



Screening of antidiabetic activity of leaf extracts of *Crotalaria Pallida* 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 tribes are from villages in the koraput district, which are surrounded by the forest and located in the state of Odisha, India. In this districts, villagers consume decoctions from the young leaves of these plants early in the morning for the treatment of ailments like anthelmintic, fever, wound healing etc. the plant all so possess anti microbial, antioxidant, & anti inflammatory properties. the present investigation we have screened the ethanol, ethyl acetate, n-butanol and pet. ether extracts of the leaves of the plant *Crotalaria pallida* 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 *C. pallida*, ethanol extract of leaves exhibited highly significant antidiabetic activity are comparable with the standard drug (Glibenclamide). 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*

Keywords: *Crotalaria pallid*, Antidiabetic Activity, Glibenclamide

INTRODUCTION

The World Health Organization recommends the use of traditional and plant based medicines for the management of diabetes mellitus [1]. 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 [2]. About 80% of people in developing countries depend on traditional systems of medicine for primary health care [3]. Diabetes mellitus is the most common endocrine disorder. More than 150 million people are suffering from it World wide [4]. and it is likely to increase to 300 million by the year 2025. 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 [5]. The recommendation made by WHO on diabetes mellitus, investigation on hypoglycemic agents from medicinal plants have become more important [6]. The tribal areas of Baipariguda, Koraput (District) of Eastern Orissa (India) due to its unique varieties geographical and

climatic factors has had a rich variety of medicinal plant. *Crotalaria pallida* (family: Fabaceae) also known as jhunjhunka (Oriya) were frequently distributed and extensively used traditionally by the tribal people. *Crotalaria pallida* Aiton is a species that belongs to the Fabaceae family, popularly known as “rattle or rattlesnake” due to the sound of their fruits when dry [7]. *Crotalaria* is one of the largest genera in tropical Africa. The genus includes 690 species that are mainly situated in Africa and Madagascar. The species have also been found throughout in India. [8] This is an erect shrub, annual short-lived perennial herb of 1.5 m or more tall. Taproot white or brown and stem grooved, solid, glabrous. Leaves trifoliolate, alternate spiral, stalked, leaflets elliptic, more than 2 cm long/ wide, hairy on upper surface, margin entire, apex obtuse base acute, pinnately veined. Flowers bisexual, grouped together in a terminal raceme, stalked, petals 5, yellow. Fruit rounded. This species is used in traditional medicine, the plant is used to treat urinary problems and fever, a poultice of the roots is applied to swelling of joints and fever and its leaves as vermifuge [9]. Mikirs of Assam take about 20 ml. extract of leaves in early morning to kill intestinal worms. [10]. Pharmacological studies have demonstrated it also present anti-inflammatory, antimicrobial, antioxidant, antibacterial & antifungal function [11-15]. The phytochemical screening showed that *C. pallida* leaves contain alkaloids, flavonoids, terpenoids, saponins, phenols, steroids and tannins

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 *Crotalaria pallida* were collected from the tribal belts of the local area of Baipariguda of Koraput district. (India) in the month of November 2011. 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-198/11, dated (16.12.2011)). 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 by grinding in mechanical grinder. 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 750 g coarse powder was extracted with 1200 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 [16,17].

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 extract of *C. pallida*. 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 *Crotalaria pallida* 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 [14]. Acute toxicity study revealed that no mortality was found in any solvent extract at any dose in Swiss albino mice, which confirmed that *C. pallida* leaves extract would be non-toxic in living body but where as the LD50 of the extracts was found to be (LD50 >

1000mg/kg). i.e., 300 mg/kg b.w. was selected as the therapeutic dose for the evaluation of antidiabetic activity[18,19]

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.[20,21] Wister 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).[31-33] 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 h 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.

Table 1: Antidiabetic activities of leaves extracts of *crotalaria pallid* by alloxan induced diabetic model

Groups	Blood glucose level (mg/dL) (X±S.D.)				
	Basal valu	1st day	3rd day	5th day	7th day
I	70 ± 0.24	87± 0.34	83± 0.57	88.±0.23	79 ± 0.17
II	334 ±0.53	334 ± 0.72	324.±0.81	305±0.46	300±0.86
III	273 ±0.86	241 ± 0.92	163 ±0.52	133 ±0.87	117±1.03*
IV	378 ±1.12	267 ±1.17	160 ±1.03	123±1.11	93±1.18***
V	354±1.17	203±0.83	167±1.13	136± 0.88	127±1.11*
VI	363±1.13	246±1.11	177±0.73	153±1.17	98±0.97**
VII	345±0.87	233±1.03	147±0.92	127±0.81	116±1.12*

Each values is represented as mean±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.)

Statistical Analysis

The observation values are reported as mean ±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 then 5% (p < 0.05) was considered statistically significant .

RESULTS AND DISCUSSION

The extracts produced a significant antidiabetic effect on first, third, fifth and seventh days at 300 mg/Kg body weight which showed in [Table 1]. Among the different extracts of *C.pallida*, significant antidiabetic activity was noticed 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 in the folklore medicines. The antidiabetic activity of the extracts is in the order of ethanol> n-butanol> petroleum ether> ethyl acetate

CONCLUSION

Based on the results of the present study, we conclude that the different extracts of *Crotalaria pallid* 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.

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