

Evaluation of anti-inflammatory and antidiabetic activity of ethanolic extracts of *Desmodium pulchellum* Benth. (Fabaceae) barks on albino wistar rats

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ABSTRACT

Effects of ethanolic extract of *Desmodium pulchellum* Benth. (Fabaceae) barks on alloxan-induced diabetic rats were investigated. In diabetic rats, blood glucose levels were reduced by 18.64 – 34.04 % on consumption of the extracts, with greater effect exhibited by the 1000mg/kg extract whereas in Glibenclamide treated diabetic rats, blood glucose levels were reduced upto 73.55%. The results suggested ethanolic extract of barks may contribute to the reduction of blood glucose levels and can be useful in the management of diabetes. The acute oral toxicity showed that the ethanolic extract of *D. pulchellum* barks was safe until 4000mg/kg body weight and no macroscopical organ abnormalities were observed in acute oral models. The investigations on Albino (Wistar) rats at dosage of 100, 200 and 400 mg/kg of ethanolic extract of *D. pulchellum* barks were made for anti-inflammatory action by using carrageenan induced paw edema and cotton pellete granuloma technique. The results of the study suggested significant dose dependent activity of extracts as compared to control group for both acute and chronic inflammation.

INTRODUCTION

Herbs are staging a comeback and herbal 'renaissance' is happening all over the globe. The herbal products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment. Over three-quarters of the world population relies mainly on plants and plant extracts for health care. An estimate suggests that about 13,000 plant species worldwide are known to have use as drug. The trend of using natural products has increased and the active plant extracts are frequently for new drug discoveries and for the presence of active phytotherapeutic materials (Das *et al* 1999). *Desmodium pulchellum* Benth. (Fabaceae), a stoutish shrub, an indigenous plant in Thailand and it is abundant in Khon Kaen.

The plant is distributed in the tropical areas and widely distributed in Bangladesh, India, Srilanka, and Southern China (Gani 2003). Traditionally the plant is used in cold and fever, malaria. Leaves are applied to ulcers. Decoction of bark is used for diarrhea, eye afflictions. Decoction of barks is used in malaria, swelling and rheumatism. The plant is used by chineses for traditional remedy of schistosomiasis. Some chemical constituents like, alkaloids, bufotenin, Indole-3 alkaline bases, tryptamine derivatives have been isolated from the whole plant (Ghosal *at al.*, 1966; Hjort *et al.*, 1989). Pharmacological investigations of the plant have not been thoroughly explored. The plant possesses antihelminthic, behavioral and anti- hepatofibrotic activities (Chitcharonthum *et al.*, 1990). In the traditional practice the barks of this plant has been used to control swelling and rheumatism. So, the anti-inflammatory activity of *D. pulchellum* barks is speculative and has not yet been documented. Diabetes mellitus is a serious endocrine syndrome and complex chronic condition that is a major source of ill health worldwide.

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This metabolic disorder is characterized by hyperglycaemia and disturbances of carbohydrate, protein and fat metabolisms, secondary to an absolute or relative lack of the hormone insulin (Apparao *et al.*, 2003). Diabetes mellitus is considered to be a serious in many countries. It is traditional to use medicinal plants to control diabetes (Pari & Ramakrishnan, 2001). The synthetic hypoglycaemic agent can produce serious side effect and are not suitable in pregnancy whereas drug derived from plants are frequently consider to be less toxic with fewer side effects & affordable in price (Momin 1987). Therefore the search for effective and safer hypoglycaemic agent has become area of research. The present study has been undertaken to study the action of anti-inflammatory and antidiabetic efficacy of ethanolic extract of *D. pulchellum* barks in rat models.

MATERIALS AND METHODS

Collection and Identification of Plant materials

Desmodium pulchellum Benth. (Fabaceae) fresh barks (collected from Bandarban, Bangladesh in July, 2011) were authenticated by the taxonomist Dr. Jasim Uddin, Associate Professor, Department of Botany, University of Dhaka and the identification number was documented as Accession no DUSH 4686 and Call no 01.

Preparation of plant extract

The barks of *Desmodium pulchellum*, washed with distilled water to remove dirt and soil, then the barks were cut into small pieces and then dried. The dried materials were powdered and passed through a 10-mesh sieve. The coarsely powdered material was extracted with ethanol. The extracts were filtered, pooled and concentrated under reduced temperature on a rotary evaporator. The extract was stored in a refrigerator and used for the present study.

Test animals

Albino (Wistar) rats 180-220 gm of either sex were used for the study. But, we selected only the male animals for antidiabetic activity since the females were reported, to be protected from lipid-Induced reduction in insulin action (Hevenor *et al.*, 2002). The animals were kept in the standard polypropylene cages and provided with food and water ad libitum. The animals were housed under standard environmental conditions with controlled conditions of temperature (23 ± 2 °C), humidity (50 ± 5 %) and 12 hour light-dark cycles. The animals were acclimatized for a period of 14 days prior to perform the experiments pharmacology. The experiments were performed according to the current guidelines for the care of the laboratory animals (Zimmerman 1983).

Acute toxicity study

The Oral acute toxicity of ethanol extract of *D. pulchellum* barks was determined in albino mice, maintained under standard conditions (Ecobichon 1997). The animals were fasted

overnight prior to the experiment. Fixed dose (OCED Guideline no. 420) method of CPCSEA was adopted for toxicity studies (Prema 2003). The tested extract was administrated orally. No mortality was observed at a dose of 4000mg/kg.

Anti-inflammatory activity

Anti-inflammatory activity was evaluated by two models: carrageenan induced paw edema and cotton pellet granuloma.

Carrageenan-induced paw edema

The five groups of rats, six in each group was included in this study. Inflammation was induced by injecting 0.1ml of 1% w/v carrageenan sodium salt subcutaneously in the sub-plantar region of the rat right hind paw (Winter *et al.*, 1962). The *D. pulchellum* barks extract (100, 200, 400mg/kg) or Diclofenac sodium (5 mg/kg) was administered orally 1 hour before carrageenan injection while control group received only saline at the doses of 10ml/kg body weight. The hind paw volume was measured plethysmometrically before and after the carrageenan injection, at hourly intervals for 3hr.

$$\% \text{ of inhibition of edema} = (V_c - V_t) / V_c \times 100$$

Where, V_t = mean paw volume of test group & V_c = mean paw volume of control group.

Cotton pellet granuloma

The five groups of rats, six in each group was included in this study. For cotton pellet granuloma, a 50 mg sterilized cotton pellet was implanted subcutaneously on the back of neck in rats under ether anesthesia. Animals in the control group received only the vehicle at the dose of 10ml/kg. Animals in treated group received the extract at the doses of 100, 200 and 400 mg/kg body weight once daily for 14 consecutive days. Diclofenac sodium (5mg/kg) was given as reference drug. On the 14th day the animals were sacrificed with ether, the pellets granulomas were removed, fixed from extraction tissue, dried overnight at 55 ± 0.5 °C and weighed (Winter *et al.*, 1957).

Induction of Diabetes

Hyperglycemia was induced in overnight fasted Albino Waster rats by a single intraperitoneal injection of freshly prepared Alloxan Monohydrate in sterile saline at a dose of 120mg/kg body weight (Ju *et al.*, 2008). After 5 days of Alloxan injection, the diabetic rats (glucose level >250 mg/dl) were selected and grouped for the study.

Evaluation of antidiabetic activity

Diabetes was induced in Albino Wistar rats by intraperitoneal administration of ice cold aqueous Alloxan Monohydrate (Rao 1999). The fasting blood sugar levels of each of the rats were checked every day with an autoanalyzer (Glucometer, Bioland G-423 S) glucose kit. After 8 days, animals with fasting blood sugar levels of 250 mg/dl and above were considered to be diabetic and were used for the study. The selected rats were divided into five groups of six rats each like Group I

(Diabetic untreated rats received Tween-80 solution), Group II, III & IV (Diabetic rats treated with ethanol extract of *D. pulchellum* root extract at the dose of 250, 500 & 1000mg/kg) and Group V (diabetic rats treated with standard reference drug Glibenclamide 5mg tablet of Daonil from Sanofi-Aventis at a dose of 5mg/kg). After the administration of drug and extracts blood glucose levels of the rats were measured at hourly intervals of 0, 1, 2 and 3 hours. Blood samples were collected by tail snip and the blood glucose measured with an autoanalyzer glucose kit (Glucometer, Bioland G-423 S). At the end of the experiment, percentage reduction of the glucose levels of the rats at the 3rd hour was calculated using the following formula:

$$\text{Percentage Reduction} = \frac{[\text{BGL at 0 hr} - \text{BGL at 3}^{\text{rd}} \text{ hr}]}{\text{BGL at 3}^{\text{rd}} \text{ hr}} \times 100\%$$

BGL = Blood Glucose Level

Statistical Analysis

The result were expressed as Mean \pm SEM. Statistical Analysis was performed with one way analysis of variance (ANOVA) followed by student's *t* test. $P < 0.05$ were considered to be statistically significant.

RESULT AND DISCUSSION

The anti-inflammatory activity of *D. pulchellum* barks at different doses employed for screening of different phases of inflammatory process. The development of carrageenan induced edema is believed to be biphasic of which the first phase is mediated by release of histamine, serotonin and kinins in the first hour after injection of carrageenan and the second phase is related to release of prostaglandin like substances in 2-3 hours (Brooks & Day 1991). The root extract predominantly inhibits the release of prostaglandin like substances from phlogenic stimuli indicated by

the results of the present study. The result of anti-inflammatory effects of *D. pulchellum* root on carrageenan induced paw has been shown in Table 1.

The cotton pellet granuloma bioassay is considered as a model for studies of chronic inflammation and considered as a typical feature of established chronic inflammatory reaction (Spector 1996). Ethanolic extract of *D. pulchellum* barks exhibited significant reduction in the granuloma formation in the cotton pellet-induced granuloma in rats. This reflected that the extracts are effective in chronic inflammatory conditions which is presented in Table 2.

Antidiabetic Activity of Diabetic Induced Rats

Elevation of blood glucose level by Alloxan, a β -cytotoxin, due to reduced synthesis and release of insulin as a result of massive destruction of β -cells of the islets of Langerhans confirmed the induction of diabetes in alloxan-induced experimental rats (Lazarow 1964). In the present study, hyperglycemia produced by alloxan monohydrate was significantly lowered (Table 3) by administration of ethanol extract of *D. pulchellum* barks in a dose of 250, 500 and 1000 mg/kg body weight after 3 hour of treatment. Table 3 shows the blood glucose levels of diabetic control, ethanol extract of *D. pulchellum* barks and glibenclamide-treated rats. In diabetic control rats, the increase in blood glucose concentration was observed after 1 h. The blood glucose concentration remained high over the next hour. Ethanol extract of *D. pulchellum* barks and glibenclamide treated rats showed significant decrease in blood glucose concentration at hourly intervals when compared with diabetic control rats. The antidiabetic studies of the ethanol extract of *D. pulchellum* barks on alloxan induced diabetic rats showed highly significant antidiabetic effect with minimal toxicity.

Table. 1: Effect of ethanolic extract of *D. pulchellum* barks on carrageenan induced paw edema in rats.

Treatment	Dose (mg/kg)	Paw volume ml (Mean \pm SEM)				% inhibition after 3 rd hour
		0 hrs	1 hrs	2 hrs	3 hrs	
Control		0.81 \pm 0.04	1.24 \pm 0.04	1.31 \pm 0.04	1.36 \pm 0.04	-
Diclofenac	5	0.82 \pm 0.02	1.01 \pm 0.02	0.95 \pm 0.04	0.91 \pm 0.04	33.08
<i>D. Pulchellum</i> Barks	100	0.79 \pm 0.03	1.15 \pm 0.03	1.12 \pm 0.02	1.09 \pm 0.02	19.85*
<i>D. pulchellum</i> barks	200	0.81 \pm 0.03	1.14 \pm 0.01	1.09 \pm 0.02	1.04 \pm 0.02	23.52 *
<i>D. pulchellum</i> barks	400	0.80 \pm 0.01	1.11 \pm 0.04	1.05 \pm 0.03	1.01 \pm 0.02	25.74 *

Values are Mean \pm SEM (n = 6). * $p < 0.05$ as compared to carrageenan control

Table. 2: Effect of ethanolic extract of *D. pulchellum* barks on cotton pellet induced granuloma in rats.

Treatment	Dose (mg/kg/day)	Mean weight of Granuloma (mg) (Mean \pm SEM)
Control	--	105.23 \pm 1.37
Diclofenac	5	63.88 \pm 1.51
<i>D. pulchellum</i> barks	100	95.44 \pm 1.42 **
<i>D. pulchellum</i> barks	200	83.29 \pm 1.32 **
<i>D. pulchellum</i> barks	400	74.55 \pm 1.17 **

Values are Mean \pm SEM (n= 6); ** $p < 0.05$ as compared to control

Table. 3: Effect of ethanol extract of *D. pulchellum* barks (EEDPB) on the fasting blood glucose levels of Alloxan induced diabetic rats:

Group	Treatment	Dose (mg/kg body weight)	Fasting Blood Glucose Level (mg/ml) (Mean \pm SE)				% reduction at the 3 rd hour
			0 hr	1 hr	2 hr	3 hr	
I	Diabetic untreated		322.7 \pm 7.3	328.3 \pm 6.1	329.7 \pm 4.9	327.4 \pm 5.7	-
II	Diabetic treated with EEDPB	250	337.8 \pm 6.1	311.4 \pm 5.3	294.4 \pm 4.9	274.7 \pm 4.5	18.68
III	Diabetic treated with EEDPB	500	325.7 \pm 7.1	293.4 \pm 4.3	271.5 \pm 3.9	249.3 \pm 4.1	23.46***
IV	Diabetic treated with EEDPB	1000	340.4 \pm 6.1	305.7 \pm 4.4	277.2 \pm 3.9	224.5 \pm 3.9	34.04***
V	Diabetic treated with Glibenclamide	05	338.5 \pm 5.2	181.7 \pm 2.3	108.3 \pm 1.7	89.5 \pm 1.4	73.55***

Values are Mean \pm SEM (n= 6); *** $p < 0.05$ as compared to control

CONCLUSION

The study reveals the protective potential of *D. pulchellum* against an unpleasant sensory or emotional experience associated with actual or potential tissue damage or prescribed in terms of such damage (inflammation). Thus from the results obtained in the present investigation, it may be further concluded that the ethanolic extract of *D. pulchellum* barks possesses a potent anti-inflammatory activity against both exudative and proliferative phases of inflammation. The study also revealed that the ethnopharmacological claim to use as anti-inflammatory agents. Ethanolic extract of barks of *D. pulchellum* exhibited significant anti hyperglycemic activities in alloxan-induced diabetic rats. Further studies will be focused on determination of the mechanism of action, as well as on the isolation of bioactive principles.

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