

## ANTIDIABETIC ACTIVITY OF DIFFERENT EXTRACTS OF *DALBERGIA SISSOO* DC. STEM BARK ON STREPTOZOTOCIN-NICOTINAMIDE INDUCED TYPE 2 DIABETIC RATS

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**ABSTRACT**

**Objective:** To investigate the antidiabetic potential of alcoholic and aqueous stem bark extract of *Dalbergia sissoo* DC. and their fractions on streptozotocin-nicotinamide induced type 2 diabetic rats.

**Methods:** Oral glucose tolerance test was done by inducing hyperglycemic state via administration of glucose in water (2g/kg). Single dose of different extracts of *Dalbergia sissoo* DC. was administered to normoglycemic and hyperglycemic rats. Type2 diabetes was induced by single intraperitoneal injection of nicotinamide (110mg/kg) followed by streptozotocin (65mg/kg). The study includes estimations of blood glucose levels, lipid profile, liver glycogen, body weight and antioxidant status in normal and diabetic rats.

**Results:** Studies showed that alcoholic extracts (250 and 500mg/kg respectively) and aqueous extract (400mg/kg) significantly reduced the blood glucose level ( $P < 0.05$ ) where as hexane soluble extract and butane soluble extract did not reduced the blood glucose level significantly ( $P > 0.05$ ) when compared with glibenclamide. Alcoholic extracts and aqueous extracts significantly restored the lipid profile and showed improvement in liver glycogen, body weight and antioxidant status in diabetic rats.

**Conclusion:** Present findings demonstrated the significant antidiabetic activity of alcoholic and aqueous bark extract of *Dalbergia sissoo* DC. Chlorogenic acid and polyphenols present in seeds is reported to have antidiabetic effect.

**Keywords:** *Dalbergia sissoo* DC., antidiabetic, streptozotocin, oral glucose tolerance test, streptozotocin induced diabetes.

**INTRODUCTION**

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia and altered metabolism of lipids, carbohydrates and proteins with an increased risk of long term complications, including vascular disease [1]. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart and blood vessels [2]. The global figure of people with diabetes is set to rise from the current estimate of 150 million to 220 million in 2010 and 300 million in 2025 [3]. Between 2010 and 2030, there will be 69% increase in number of adults with diabetes in developing countries, and 20% increase in developed countries [4]. The number of people diagnosed with type 2DM globally is estimated to be at 2%-3% of the world population and is rising at a rate of 4%-5% per year [5].

At present, nearly 222 clinical trials investigating the effects of antidiabetic plants on diabetic patients are undergoing [6]. However from 1950 to 1970, only five drugs of plant origin successfully tested in clinical phases and came in to market [7]. Therefore, it is necessary to search for new drugs and interventions that can be used to manage this metabolic disorder.

*Dalbergia sissoo* DC. is an erect to large-sized deciduous tree. It grows up to a height of 25 meter and 2-3 meter in diameter. It has leathery leaves which are up to 15 cm long. The **leaves** are imparipinnate; leaflets are 3-5, alternate, 2.5-3.6 cm in diameter, broad ovate, acuminate, glabrescent, petiolules 3-5 mm long. The **flowers** are whitish pink in colour. Leaves of plant contain trisaccharides [8], oligosaccharides [9], phenols [11-12], neoflavenes[13]. **Flower** contain tectorigeninbiochanin [10]. **Stem Bark** contain flavonoids [15], dalbergichromene [16] cinnamylphenols[17], 4-phenyl chromene, dalbergichromene[14]. **Root bark** contains chalcone (2,3-dimethoxy-4'- $\gamma,\gamma$ -dimethylallyloxy-2'-hydroxychalcone)[21], isoflavone (7- $\gamma,\gamma$ -dimethylallyloxy-5-hydroxy-4'-methoxyisoflavone),biochanin A [21], flavone, 7-hydroxy-6-methoxyflavone [21], retenoid, dehydroamorphigenin [21]. **Heart wood** contains 4-phenyl chromene, dalbergichromene [17], chalcones [isoliquiritigenin], isosalipurposide, amino acids (glycin, alanine, threonine, isolucine, phenylalanine)[18], myristic acid, palmitic acid, stearic acid, arachidic acid, linoleic acid, oleic acid [19], dalbergin [20], dalbergenone [23].

To our knowledge, there are no available reports on the antidiabetic effect of the stem bark of this plant. Hence, the present study was

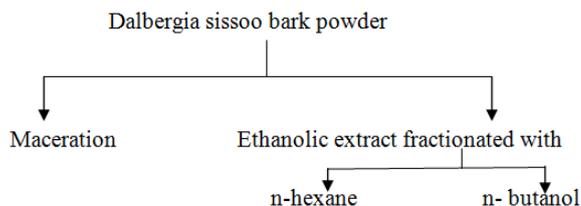
carried out to determine the effect of extract of *Dalbergia sissoo* DC. on blood glucose level in STZ-induced diabetic rats. In this investigation, Glibenclamide is used as the reference drug.

**MATERIALS AND METHODS****Chemicals and standard drugs**

Streptozotocin (Sigma-Aldrich Co., Bangalore), glibenclamide, heparin, EDTA, n-butanol, acetic acid, n-hexane, petroleum ether, ethyl acetate, glucose standard, citric acid, sodium citrate, tris hydrochloride, buffer tablet, sodium lauryl sulphate, thiobarbituric acid, trichloroacetic acid, triton-X, glycogen, ethanol, Tween 80, carboxy methyl cellulose, Ellman's reagent (5,5'-dithiobis-(2-nitrobenzoic acid); DTNB), sodium sulphate, methanol, pyridine, anthrone, thiourea, benzoic acid. Solvents were purchased from SD Fine Chemicals Ltd., Mumbai, India. All the chemicals used were of analytical grade.

**Plant material**

*Dalbergia sissoo* DC. bark was collected in November 2011 from the local area of Hisar, Haryana. Identified by Dr. H.B. Singh, Chief Scientist & Head, Raw Materials, Herbarium and Museum Division, National Institute of Science Communication And Information Resources (NISCAIR), New Delhi, vide reference no. NISCAIR/RHMD/Consult/-2012-13/2029/37. Dated : June 21, 2012.

**Preparation of extract**

The bark was dried at  $40 \pm 1^\circ\text{C}$ , grounded into a granulated powder. The ethanol extract was obtained by extracting 4 kg of defatted root powder with ethanol (95%) at  $50^\circ\text{C}$  for 72 h in soxhlet apparatus followed by filtration and concentrated in rotary vacuum evaporator at  $50 \pm 5^\circ\text{C}$  to its one third volume. The concentrate was partitioned with n-butanol (n-butanol Extract; BS) and n-hexane (n-

hexane Extract; HS) and the respective layers were separated out and dried on water bath at 30 °C till dryness (BS, 20.25 gm, Extract HS, 10.25 gm). All the extracts were stored at temperature below 10 °C and were freshly prepared with 2% Tween 80 for pharmacological experiments.

#### Aqueous Extraction

The coarsely powdered shade dried bark of the plant *Dalbergia sissoo* DC. (2Kg) was extracted with distilled water by maceration for 7 days. The solution obtained was filtered and the solvent was removed by distillation and concentrated under reduced pressure.

#### Preliminary phytochemical screening

*Dalbergia sissoo* DC. was subjected to qualitative chemical screening to identify the various major classes of active chemical constituents, namely tannins, steroid, terpenoids, saponins, flavonoids, and alkaloid [22-25].

#### Animals

This study was carried out in Guru Jambheshwar University of Science and Technology, Hisar, Haryana, India. Healthy adult male Albino Wistar rats (150–200 g), in-house bred at the Lala Lajpat Rai University of Veterinary and Agricultural sciences, Hisar, India; were used for the study. Rats were housed in polypropylene cages lined with husk in standard environmental conditions (temperature 25 ± 2°C, relative humidity 55 ± 10% and 12:12 light:dark cycle). The rats were fed on a standard pellet diet (Amrut rat and mice feed, Sangli, India) *ad libitum* and had free access to water. The experiments were performed after approval of the protocol by the Institutional Animal Ethics Committee (IAEC) and animal care was taken as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Govt. of India (Registration No. 0436).

#### Acute toxicity study

The doses for the study were fixed based on Irwin test for the extracts at 1, 2, 3, 4 and 5g/kg [26]. The extracts were dissolved in a vehicle containing 4% Tween-80. Non-diabetic, male rats weighing 150±5 g were used in this study. Three rats were used for each group. On the day preceding the experiment, the rats were appropriately grouped and placed in the experiment room for acclimatization. On the morning of the experiment day, food and water were removed from the cages. Then the rats were treated orally with the vehicle or the extracts. At 0, 15, 30, 60, 120, 180 min and 24 h after treatment of the extracts behavioural alterations were observed. 1/10th–1/20th of the dose in which no behavioural alterations were glucose levels significantly (data not shown). Hence, aqueous extract at 400 mg/kg, butanol soluble(BS) extract at 400mg/kg, hexane soluble(HS) extract at 400mg/kg and residual ethanol (RES) soluble extract at 250mg/kg and 500mg/kg respectively were selected for further studies [27].

#### Experimental design

Antidiabetic activity of *Dalbergia sissoo* extracts was assessed in normal, glucose-loaded hyperglycemic and streptozotocin-induced diabetic rats. In all studies, the animals were fasted overnight for 16 h with free access to water throughout the duration of the experiment.

#### Induction of experimental diabetes

Experimental diabetes was induced by single intraperitoneal injection of 60mg/kg of streptozotocin (STZ), freshly dissolved in cold citrate buffer, pH 4.5 (28-29) after 15 min of i.p. injection of nicotinamide (110 mg/kg) prepared in normal saline. Rats with marked hyperglycemia (fasted blood glucose level greater than 200 mg/dL) after one week of administration of STZ were used for the study.

#### Acute hypoglycemic effect of *Dalbergia sissoo* DC. ethanolic extract on normoglycemic rats

Acute hypoglycemic studies were performed in overnight fasted normal rats. Normal rats were divided into seven groups, each consisting of six rats. Animals in group second, third and fourth were

treated orally with aqueous extract(AE), hexane soluble(HS) and butane soluble(BS) extract of *Dalbergia sissoo* DC. each at a dose of 400mg/kg respectively. Group fifth and sixth were treated with residual ethanol soluble (RES) extract at a dose of 250mg/kg and 500mg/kg respectively. Group seven (positive control) treated with glibenclamide (600µg/kg). Control animals were administered with equal volume of water. Blood was withdrawn from the retro orbital plexus at 0, 30, 60, 90 and 120 min of glucose administration and glucose levels were estimated within 1 h, by GOD-POD method

#### Oral glucose tolerance test (OGTT) in normal rats

Oral glucose tolerance test was performed in overnight fasted normal rats. Normal rats were divided into seven groups, each consisting of six rats. Animals in group second, third and fourth were treated orally with aqueous extract(AE), hexane soluble(HS) and butane soluble(BS) extract of *Dalbergia sissoo* DC. each at a dose of 400mg/kg respectively. Group fifth and sixth were treated with residual ethanol soluble (RES) extract at a dose of 250mg/kg and 500mg/kg respectively. Group seven (positive control) treated with glibenclamide (600µg/kg). Glucose (2g/kg) was fed 30 min after the administration of extracts [30]. Control animals were administered with equal volume of water. Blood was withdrawn from the retro orbital plexus at 0, 30, 60, 90 and 120 min of glucose administration and glucose levels were estimated within 1 h, by GOD-POD method.

#### Oral glucose tolerance test (OGTT) in diabetic rats

Overnight fasted diabetic rats were separated in 7 groups of 6 rats each. Grouping was done in the same manner as in oral glucose tolerance test in normal rats. Animals of all groups were administered with glucose (2g/kg) orally by means of gastric intubation. Control animals were administered with equal volume of water only, blood samples were withdrawn from the retro orbital plexus of eye of each animals just after oral glucose administration 0, 30, 60, 90 and 120 min for the assay of glucose.

#### Evaluation of extract in streptozotocin-induced diabetic rats

The rats were divided into eight groups of six rats in each group:

Group 1 (NC): Normal rats treated with vehicle alone (1% tween 80, 1ml per orally); Group 2 (DC): Diabetic rats treated with vehicle alone (1% tween80,1ml per orally); Group 3 (D+AE 400): Diabetic rats treated with aqueous *Dalbergia sissoo* DC. extract at the dose 400mg/kg; Group 4 (D+HS 400): Diabetic rats treated with hexane soluble *Dalbergia sissoo* DC. extract (HS) at the dose of 400mg/kg; Group5 (D+BS 400): Diabetic rats treated with butane soluble *Dalbergia sissoo* DC. extract (BS) at the dose of 400mg/kg; Group 6 (D+RES 250): Diabetic rats treated with residual ethanol soluble *Dalbergia sissoo* DC. extract at the dose 250mg/kg; Group 7(D+RES 500): Diabetic rats treated with residual ethanol soluble *Dalbergia sissoo* DC. extract at the dose 500mg/kg Group 8(D+Glibenclamide): Diabetic rats treated with glibenclamide at the dose of 600µg/kg [31]

All rats except normal and diabetic control group were administered single dose of drug (orally) daily for 21 days. Normal and diabetic control group rat received equal volume of vehicle only. The day of administration of first dose was considered the zero day of treatment. Blood samples were collected by retro-orbital plexus of eye under and fasting blood glucose levels were determined by glucose oxidase method on day 0<sup>th</sup>, 7<sup>th</sup>, 14<sup>th</sup> and 21<sup>st</sup> day with commercially available biochemical kit. Body weight of rats was taken on day 0<sup>th</sup>(day when diabetes is induced), 10<sup>th</sup>, 20<sup>th</sup> and 28<sup>th</sup> day. At the end of the experimental period, the animals were deprived of food overnight and then sacrificed by cervical decapitation. Blood was collected in tube containing heparin for the estimation of blood glucose and other parameters.

#### Biochemical analysis

Blood glucose level and plasma cholesterol levels were measured by commercial supplied biological kits, Erba Glucose Kit (GOD-POD Method) and Erba Cholesterol Kit (CHOD-PAP Method) respectively using Chem 5 Plus-V2 Auto-analyser (Erba Mannheim, Germany). Glucose and cholesterol values were calculated as mg/dl blood sample. Glycosylated hemoglobin was measured

using commercial supplied biological kit (Erba Diagnostic) in plasma sample using Chem 5 Plus-V2 Auto-analyser (Erba Mannheim, Germany). Values are expressed as the percent of total hemoglobin. Malondialdehyde (MDA), an index of free radical generation/lipid peroxidation, was determined as described by Okhawa *et al.* (1979) [32]. Glutathione level was measured by method of Sedlak and Lindsay (1968). Liver was dissected out, washed in ice-cold saline, patted dry, weighed and subjected for bio-chemical estimation using anthrone reagent [33].

#### Statistical analysis

All values are expressed as mean  $\pm$  S.E.M. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Dunnett's tests. The results were considered statistically significant if probability factor,  $P < 0.05$ .

## RESULTS

Phytochemical screening of the extracts of *Dalbergia sissoo* DC. showed the presence of various chemical constituents, mainly alkaloids, saponins, polysaccharides, flavonoids, polyphenols. The results obtained were comparable and satisfied the standard literature.

#### Acute hypoglycemic effect of *Dalbergia sissoo* DC. extract on normoglycemic rats

The effect of the treatment with *Dalbergia sissoo* DC. extract on the blood glucose level in normal fasted rats is shown in Table 1. In normoglycemic rats, none of the *Dalbergia sissoo* DC. extracts reduced the plasma glucose in normal rats. However, the rats treated with glibenclamide showed a marked reduction in blood glucose levels ( $P < 0.05$ )

**Table 1: It shows the acute hypoglycemic effect of *Dalbergia sissoo* DC. extracts on normoglycemic rats**

Treatment	Mean blood glucose concentration (mg/dl) $\pm$ S.E.M				
	0hr	1hr	2hr	3hr	4hr
Control	72.43 $\pm$ 3.80	73.12 $\pm$ 3.18	73.16 $\pm$ 3.04	72.98 $\pm$ 2.93	73.14 $\pm$ 3.16
AE 400mg/kg	75.16 $\pm$ 3.58	74.13 $\pm$ 2.67	73.18 $\pm$ 2.2	70.19 $\pm$ 2.45	74.13 $\pm$ 2.36
HS400mg/kg	73.42 $\pm$ 3.45	72.19 $\pm$ 2.18	72.12 $\pm$ 3.65	72.10 $\pm$ 3.56	73.13 $\pm$ 3.12
BS400mg/kg	74.18 $\pm$ 3.37	74.01 $\pm$ 2.24	73.82 $\pm$ 2.43	72.45 $\pm$ 2.45	73.89 $\pm$ 3.56
RES 250 mg	74.32 $\pm$ 3.98	72.12 $\pm$ 2.59	69.12 $\pm$ 3.26	65.14 $\pm$ 2.79	72.41 $\pm$ 2.18
RES500mg/kg	76.13 $\pm$ 2.59	71.84 $\pm$ 2.67	69.14 $\pm$ 2.37	62.45 $\pm$ 1.93	70.27 $\pm$ 1.08
Glibenclamide	76.14 $\pm$ 2.20	69.67 $\pm$ 1.15*	64.13 $\pm$ 2.07*	60.98 $\pm$ 1.86*	69.17 $\pm$ 1.98*

The values are expressed as mean  $\pm$  SEM. n=6 animals in each group. Statistical significant test for comparison was done by ANOVA, followed by Dunnett's t-test. The blood glucose values of groups are compared with normal control animals, values \*\*\* $p < 0.001$ , \*\* $p < 0.01$ , \* $p < 0.05$

#### Effect of *Dalbergia sissoo* DC. extract in oral glucose tolerance test(OGTT) in normal rats

Administration of RES extract at 500mg/kg half an hour prior to the glucose administration showed significant reduction ( $p < 0.05$ ) in blood glucose levels nearly by 33%. However, significant reduction in total glucose was not observed with aqueous extract (AE), Hexane soluble extract (HS, butane soluble extract (BS) and RES 250mg/kg. The values of HS, BS are very insignificant, therefore are not shown in the graph. Glibenclamide showed a significant decrease ( $P < 0.01$ ) in blood glucose levels when compare with vehicle control.

#### Effect of *Dalbergia sissoo* DC. extracts in oral glucose tolerance test (OGTT) in diabetic rats

Administration of RES extract at 250 and 500mg/kg half an hour prior to the glucose administration showed significant reduction of ( $p < 0.05$  and  $p < 0.001$  respectively) in blood glucose levels. However, aqueous extract (AE), Hexane soluble extract(HS), butane soluble extract(BS) shows no reduction. The values of HS, BS are very insignificant, therefore are not shown in the graph. Glibenclamide showed a significant decrease ( $P < 0.01$ ) in blood glucose levels when compare with vehicle control.

#### Effect of *Dalbergia sissoo* DC. on n-STZ diabetic rats on acute antihyperglycemic model

Administration of residual alcoholic extract of *Dalbergia sissoo* at a dose 250mg/kg and 500mg/kg b. wt. p. o. to STZ diabetic rats showed reduction in blood glucose level (BGL) by 5.7% and 22.78% respectively at 60 min. When the aqueous extract is administered at 400 mg/kg then the BGL was decreased by 6.27%.at 60 min. Maximum reduction in blood glucose level is observed at 60 min.

#### Chronic hypoglycemic effect of *Dalbergia Sissoo* DC. extract in n-STZ diabetic rats

After 21 days of treatment with *Dalbergia sissoo* RES extract at 250 and 500 mg/kg, the blood glucose level is significantly reduced by 52% and 58.21%, respectively when compared to the diabetic control. The plasma glucose levels of aqueous extract is significantly

reduced by 49.15%. In glibenclamide treated rats the plasma glucose level was reduced by 68.28% compared to diabetic control rats. The blood glucose level of n-hexane soluble and n-butanol soluble extract is reduced by 9.4% and 10.86%.

#### Effect of *Dalbergia Sissoo* DC. extract on body weight

The body weight of diabetic rats become less as compared to the initial wt. as shown in the Table 2. In case of RES and AE treated groups both at 250mg/kg, 500mg/kg, 400mg/kg respectively b.wt there was a significant gain in the body wt. of animals when compared to the diabetic control group. The weight gain in the extract treated rats was comparable to the glibenclamide treated rats ( $p < 0.01$ ).

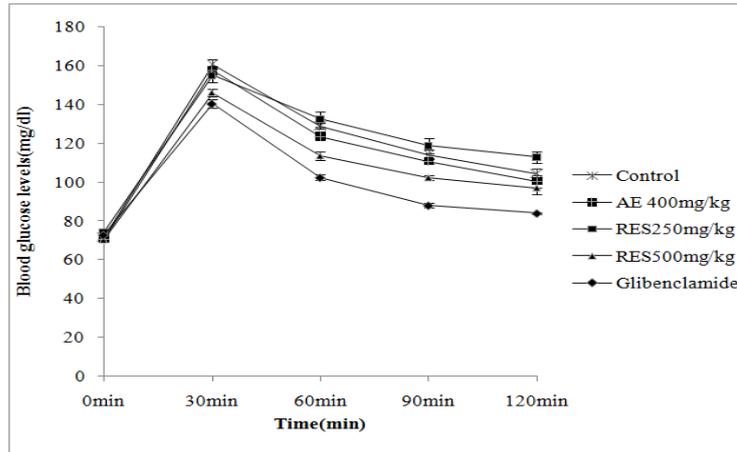
**Effect of *Dalbergia sissoo* DC. extracts on serum insulin in STZ-induced diabetic rats:** STZ caused a significant decrease in serum insulin levels. Of all the doses, RES500mg/kg showed maximum increase which was comparable to Glibenclamide (Table 3).

**Effect of *Dalbergia sissoo* DC. extracts on serum lipids in STZ-induced diabetic rats:** HS and BS were least effective and insignificant in reducing serum lipids. RES at the doses of 500mg/kg was more effective than 250mg/kg in reducing the cholesterol levels (Table 3).

**Effect of *Dalbergia sissoo* DC. extracts on glycogen content in STZ-induced diabetic rats:** Administration of RES at the doses of 250mg/kg and 500 mg/kg respectively and AE 400mg/kg for 21 days resulted in significant ( $P < 0.01$ ) increase in the glycogen levels in the liver. However, with none of the dose levels, the values were restored to normal (Table 3).

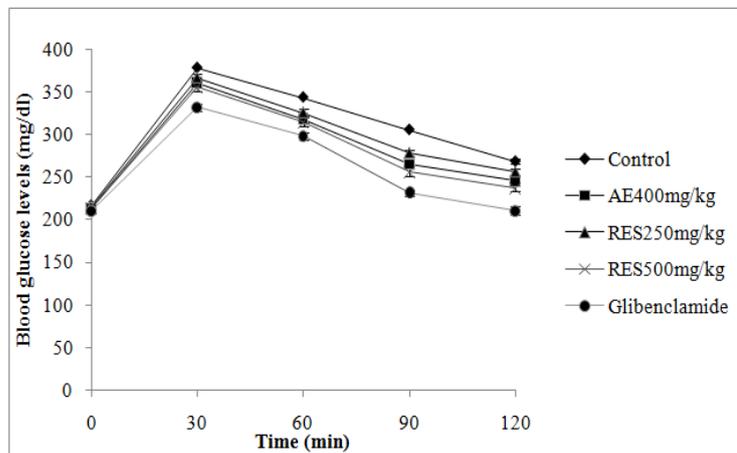
#### Acute oral toxicity study:

In acute toxicity study, extract treated animals did not show any change in their behavioral pattern. There was no significant difference in the body weights and food consumption when compared to the vehicle treated group. Also, no gross pathological changes were seen. Thus, it was concluded that RES was safe at 2000 mg/kg.



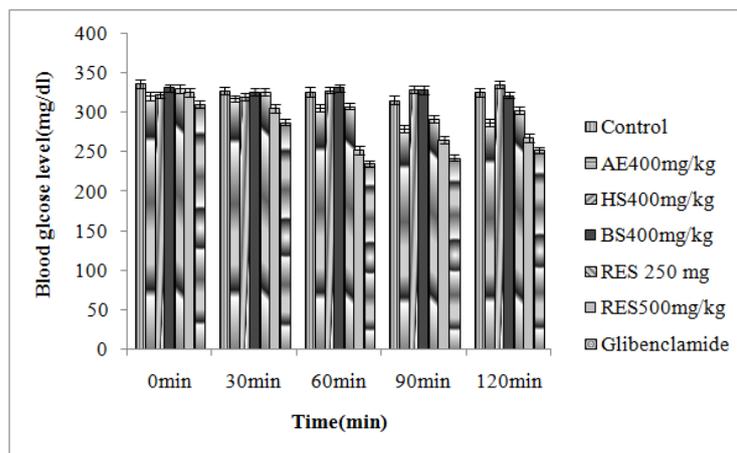
**Fig. 1:** It shows the effect of *Dalbergia sissoo* DC. extracts in oral glucose tolerance test (OGTT) in normal rats

The values are expressed as mean ± SEM. n=6 animals in each group. Statistical significant test for comparison was done by ANOVA, followed by Dunnett's t-test. The blood glucose values of groups are compared with normal control animals, values \*\*\*p<0.001, \*\*p<0.01, \*p<0.05



**Fig. 2:** It shows the effect of *Dalbergia sissoo* DC. extracts in oral glucose tolerance test (OGTT) in diabetic rats

The values are expressed as mean ± SEM. n=6 animals in each group. Statistical significant test for comparison was done by ANOVA, followed by Dunnett's t-test. The blood glucose values of groups are compared with normal control animals, values \*\*\*p<0.001, \*\*p<0.01, \*p<0.05



**Fig. 3:** It shows the effect of *Dalbergia sissoo* DC. on n-STZ induced diabetic rats on acute antihyperglycemic model.

The values are expressed as mean ± SEM. n=6 animals in each group. Statistical significant test for comparison was done by ANOVA, followed by Dunnett's t-test. The blood glucose values of groups are compared with normal control animals, values \*\*\*p<0.001, \*\*p<0.01, \*p<0.05

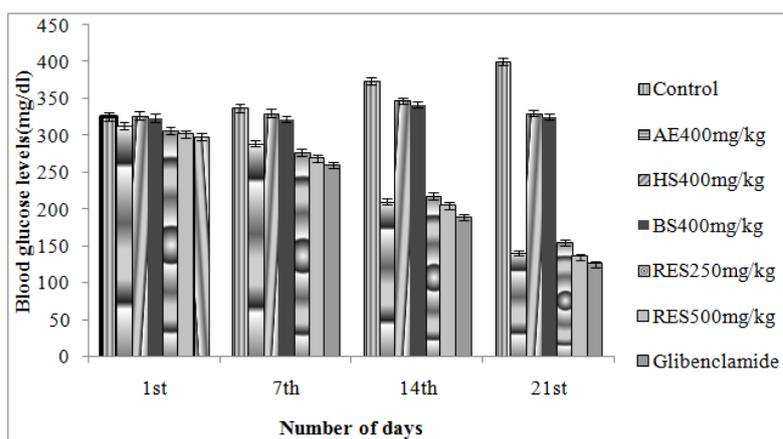


Fig. 4: It shows the Chronic hypoglycemic effect of *Dalbergia sissoo* DC. extract on n-STZ diabetic rats

The values are expressed as mean  $\pm$  SEM. n=6 animals in each group. Statistical significant test for comparison was done by ANOVA, followed by Dunnett's t-test. The blood glucose values of groups are compared with normal control animals, values \*\*\*p<0.001, \*\*p<0.01, \*p<0.05

Table 2: It shows the Effect of *Dalbergia sissoo* DC. extract on body weight

S. No.	Treatment	Initial wt.	Final wt.
1.	Normal	155.67 $\pm$ 0.76	185.33 $\pm$ 0.87
2.	Diabetic control	158.17 $\pm$ 0.70	139.00 $\pm$ 0.77
3.	AE400mg/kg	160.42 $\pm$ 0.33	172.67 $\pm$ 1.23**
4.	RES250mg/kg	159.44 $\pm$ 0.67	169.83 $\pm$ 1.12**
5.	RES500mg/kg	162.33 $\pm$ 0.32	177.83 $\pm$ 1.14**
6.	Glibenclamide	158.00 $\pm$ 0.77	168.33 $\pm$ 1.35**

The values are expressed as mean  $\pm$  SEM. n=6 animals in each group. Statistical significant test for comparison was done by ANOVA, followed by Dunnett's t-test. The blood glucose values of groups are compared with normal control animals, values \*\*\*p<0.001, \*\*p<0.01, \*p<0.05

Table 3: It shows the effect of *Dalbergia sissoo* DC. extracts on various blood parameters.

Group treatment	Glycosylated haemoglobin (mg/g)	Serum insulin ( $\mu$ U/ml)	Liver glycogen (mg/g)	Plasma malondialdehy-de (nmol/ml)	Plasma glutathione (mg/ml)	Total cholesterol
Normal control	0.24 $\pm$ 0.008	19.01 $\pm$ 0.45	15.45 $\pm$ 0.82	1.91 $\pm$ 0.30	37.45 $\pm$ 1.31	65.98 $\pm$ 0.78
Diabetic control	0.62 $\pm$ 0.007	8.41 $\pm$ 0.42	8.34 $\pm$ 0.74	5.23 $\pm$ 0.82	13.88 $\pm$ 2.13	94.18 $\pm$ 0.67
Diabetic+ Glibenclamide	0.23 $\pm$ 0.005**	12.33 $\pm$ 0.33**	12.23 $\pm$ 0.3**	1.99 $\pm$ 0.30**	27.76 $\pm$ 1.33**	73.42 $\pm$ 0.66**
Diabetic+AE (400mg/kg)	0.36 $\pm$ 0.004**	13.33 $\pm$ 0.66**	11.3 $\pm$ 0.32**	2.79 $\pm$ 0.76**	22.16 $\pm$ 1.34**	78.55 $\pm$ 0.54**
Diabetic+RES (250mg/kg)	0.28 $\pm$ 0.004**	16.33 $\pm$ 0.33**	13.58 $\pm$ 0.28**	2.05 $\pm$ 0.45**	27.76 $\pm$ 1.45**	69.19 $\pm$ 0.76**
Diabetic+RES (500mg/kg)	0.25 $\pm$ 0.004**	17.66 $\pm$ 0.33**	14.05 $\pm$ 0.67**	2.59 $\pm$ 0.33**	36.65 $\pm$ 1.28**	67.13 $\pm$ 0.54**

The values are expressed as mean  $\pm$  SEM. n=6 animals in each group. Statistical significant test for comparison was done by ANOVA, followed by Dunnett's t-test. The blood glucose values of groups are compared with normal control animals, values \*\*\*p<0.001, \*\*p<0.01, \*p<0.05

## DISCUSSION

This study was undertaken to evaluate the antihyperglycemic activity *Dalbergia sissoo* stem bark in streptozotocin-induced diabetic rats. Streptozotocin induced diabetes is a well documented model of experimental diabetes. Previous reported literature indicates that the type of diabetes and characteristics differ with the employed dose of STZ and animal and species used [34-35]. It has been stated that STZ diabetic animals may exhibit most of the diabetic complications mediated through oxidative stress [36-37]. Studies also suggest free radical involvement in pancreatic cell destruction [38]. Glibenclamide is often used as an insulin stimulant in many studies and also used as a standard antidiabetic drug in STZ-induced moderate diabetes to compare the antidiabetic properties of a variety of hypoglycemic compounds [39]. After 3 weeks supplementation of different stem bark extract of *Dalbergia sissoo* DC. resulted significant diminution of fasting blood glucose level in respect to diabetic rat, but no significant alteration of fasting blood glucose level to the control, which clearly explain the

antidiabetogenic action of this extract. It may be due to its protective action against STZ-mediated damage to the pancreatic beta cells and also possibly because of regeneration of damaged beta cells and also possibly because of inhibition of gluconeogenesis and glycogenesis [40].

STZ-induced diabetes is characterized by severe loss in body weight [41] and this was also observed in the present study. The characteristic loss of body weight is mainly due to increased muscle wasting in diabetes [42]. *Dalbergia sissoo* DC. stem bark extract and glibenclamide administration controlled this loss in body weight. However, it did not normalize the body weight completely as it remained lesser than normal control rats. The decrease in body weight observed in diabetic rats might be the result of protein wasting due to unavailability of carbohydrate for utilization as an energy source [43]. When diabetic rats were treated with *Dalbergia sissoo* extract, the weight loss was reversed. The capability of *Dalbergia sissoo* DC. to protect the body from weight loss seems to be a result of its ability to reduce hyperglycemia. Diabetes mellitus impairs the normal capacity of the liver to synthesize glycogen. The

conversion of glucose into glycogen in liver depends on concentration of glucose and availability of insulin which stimulates glycogen synthesis, which occur in presence of enzyme glycogen synthase and glycogen phosphorylase. Synthase phosphatase activates glycogen synthase resulting in glycogenesis and this activation appears to be defective in diabetes [44]. Skeletal muscle is also a major site of insulin-stimulated glucose uptake [45]. Decrease in hepatic glycogen was observed in this study. Treatment with *Dalbergia sissoo* extracts significantly increased liver glycogen indicating that the defective glycogen storage of the diabetic state was corrected by the extract.

Hypercholesterolemia is primary factor involved in the development of atherosclerosis and coronary heart disease which are the secondary complications of diabetes [46]. Increased fatty acid concentrations also increased the  $\beta$ -oxidation of fatty acids, producing more acetyl-CoA and cholesterol in diabetics. The hypocholesterolaemic activity of *Dalbergia sissoo* extracts after subchronic administration may be due to a number of mechanisms, including **a**) stimulation of cholesterol-7-alpha-hydroxylase (CYP7A1), which converts cholesterol into bile acids; **b**) inhibition of HMG-CoA reductase; and/or **c**) inhibition of cholesterol absorption from the intestines due to the formation of complexes with compounds such as glycosides and saponins [47-50].

Hexokinase plays an important role in the maintenance of glucose homeostasis [51]. The activity of hexokinase significantly decreased in the liver of diabetic rats [52]. Administration of *Dalbergia sissoo* to diabetic rats resulted in significant reversal in the activity of hexokinase. The increased activity of hexokinase causes an increase in glycolysis and utilization of glucose for energy production. Administration of *Dalbergia sissoo* DC to diabetic rats resulted in a significant increase in the level of plasma insulin that, in turn, favoured glycolysis. Our findings coincided with previous reports that the increased activities of gluconeogenic enzymes were shown to be reduced after treatment with other medicinal plants [53-54].

Recently, it was reported that the sissoo extracts, exhibited significant radical scavenging activity and thus antioxidant activity and the present finding indicates that administration of *Dalbergia sissoo* bark extract confirms the possibility that the major function of the extract is on the protection of vital tissues including the pancreas, thereby reducing the causation of diabetes in these animals [55]. Therefore, protective effect of sissoo extract on pancreas of STZ induced diabetic rats could be attributed directly to scavenging activity and for more extent to the regenerative properties of the extract.

Glycosylated hemoglobin was found to increase in diabetic patients up to 16% and the level of HbA1c is monitored as a reliable index of glycemic control in diabetes. In case of RES treated rats (500 mg/kg, p. o.), glycosylated hemoglobin was reduced to (0.25±0.004) mg/kg which was comparable to reference drug glibenclamide (0.23±0.006). Treatment with RES and AE at 250 mg/kg, 500 mg/kg and 400mg/kg doses, reduced cholesterol level significantly as shown in Figure 5.9. This cholesterol reducing activity may be explained on the basis of improved glycemic control.

Hepatic glycogen content was found to low (8.34±0.74mg/g) in diabetic rats when compared to normal rats (15.45±0.82mg/g). On treatment with RES at a dose 250 mg/kg and 500 mg/kg, liver glycogen level was increased to 58.50% and 94.17% respectively. Decrease in the level of glutathione (GSH) in diabetic rats (13.88±2.13 mg/dl) compared to normal rats (37.45± 1.31mg/dl) give evidences for altered antioxidant system during diabetes. Reduced glutathione has an important role in regulation of cellular redox state and therefore imbalance in reduced glutathione to oxidized glutathione is a putative indicator of cellular oxidative stress [56]. RES (500mg/kg p. o.) increased the level of GSH near to standard drug glibenclamide (27.76±1.45mg/ml).

In the study, reduced glycosylated haemoglobin level and improved glutathione level in extract treated rats was observed and gives a negative correlation between GSH and HBA1c in diabetic animal as reported by Giugaliano et al, 1996, which confirmed the link between hyperglycemia and GSH depletion. Indeed, in

hyperglycemic condition, glucose is preferentially used in polyol pathway [57], which consumes NADPH necessary for GSH regeneration by the GSH-Reductase enzyme. Hyperglycemia is, therefore, indirectly the cause of GSH depletion. As GSH is an important antioxidant molecule, its depletion leads to the increased of oxidative stress. Dose at 500mg/kg p. o. dose showed marked reduction in the plasma MDA level (2.59± 0.30 nmol/ml) than reference antihyperglycemic drug glibenclamide (1.99 ±0.33 nmol/ml). Aqueous extract showed reduction up to (2.79±0.3) which is comparable to RES 250mg/kg. The insulin levels was found to be low in n-STZ rats when compared to normal rats. RES extract at dose level 500 mg/kg b. wt. increased the insulin levels significantly when compared diabetic control rats (p < 0.01). Significant reduction (P<0.05) was also observed in aqueous extract(AE) and residual ethanolic extract (RES) at 250mg/kg.

Present study indicates that ethanolic and aqueous extract produced antihyperglycemic effects in experimental diabetes by providing a regenerative modification against damage caused by STZ to endocrine cells of the pancreas. However, ethanolic extract of sissoo may also exert its hypoglycemic action by mechanisms such as stimulation of glucose uptake by peripheral tissues, inhibition of insulinase activity in both liver and kidney, inhibition of endogenous glucose production or inhibition of renal glucose reabsorption.

## CONCLUSION

The ethanolic extract of *Dalbergia sissoo* DC. stem bark has potential antihyperglycemic activity in n-STZ induced diabetic rats. Ethanolic and aqueous extract of bark exerts glucose lowering effect in diabetic rats. Extract also normalized the altered diabetic parameter viz. glycosylated hemoglobin, plasma cholesterol, malondialdehyde and glutathione. Present study indicates that DS extracts exerts their hypoglycemic action by mechanisms such as stimulation of glucose uptake by peripheral tissues, inhibition of insulinase activity in both liver and kidney, inhibition of endogenous glucose production or inhibition of renal glucose reabsorption This summed effect seems to have a promising value for the development of a potent phytomedicine for diabetes.

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