In the acute oral toxicity

test dose of 5000 mg/kg of *D. sissoo* did not cause mortality in mice and rats during 14-days observation. The mice and rats did not show any signs of toxicity or change in general behavior or other physiological activities.

2.3.2. Experimental induction of diabetes mellitus

The rats were injected alloxan monohydrate dissolved in sterile normal saline at a dose of 150 mg /kg body weight, intraperitoneally. Since alloxan is capable of producing fatal hypoglycaemia as a result of massive pancreatic insulin release, rats were treated with 20% glucose solution (15–20 ml) intraperitoneally after 6 h. The rats were then kept for the next 24 h on 5% glucose solution bottles in their cages to prevent hypoglycaemia [14,15,16]

2.3.3. Chronic treatment model

Rats were divided into five groups of six rats ($n = 6$) each. Groups 1 & 2 served as control and diabetic untreated control respectively. Group 3 and 4 was treated with the ethanolic extract of *D. sissoo* at of 250 and 500 mg/kg per oral/day. Group 5 served as standard and was treated with 10 mg/kg/day glibenclamide for 21 days. Blood glucose levels and body weight were measured on day 1, 7, 14 and 21 of the study. Finally on day 21, blood was collected to perform various parameters. Total cholesterol (TC) and Triglyceride (TG) were estimated by methods [17, 18] respectively.

2.3.4. Hepatic antioxidant enzymes assay (estimation of MDA, GSH, SOD, and CAT)

Ten percent liver homogenate was prepared in ice-cold 0.15 M KCl, centrifuged at 12,000 rpm for 45 min in Sigma Laboratory centrifuge. The clear supernatant thus obtained was used for the assay of lipid peroxidation [19] the levels of glutathione (GSH), [20] superoxide dismutase (SOD) and catalase (CAT) enzymes [21, 22].

2.3.5. Histological analysis:

On day 21, when the animals were sacrificed, the pancreas tissues were removed and stored in 10% formalin after washing with normal saline. The tissues embedded in paraffin and sectioned with 5 μ m thickness and then stained with hematoxylin eosin for microscopic assessment. For the quantitative analysis of pancreatic islets, the number of pancreatic islets was counted under microscope (40 \times).

2.4. Statistical analysis

The results are expressed as mean \pm SEM. Statistical analysis was done using INSTAT graph pad software. Comparison between the control and diabetic control group was made with unpaired Students *t* test. Comparison between test groups and diabetic control was made with one way analysis of variance (ANOVA) followed by Dunnett's test.

RESULTS

3.1 Acute oral toxicity test

The single dose of *Dalbergia sissoo* orally did not produce any signs of toxicity or mortality within 04 hours in all animals, when observed continuously and was found to be safe at 5000 mg/kg in mice. The OECD guidelines AOT-425 was followed for estimation of acute toxicity study in mice.

3.2 Effect of DS treatment on body weight

After 21 days of diabetes induction, vehicle treated diabetic rats showed very significant ($p < 0.001$) decrease in body weight as compare to control animals. DS500mg/kg and

Gliben10mg/kg treated animals showed significant ($p<0.01$) increase in body weight after 21 day of treatment and DS 250 mg/kg also showed less significant($p<0.05$) after 14 days as compare to vehicle treated diabetic rats.

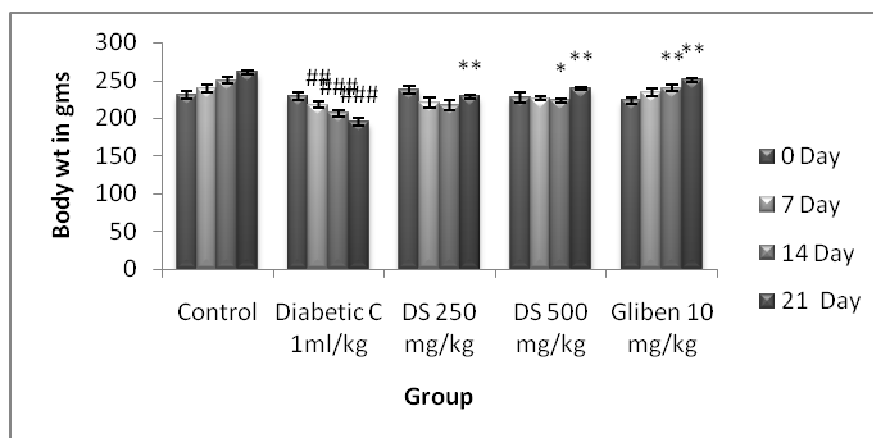


Figure 1: Graphical representation of effect on body weight of *Dalbergia sissoo* against Alloxan induced diabetes mellitus in rats.

Results are expressed as Mean \pm SEM ($n=6$). The unpaired Student's *t*-test was used for analyzing the data between two groups where as multiple comparisons using One-way Analysis of Variance (ANOVA) followed by Dunnett's-test.; ## $p<0.01$, ### $p<0.0001$ compared with control; * $p<0.05$, ** $p<0.01$ compared with diabetic control

3.3 Effect of DS treatment on blood glucose levels

After 21 days of diabetes induction, vehicle treated diabetic rats showed very significant ($p<0.001$) increase in plasma glucose level as compare to control animals. DS 500 and Gliben10 mg/kg treated animals showed significant ($p<0.01$) reduction in plasma glucose level from 7th day onwards, while DS 250 mg/ kg treated animals showed significant ($p<0.01$) reduction in plasma glucose level from 14th day onwards.

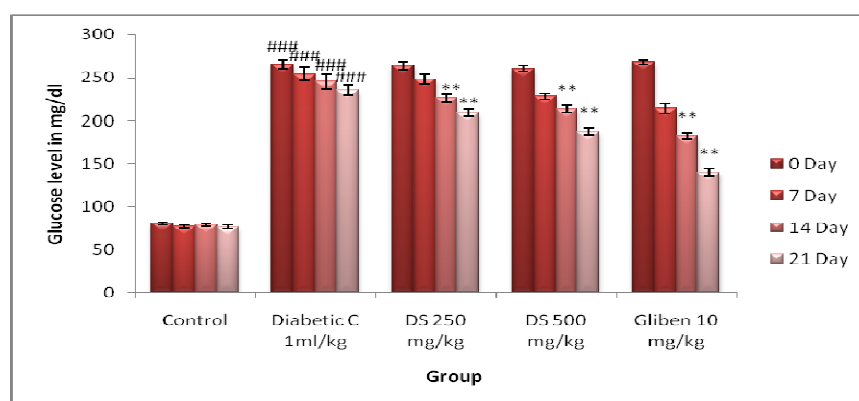


Figure 2: Graphical representation of effect on blood glucose levels of *Dalbergia sissoo* against Alloxan induced diabetes mellitus in rats.

Results are expressed as Mean \pm SEM ($n=6$). The unpaired Student's *t*-test was used for analyzing the data between two groups where as multiple comparisons using One-way Analysis of Variance (ANOVA) followed by Dunnett's-test.; ## $p<0.01$ compared with control; * $p<0.05$, ** $p<0.01$ compared with diabetic control

3.4 Effect of DS treatment on triglyceride, Cholesterol, HDL, VLDL and LDL levels

After 21 days of diabetes induction, vehicle treated diabetic rats showed very significantly ($p<0.001$) increase in serum TG, TC, VLDL and LDL level except significantly ($p<0.01$) decrease level of HDL as compare to control animals. DS 250 mg/kg, 500 mg/kg and

Glibenclamide 10 mg/kg treated animals showed significant ($p<0.01$) reduction in serum TG, TC, VLDL and LDL level, while DS 250 mg/kg treated animals showed less significant ($p<0.05$) reduction in serum TC and LDL level as compare to vehicle treated diabetic control. DS 250, 500 and Glibenclamide 10 mg/kg treated animals showed significant ($p<0.01$) increase in serum HDL level as compare to vehicle treated diabetic control.

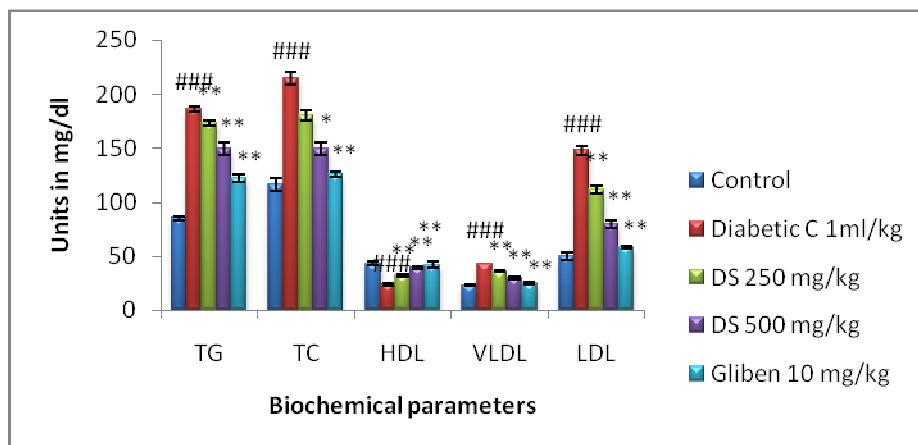


Figure 3: Graphical representation of effect on triglyceride, Cholesterol, HDL, VLDL and LDL levels of *Dalbergia sissoo* against Alloxan induced diabetes mellitus in rats.

Results are expressed as Mean \pm SEM ($n=6$). The unpaired Student's *t*-test was used for analyzing the data between two groups where as multiple comparisons using One-way Analysis of Variance (ANOVA) followed by Dunnett's test.; ### $p<0.0001$ compared with control; * $p<0.05$, ** $p<0.01$ compared with diabetic control

Table 1: Effect of *Dalbergia sissoo* treatment on antioxidant enzyme levels against Alloxan induced diabetes mellitus in rats.

Groups	Antioxidant Enzymes			
	LPO (nM of MDA/ g of tissue)	GSH (μ g of GSH/g of tissue)	Catalase (μ M of H_2O_2 /g of tissue/min)	SOD (units/ mg of tissue)
Control	11.66 \pm 1.10	52.48 \pm 2.4	12.1 \pm 1.48	65.56 \pm 1.51
Diabetic Control DW 1ml/kg	22.98 \pm 1.35 ###	22.5 \pm 2.7 ##	4.99 \pm 0.99 ##	29.48 \pm 4.15 ###
DS 250 mg/kg	17.83 \pm 1.05 *	32.51 \pm 1.76 *	8.45 \pm 0.96	44.33 \pm 2.74 *
DS 500 mg/kg	16.02 \pm 1.0 **	37.7 \pm 2.63**	9.52 \pm 0.73 **	45.47 \pm 3.34 **
Glibenclamide 10 mg/kg	13.57 \pm 1.91**	43.06 \pm 3.41 **	10.62 \pm 0.81 **	49.44 \pm 4.38**

Results are expressed as Mean \pm SEM ($n=6$). The unpaired Student's *t*-test was used for analyzing the data between two groups where as multiple comparisons using One-way Analysis of Variance (ANOVA) followed by Dunnett's test.; ## $p<0.001$, ### $p<0.0001$ compared with control; * $p<0.05$, ** $p<0.01$ compared with diabetic control

3.5 Effect of DS treatment on antioxidant enzyme levels

The effect of administration of DS on MDA, GSH, CAT and SOD in liver tissue of different groups of rats. There was very significant ($p<0.0001$) elevation in tissue MDA in vehicle treated diabetic rats as compared to normal rats. Treatment with DS 500 and glibenclamide 10 mg/kg for 21 days resulted in significant ($p<0.01$) as well as DS 250 mg/kg for 21 days resulted in less significant ($p<0.05$) decrease in liver tissue MDA level. GSH, CAT and SOD contents in diabetic control rats were significant ($p<0.01$) depleted in liver tissue. DS 500 and Glibenclamide 10 mg/kg treatment leads to significant ($p<0.01$) restored GSH, CAT and SOD and DS 250 mg/kg for 21 days resulted in less significant ($p<0.05$) restored in liver tissue GSH, CAT and SOD levels as compared with vehicle treated diabetic control rats.

3.6 Effect of DS on Histopathological study

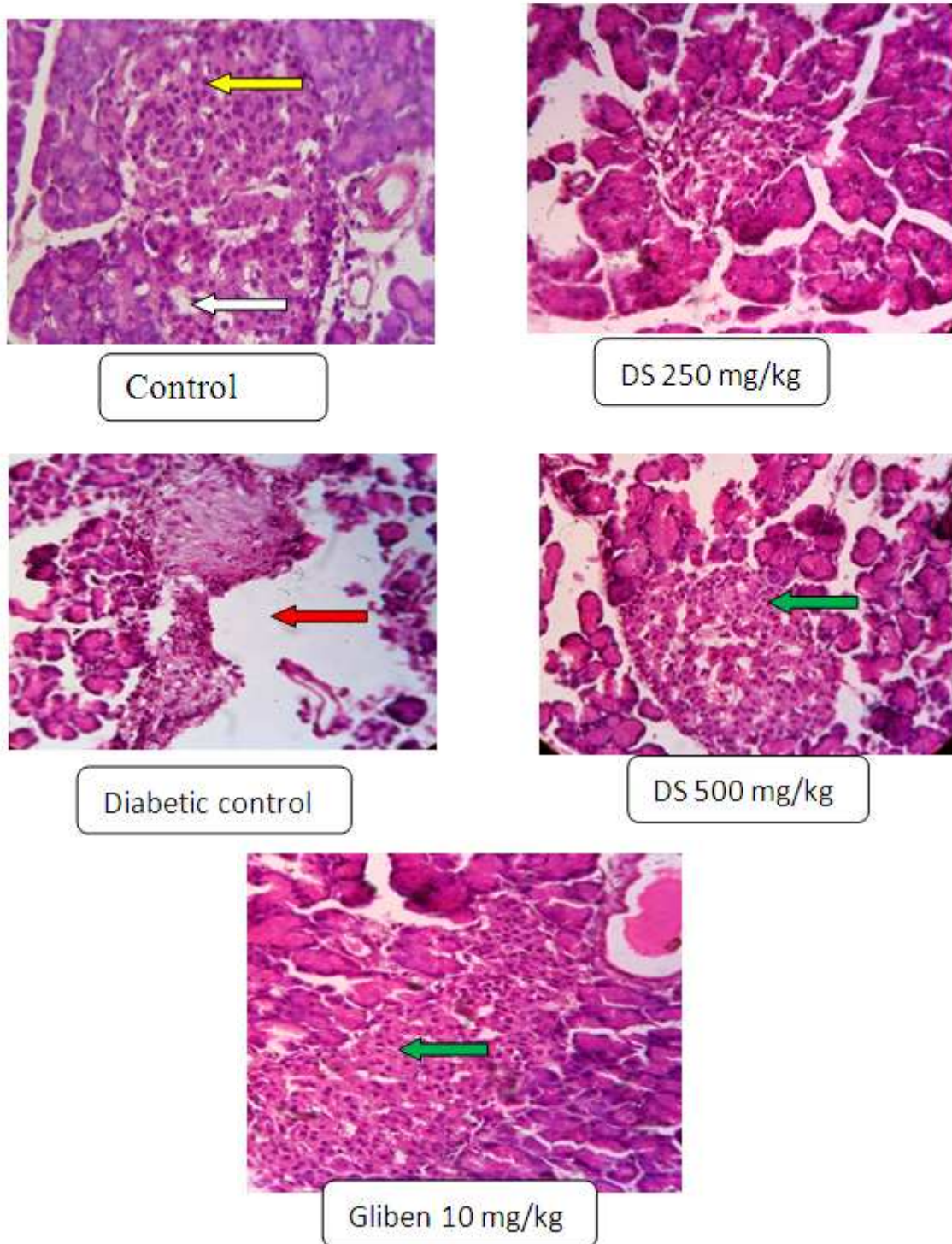


Figure 4: Effect of *Dalbergia sissoo* treatment on Histopathological study against Alloxan induced diabetes mellitus in rats.

DISCUSSION

Alloxan is widely used to induce diabetes in experimental animals by generation of reactive oxygen species that causes damage to β - cells [23]. Alloxan and the product of its reduction,

dialuric acid, establish a redox cycle with the formation of superoxide radicals [24, 25]. These radicals undergo dismutation to hydrogen peroxide. There after highly reactive hydroxyl radicals are formed by Fenton reaction. The actions of reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration cause rapid destruction of β -cells and thus increase the blood sugar level [26].

In the present investigation, statistical analysis revealed that the 21 days treatment with 250, 500 and Glibenclamide 10mg/kg showed significant decrease in glucose, cholesterol, triglyceride VLDL, LDL and increase in body weight and HDL level, thereby exhibited significant antidiabetic activity.

The present data indicate that Alloxan-induced diabetes disrupts actions of antioxidant enzymes [27]. The decreased activities of these enzymes may be due to the production of reactive oxygen species (ROS) such of superoxide ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH) that reduces the activity of these enzymes [28, 29]

The reduction in body weight [30, 31] is typical with alloxan and hence enhanced body weight is indicative toward more potent activity.

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