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Research Article

ANTIDIABETIC ACTIVITY OF ETHANOLIC EXTRACT OF *DALBERGIA SISSOO* L. LEAVES IN ALLOXAN-INDUCED DIABETIC RATS

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ABSTRACT

This research was performed to characterize the hypoglycemic effect of ethanolic extract of *Dalbergia sissoo* L. leaves in alloxanized diabetic rats. The ethanolic extract of *Dalbergia sissoo* L. leaves was administered orally at different doses (250 and 500 mg kg^{-1}) to normal rats. The dose of 500 mg kg^{-1} was found to be more effective dose in oral route and it decreases Blood Glucose Level (BGL) by 38.2 % in normal healthy rats after 1 day of administration. After daily treatment with the both dose (250 and 500 mg kg^{-1}) of ethanolic *Dalbergia sissoo* extract for 21 days to severely Diabetic (FBG 300-350 mg dL^{-1}) rats, the BGL reduced to 125 mg dL^{-1} by 250 mg kg^{-1} and 104 mg dL^{-1} by 500 mg kg-1. The findings of our study indicate the hypoglycemic and potential antihyperglycemic nature of the extract. It is also much effective when compare with the standard drug Glibenclamide. It reduces blood glucose level up to 189.2, 115.2, 104.6 mg dL^{-1} at successive days of 7, 14, 21, at the dose of 500 mg kg^{-1} in rats compare with standard drug which reduces blood glucose level up to 250.2, 141.2, 120.4 mg dL^{-1}. We found that the ethanolic extract of plant *Dalbergia sissoo* leaves is 12 % more effective in reducing the BGL compare to standard drug (Glibenclamide).

Key words: - *Dalbergia sissoo* L. leaves, Antidiabetic activity, Herbal medicine.

INTRODUCTION

Diabetes mellitus is a heterogeneous metabolic disorder as old as mankind and its incidence is considered to be high (4-5%) all over the world [(1)]. Diabetes is a chronic disorder of carbohydrate, fat and protein metabolism characterized by elevation of both fasting and post-paradinal blood sugar levels. The synthetic oral hypoglycemic agents can produce serious side effects [(2,3)]. In addition they are not considered safe for use during pregnancy [(4)]. Furthermore, after the recommendation made by [(5)] on diabetes mellitus investigation on hypoglycemic agents from medicinal plants have become important. Plants have played a major role in the introduction to new therapeutic agents. A multitude of herbs, spices and other plant materials have been described for the treatment of diabetes throughout the world [(6,7,8,9)]. The medicinal plants might prodive a useful source of new oral hypoglycemic compounds for development of pharmaceutical entities or as a dietary adjunt to existing therapies [(10)]. Few of the plants used for the treatment of diabetes have received scientific or medical scrutiny and even the WHO expert committee on diabetes recommends that this area warrant further attention[(5)]. Despite the presence of known antidiabetic medicines in the pharmaceutical market, screening for new antidiabetic sources from natural plants is still attractive because they contain substances that have an alternative and safe effect on diabetes mellitus.

Dalbergia sissoo is a medium to large-sized deciduous tree, growing upto 30 m in height under favoranle conditions. This plant belongs to the Leguminosae family and widely planted outside its natural range. It has been established in irrigated plantations, along road sides and canals, and around farms and orchards as windbreaks. The Sissoo plant is a folk remedy for excoriations, gonorrhea and skin ailments [(11)]. Ayurvedics prescribe the leaf juice for eye ailments, considering the wood and bark abortifacient, anthelmintic, antipyretic, apertif, aphrodiasiac, expectorant and refrigerant. They use the wood and bark for anal disorders, blood diseases, burning sensations and dysentery, dyspepsia, leucoderma and skin ailments. Yunana use the wood for blood disorders, burning sensations, eye and nose disorders, scabies, scalding urine, stomach problems, and syphilis. The alternative wood is used in India for doils, eruptions, leprosy and nausea [(12)]. Dried leaves of *Dalbergia sissoo* is reported to have antibacterial, anti protozoal, anti inflammatory activity [(13)]. The plant having the isoflavones irisolidone, biochanin-A, muningin, tectorigenin, prunetin, genestein, sissotrin and prunetin-4-O-galactoside. The flavone norartocarpotin and F3-amyrin, F3-sitosterol and stigmasterol were isolated and identified from the green branches of aerial parts of *Dalbergia sissoo* [(14)]. This study was designed to elucidate the hypoglycemic effect of the ethanolic extract of *Dalbergia sissoo* leaves in normal and alloxanized diabetic rats.

MATERIALS AND METHODS

The *Dalbergia sissoo* was used in this study was collected from medical region Jhansi (India) and was authenticated by Dr. D.K. Srivastava, Scientist, College of Agriculture ICAR Devision, Indore, M.P. (India). The plant sample was submitted to the Department of Pharmacognosy, B.U. Jhansi, with reference No. BU/Pharma/07/1807.

Preparation of Extract

In the continuous hot extraction method the plant leaves extracted in Ethanol in 3 regular days at the temperature of 78-80^{0}C. The mixture was subsequently filtered and concentrated under reduced pressure at 40^{0}C. The extract yield was 26% w/w.

Animals

Male Wistar rats 200-250 g obtained from Institute of Pharmacy, Bundelkhand University, Jhansi, India were housed in animal house (App. No. 716/02/a/CPCSEA) with 12/12 h light/dark cycle at 21±2^{0}C and fed with laboratory pellet chow and given water ad libitum. Animals were acclimatized to their environment for one week prior to experimentation. Investigations using experimental animals were conducted in accordance with the inernationally accepted principles for laboratory animal use and care as found in the United States Guidelines (United States National Institutes for Health Publication No. 85-23, revised in 1985) and our ethical committee on animal care approved the protocol.

Induction of diabetes in rats

After 15 h fasting, rats were injected intraperitoneally with alloxan monohydrate (Sigma chemicals, USA) dissolved in sterile normal saline at a dose of 120 mg kg^{-1} body weight. The confirm diabetes, glycemia was daily determined after the administration of the last

alloxan dose. Depending on their Fasting Blood Glucose (FBG) level the animals were divided arbitrarily in to 2 groups [9]: Mild Diabetic (MD) animals with FBG of 120-250 mg dL^{-1}, Severely Diabetic (SD) animals showing FBG of 250-300 mg dL^{-1}.

Estimation

Blodd glucose was estimated by using one touch glucometer (Accu-chek sensor) of Roche Diagnostic, Germany for regular checkup. Blood sample were collected from tail veins.

Biological assays

Oral glucose tolerance test (OGTT)

Animals were fasted 18 hrs. before the day of experiment with free access to water and were separated in 5 groups of 10 rats each. Animals of all groups were treated with an oral D-glucose load of 2 gm kg^{-1} by means of cannula, Group third and fourth were treated orally with ethanolic extract at a dose of 250 mg kg^{-1} b.w. and 500 mg kg^{-1} b.w. each for 30 min before the oral administration of oral glucose load. Control animals were treated with vehicle, blood sample were withdrawn from the cordal (tail) vein of each animals just after oral glucose administration (0 min), 30 min, 90 min and 120 min after glucose challenge and the fifth group received glibenclamide (200 mg kg^{-1}) as positive control.

Statistical analysis

All biochemical results were expressed as mean ± SEM. Significant differences among the groups were dtermined by one-way Analysis of Variance (ANOVA) followed by Dunnett-t test. Statistical significance was considered at $P < 0.05$.

RESULT

The plant extract of *Dalbergia sissoo* leaves (ethanolic extract) showed antidiabetic activity by reducing blood glucose level significantly. It is also much effective when compare with the standard drug Glibenclamide. It reduces blood glucose level up to 189.2, 115.2, 104.6 mg dL^{-1} at successive days of 7, 14, 21, at the dose of 500 mg kg^{-1} in rats compare with the standard drug which reduces blood glucose level upto 250.2, 141.2, 120.4 mg dL^{-1}. We found that the ethanolic extract of plant *Dalbergia sissoo* leaves is 12% more effective in reducing the blood glucose level compare to the standard drug (Glibenclamide).

LD_{50}

The extract of the test substance (*Dalbergia sissoo* leaves) was found to be safe for further biological studies as no toxic effect and lethality was observed up to 3000 mg kg^{-1} per oral in rat. Only the consumption of food was increased by 20% in the dose of 2000 and 3000 mg kg^{-1} during 4 h but remaining normal afterwards.

Table 1: Shows effect of *dalbergia sissoo* leaves (ethanolic extracts) on body weight.

Treatment	Body weight in gm			
	1st Day	7th Day	14th Day	21st Day
Normal Rats	171±5.50	179±7.33	186±6.25	192±7.58
Control Diabetic Rats	155±11.66	153±13.04	146±10.37	142±7.53
Diabetic Rats + DS 1	167±5.70*	162±5.70*	175±6.12*	180±6.33*
Diabetic Rats + DS 2	138±10.30*	130±7.30*	145±12.20*	158±9.00*
Diabetic Rats + SD (Glibenclamide)	130±12.20*	140±13.30*	148±18.90*	166±16.60*

Values are Mean±SEM, (n=10), * P<0.05 when compared with control.

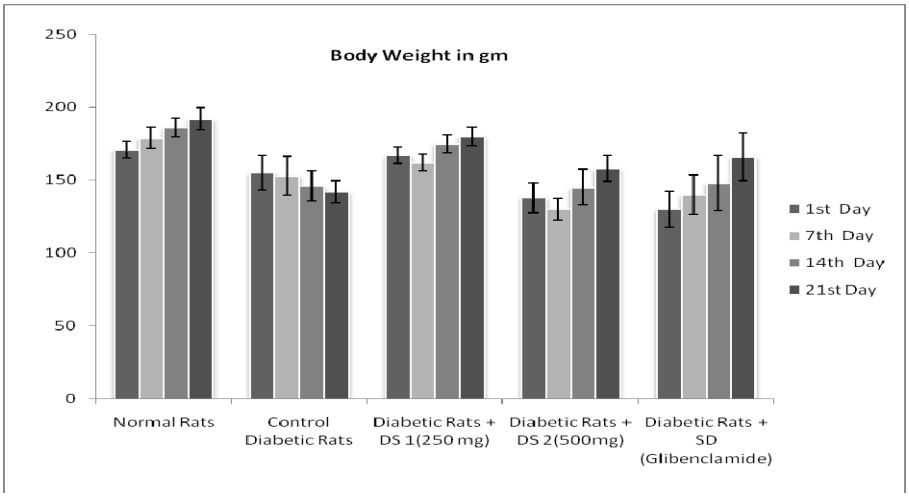

Fig. 1: It shows effect of *dalbergia sissoo* leaves (ethanolic extracts) on body weight.

Table 2: Shows effect of *dalbergia sissoo* leaves (ethanolic extract) on blood glucose level (mg dl^{-1})

Group Treatment	Blood Glucose Level (BGL) in mg dL^{-1}			
	1st Day	7th Day	14th Day	21st Day
Normal Rats	94.8±1.715	94.0±2.569	91.8±2.596	92.6±2.064
Control Diabetic Rats	356.6±3.586	313.8±4.236	160.2±11.338	125.8±3.920
Diabetic Rats + DS 1 (250 mg kg^{-1} b.w.)	235.0±4.336*	204.0±4.909*	160.2±11.338*	125.8±3.929*
Diabetic Rats + DS 2 (500 mg kg^{-1} b.w.)	220.6±2.874*	189.2±4.716*	115.2±3.216*	104.6±2.315*
Diabetic Rats + SD (Glibenclamide)	311.4±11.868*	250.2±7.453*	141.2±4.171*	120.4±2.400*

Values are Mean±SEM, (n=10), * P<0.05 when compared with control.

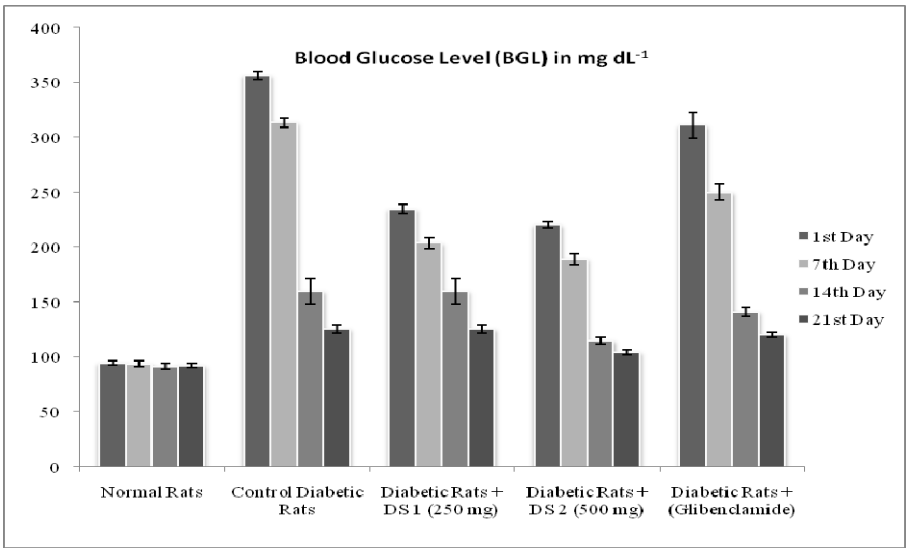

Fig. 2: It shows effect of *dalbergia sissoo* leaves (ethanolic extract) on blood glucose level (mg dl^{-1})

DISCUSSION

The results showed important reductions of blood glucose levels in healthy rats when administered the ethanolic extract of Dalbergia sissoo L. leaves by oral route.

Ethanolic sissoo extract showed a dose-dependent effect on FBG up to a dose of 500 mg kg^{-1}. The BGL decreases by 38.2 % in normal healthy rats after 1 day of administration. After daily treatment with the both dose (250 and 500 mg kg^{-1}) of ethanolic *Dalbergia sissoo* extract for 21 days to severely Diabetic (FBG 300-350 mg dL^{-1}) rats, the BGL reduced to 125 mg dL^{-1} by 250 mg kg^{-1} and 104 mg dL^{-1} by 500 mg kg-^{1}. The findings of our study indicate the hypoglycemic and potential antihyperglycemic nature of the extract. It is also much effective when compare with the standard drug Glibenclamide. It reduces blood glucose level up to 189.2, 115.2, 104.6 mg dL^{-1} at successive days of 7, 14, 21, at the dose of 500 mg kg^{-1} in rats compare with standard drug which reduces blood glucose level up to 250.2, 141.2, 120.4 mg dL^{-1}. We found that the ethanolic extract of plant *Dalbergia sissoo* leaves is 12 % more effective in reducing the BGL compare to standard drug (Glibenclamide).

The present investigation shows that in MD and SD alloxan-diabetic rats, ethanolic extract of *D. Sissoo* caused significant reductions of blood glucose levels after 2 h of extract administration. Glibenclamide (200 mg kg^{-1}) caused a lesser hypoglycemic effect than *D. Sissoo* ethanolic extract in diabetic rats after 4 h of drug administration. In addition, ethanolic extract of *Dalbergia sissoo* L. leaves caused significant hypoglycemic effect in MD and SD rats after 14 days treatment, while Glibenclamide exhibited a mild hypoglycemic activity in these animals.

The mechanism of alloxan diabetes has been the subject of many investigations and it is now generally accepted that free radicals are selectively involved in the initiation of the damage that ultimately leads to β-cell death [15,16]. Therefore, the pancreas is especially susceptible to the action of alloxan-induced free-radical damage. Many substances have been shown to ameliorate the diabetogenicity of alloxan in animals, which protect by reacting with free radicals formed from alloxan during its interaction with the β-cell, or prevent radical formation [17]. Recently, it was reported that the sissoo extract, exhibited significant radical scavenging activity and thus antioxidant activity [18] and the present finding indicates that administration of *Dalbergia sissoo* L. leaves 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.

Therefore, protective effect of sissoo extract on pancreas of alloxan-induced diabetic rats could be attributed directly to scavenging activity and for more extent to the regenerative properties of the extract. In conclusion, our study indicates that sissoo ethanolic extract produced antihyperglycemic effects in experimental diabetes by providing a regenerative modification against damage caused by alloxan to endocrine cells of the pancreas.

However, ethanolic extract of sissoo may exert its hypoglycemic action by mechanisms such as stimulation of glucose uptake by peripheral tissues, inhibition of insulinase activity in both liver and kidney [19], inhibition of endogenous glucose production or inhibition of renal glucose reabsorption.

CONCLUSION

Taken in all, the use of this plant in diabetes is then supported but the precise active substance(s), site(s) and cellular and molecular mechanism(s) of this pharmacological effect are still to be determined. In addition, the possible long-term toxic effects of ethanolic sissoo extract and its mechanism of protective effects on the pancreas also remain to be clarified.

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