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Phytochemical investigation and antidiabetic activity of Leaf extracts of *Dalbergia sissoo* (Roxb.) in alloxan induced diabetic rats

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Abstract

Diabetes mellitus is the most common endocrine disorder. The World Health Organization recommends the use of traditional and plant based medicines for the management of diabetes mellitus. The recommendation made by WHO on diabetes mellitus, investigation on hypoglycemic agents from medicinal plants have become more important. The tribes are from villages in the Koraput district located in the state of Odisha, India. In this district, villagers consume decoctions from the leaves of these plants early in the morning for the treatment of ailments like sore throats, heart problems, dysentery, syphilis, and gonorrhoea. In the present investigation we have screened the ethanol, ethyl acetate, n-butanol and petroleum ether extracts of the leaves of the plant for antidiabetic activity in alloxan induced Diabetic rats. The extracts produced a significant antidiabetic effect on first, third, fifth and seventh days at 300 mg/Kg body weight. Among all the extracts of *Dalbergia sissoo*, ethanol extract of leaves exhibited highly significant antidiabetic activity comparable with the standard drug (Glibenclamide). The observation values are reported as mean ± SEM of each observation. The significance of difference among the various treated groups and control group were analysed by means of one way ANOVA followed by Dunnett's t-test. The value of less than 5% ($p < 0.05$) was considered statistically significant.

Key words: *Dalbergia sissoo*, Antidiabetic Activity, Glibenclamide

INTRODUCTION

Herbal medicine is an alternative method for the treatment of diabetes due to their perceived effectiveness, safety, affordability, and acceptability, with minimal side effects in clinical experience, and relatively low cost [1]. About 80% of people in developing countries depend on traditional systems of medicine for primary health care [2]. Diabetes mellitus is the most

common endocrine disorder. More than 150 million people are suffering from it World wide [3]. More than one fifth of them are Indians and the International Diabetes Federation declared India "Diabetic capital of the world". Synthetic antidiabetic drugs can produce serious consequences and are not suitable for use during pregnancy. In view of the adverse effect associated with the synthetic drugs and considering natural medicine safer, cheaper and effective, traditional antidiabetic plants can be explored [4]. The tribal areas of Baipariguda, Koraput (District) of Eastern Orissa, due to its unique varieties geographical and climatic factors has had a rich variety of medicinal plant. *Dalbergia sissoo* (family: fabaceae.) also known as sisu (Oriya) is frequently distributed. And extensively used traditionally by the tribal people. The plant species are found generally in many tropical areas of the globe, particularly Africa, Asia, central and southern America where they

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are used to manage a number of ailments [5,6,7]. Some *Dalbergia* species have been investigated and found to possess antimicrobial, antioxidant, anti-inflammatory and anti-diarrhoeal activities [8,9,10,11]. Traditionally Different parts such as roots, bark, wood, leaves and seeds are being used as remedy in many diseases including skin diseases, blood diseases, syphilis, stomach problems, dysentery, nausea, eye and nose disorders, aphrodisiac, expectorant. Leaf extract has been used to treat sore throats, heart problems, dysentery, syphilis, and gonorrhoea. In India and Nepal rural people use *Dalbergia sissoo* leaves to treat animals suffering from non-specific diarrhea [12]. Chemically leaves contains sissotrin and an isoflavon-O-glycoside all so reported [13].

MATERIALS AND METHODS

Drugs and chemicals

Alloxan (Hydrate-CAS: 2244-11-3) were procured from Oxford laboratory, Maharashtra, India. The ethanol AR and ethyl acetate AR 60-80°C (Emsure® ACS) were procured from Merck Pvt. Ltd., Navi Mumbai, Maharashtra, India. n-butanol GR 80°C, petroleum ether AR 40-60°C, Loba Chemie Pvt. Ltd., Mumbai, India. All other chemicals reagents used in present work were procured from authorized dealer.

Collection of Plant Material

The leaves of *Dalbergia sissoo* were collected from the tribal belts of the local area of Patrapur of Koraput district.(India) in the month of October 2015. The plant was identified, confirmed and authenticated by the Biju Patnaik Medicinal Plants Garden and Research Centre, Dr. M. S. Swami Nathan Research Foundation, Jeypore, Koraput (District), Orissa (Letter No. MJ/SS/P-207/15, dated 10.5.2015). After authentication leaves were collected in bulk and washed under running tap water to remove adhering dirt. Then leaves were shade dried. The dried materials were made into coarse powder and stored in a closed air tight container for further use.

Preparation of Extracts

The coarse powder was taken in Soxhlet apparatus and extracted successively with ethanol, ethyl acetate, n-butanol and petroleum ether as solvent. A total amount of 650 g coarse powder was extracted with 1000 ml of each solvent. For each solvent, 10 cycles were run to obtain thick slurry. Each slurry was then concentrated under reduced pressure to obtain crude extract. All crude extracts were kept in closed air tight containers under cool and dark place for further study [14, 15, 16].

Phytochemical investigation

The crude ethanol, ethyl acetate, n-butanol and petroleum ether extracts of the leaf of *Dalbergia sissoo* were subjected to preliminary phytochemical analysis in order to detect the presence of various groups of

phytoconstituents by carrying out the chemical analysis [15, 16].

Experimental protocol

Animals were selected, weighed (25-30 g) and divided into seven groups (n=3), namely control, diabetic control, standard drug and four groups belonging to four different extracts of *Dalbergia sissoo*. All the studies conducted were approved by the Institutional Animal Ethical Committee (1200/ac/08/CPCSEA), Dadhichi college of pharmacy, Vidya vihar, Cuttack, according to prescribed guide-lines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

Acute toxicity studies

The acute toxicity was performed according to OECD 423, 2001. The selected female albino rats were used to determine the dose. The animals were divided into twelve groups of three in each. The animals were fasted overnight prior to the acute experimental procedure. Distilled water was used as vehicle to suspend the different leaf extracts of *Dalbergia sissoo* and administered orally as following doses of 100, 300, 600, 1000 and 2000 mg/kg body weight. Immediately after dosing, the animals were observed continuously for first four hours for behavioral changes and for mortality at the end of 24hrs and daily for 14 days respectively. Acute toxicity study revealed that no mortality was found in any solvent extract at any dose in Swiss albino mice, which confirmed that *Dalbergia sissoo* leaves extract would be non-toxic in living body but where as the LD50 of the extracts was found to be (LD50 > 1000 mg/kg). i.e., 300 mg/kg b.w. was selected as the therapeutic dose for the evaluation of antidiabetic activity [17, 18].

Antidiabetic activity

The antidiabetic activity was carried out on albino rats as described by the method based on alloxan induced diabetes. Here the blood sugar level of rats was raised by administration of alloxan [19]. Wistar rats were divided into seven groups of three animals in each group. The animals were fasted for 16 h with water *ad libitum*. Group I animals received 1.0ml of normal saline orally, and served as nondiabetic control, the group - II was served as diabetic control which received alloxan (150 mg/Kg) with normal saline water subcutaneously, group-III was served as standard control which received alloxan 150 mg/Kg with glibenclamide at a dose of 10 mg/Kg orally, groups-IV to VII were served as test groups which received alloxan (150 mg/kg) along with single dose (300 mg/Kg, b.w.) of ethanol, ethyl acetate, n-butanol and petroleum ether extracts respectively. Rats were made diabetic by a single intraperitoneal injection of alloxan monohydrate (150 mg/Kg). Two days after of alloxan injection, rats with plasma glucose levels of more than 200 mg/dl were included in the study and at this stage the blood glucose level of each rat was

consider as basal value in each group. Treatment with plant extracts and standard drug was started after 48 hr of alloxan injection. The blood sample were obtained through the tail vein puncturing with hypodermic needle, 0.2 ml of Blood was withdrawn from all the animals of all the groups at an interval of initial 0, 1st, 3rd, 5th and 7th hour of administration of single dose and blood glucose levels was measured using glucometer and the results were compared with standard Glibenclamide group.

Statistical Analysis

The observation values are reported as mean \pm SEM of each observations. The significance of difference among the various treated groups and control group were analysed by means of one way ANOVA followed by Dunnet's t-test. The value of less than 5% ($p < 0.05$) was considered statistically significant [20].

RESULTS

The preliminary phytochemical screening showed that the different solvent extracts of *D.sissoo* leaf contain the glycosides, steroids, terpenoids, phenols and tannins were present in all the solvent extract & flavonoid and saponins were absent. which showed in [Table1]. The extracts produced a significant antidiabetic

effect on first, third, fifth and seventh days at 300 mg/Kg body weight which showed in [Table 2]. Among the different extracts of *D.sissoo*, significant antidiabetic activity was found in animal groups treated with ethanol extract of leaves, it had exhibited highly significant antidiabetic activity. These effects are comparable with the standard drug (Glibenclamide). The activity showed by this extract is of considerable importance and justified its use in the diabetic control.

Conclusion

Based on the results of the present study, we conclude that the different extracts of *Dalbergia sissoo* leaves possesses antidiabetic activity. The ethanol extract showed most potent antidiabetic activities. However, further studies are necessary to examine underlying mechanisms of antidiabetic activities and to isolate the active compound responsible for these pharmacological activities. Hence further investigations using more experimental paradigms are warranted for further confirmation of the treatment of various ailments, diseases and disorders of this plant.

Table-1. Phytochemical screening for the different solvent extracts of *Dalbergia sissoo* leaves Extracts

	Cadiac glycoside	Flavonoids	Steroids	Terpinoids	Tannins	Saponins	Phenols
Ethanol	+++	--	++	++	+++	-	++
Ethyl-acetate	+	--	-	+	+	-	+
n-butanol	++	--	++	+	+	-	++
Petroleum ether	++	--	++	+	+	--	+

+++ , strong; ++ , moderately; + , poor presence, -- , absence

Table-2. Antidiabetic activities of leave extracts of *Dalbergia sissoo* by alloxan induced diabetic model

Groups	Blood glucose level (mg/dL) (X \pm S.D.)				
	Basal valu	1st day	3rd day	5th day	7th day
I	83 \pm 0.22	92 \pm 0.36	87 \pm 0.52	93. \pm 0.21	88 \pm 0.14
II	327 \pm 0.33	327 \pm 0 .52	318. \pm 0.73	306 \pm 0.36	300 \pm 0.76
III	267. \pm 0.76	233 \pm 0.82	157 \pm 0.52	127 \pm 0.83	114 \pm 1.06*
IV	333 \pm 1.14	258 \pm 1.17	153 \pm 1.06	118 \pm 1.12	91 \pm 1.16***
V	323 \pm 1.12	206 \pm 0.73	163 \pm 1.11	126 \pm 0.82	123 \pm 1.11*
VI	334 \pm 1.13	236 \pm 1.11	172 \pm 0.73	147 \pm 1.17	96 \pm 0.93**
VII	317 \pm 0.77	223 \pm 1.09	137 \pm 0.82	117 \pm 0.71	112 \pm 1.14*

Each values is represented as mean \pm standard deviation ($n=3$). Where $*P<0.05$, Group I-Control (Normal saline water), group II-Diabetic control (Alloxan-150 mg/kg), group III-Standard control (Glibenclamide 10 mg/kg), groups IV to VII-Alloxan (150 mg/kg) with ethanol, ethyl acetate, n-butanol and petroleum ether extracts respectively (200 mg/kg of b.w.)

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Competing interests

The authors have declared that no competing interests exist.

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