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Evaluation of Anti-inflammatory activity of Hydro-alcoholic extract *Diplocyclos palmatus* (L.) Jeffry. roots in experimental animals

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Abstract

Diplocyclos palmatus (l.) Jeffry. commony known as Shivlingi belongs to family Cucurbitaceae had been widely used for its reported biological activities in indigenous system of medicine. The present investigation was carried out to find the effect of hydro-alcoholic extract of roots of *Diplocyclos palmatus* (l.) Jeffry. for its anti-inflammatory activity. The anti-inflammatory activity was evaluated using acute inflammatory models viz., carrageenan induced paw oedema. Oral administration of the extract at the doses 100 and 200 mg/kg b.w. exhibited dose dependent and significant anti-inflammatory activity in ($p < 0.01$). Hence, present investigation established pharmacological evidences to support the folklore claim that *Diplocyclos palmatus* (l.) Jeffry. is used as anti-inflammatory agent.

Key-Words: *Diplocyclos palmatus* (l.) Jeffry., Roots, Anti-inflammatory activity

Introduction

Plants represent still a large untapped source of structurally novel compounds that might serve as lead for the development of novel drugs. Screening of the plants for their biological activity is done on the basis of either their chemotaxonomic investigation or ethnobotanical knowledge for a particular disease. Identification of a particular compound against a specific disease is a challenging long process. Importance of the plant lies in their biologically active principles. [1]

Diplocyclos palmatus is commonly known as Shivalingi, belongs to family Cucurbitaceae, is an annual, herbaceous climber, growing up to a height of 3-4 m. Stem glabrous, becoming thickened and white dotted on the ridges when older. Leaves are alternate, broadly ovate, palmately 3-7 lobed, 3.5-14 x 4-14.5 cm, lobes linear-lanceolate to elliptic, glabrous; margins often irregularly toothed petiole 1.5-9.0 cm long. Tendrils have 2 branches. Flowers small, pale yellow, unisexual, monoecious, male in sessile clusters of 2-8, along with 2-5 female flowers borne in the same axil. Calyx tube 3-4 mm long in male, 1.5-2.5 mm long in female, lobes smaller than tube. Corolla of male larger than female. Ovary ellipsoid, inferior, 5 mm long,. 2.5 mm broad, with longitudinal white stripes. Fruit solitary or in clusters of 2-5, ovoid-subglobose, 1.5-2.5 cm diameter, at maturity it is red with white stripes. Seeds 5-6 x 2.5-3 mm. Flowering and fruiting occur during August - November. [2]

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The plant is used by the various tribal communities of India in the treatment of various disease and disorders, keeping this view the present work was conceived to explore the folk lore and traditional uses of this plant. As there is no reference in literature to the anti-inflammatory aspects, it was considered worthwhile to study the anti-inflammatory activity of hydro-alcoholic extract roots of *Diplocyclos palmatus*.

Material and Methods

Selection, collection and authentication of plant/plant material

The plant parts were collected in the months of July-September 2022 from the various local sites of Rewa, M.P. and identified & authenticated by Dr. S. N. Dwivedi, Retd. Prof. and Head, Department of Botany, Janata PG College, A.P.S. University, Rewa, M.P. and was deposited in our Laboratory, Voucher specimen No. PCog/DP/028.

Preparation of Extract

Successive Extraction of Plant Material

Sample were shattered and screened with 40 mesh. The shade dried coarsely powdered roots of *D. palmatus* (250gms) were loaded in Soxhlet apparatus and was extracted with ethanol and water (9:1) until the extraction was completed. After completion of extraction, the solvent was removed by distillation. The extracts were dried using rotator evaporator. The residue was then stored in dessicator and percentage yield were determined. [3]

Pharmacological Screening

Procurement of experimental animals

The mice were used for acute toxicity study as per OECD guidelines 423. The animals were fed with standard pellet diet (Hindustan lever Ltd. Bangalore) and water *ad libitum*. All the animals were housed in polypropylene cages. The animals were kept under alternate cycle of 12 hours of darkness and light. The animals were acclimatized to the laboratory condition for 1 week before starting the experiment. The experimental protocols were approved by Institutional Animal Ethics Committee after scrutinization.

Anti-inflammatory Activity [4-5]

Carrageenan induced paw oedema

Animals

Female Wistar rats of (200-250 gm) were procured from Veterinary College, Mhow, Indore, (M.P.) maintained under ideal feeding and management practices in the laboratory. The animals were fed with standard pellet diet (Hindustan lever Ltd. Bangalore) and water *ad libitum*. All the animals were housed in polypropylene cages. The animals were kept under alternate cycle of 12 hours of darkness and light. The animals were acclimatized to the laboratory condition for 1 week before starting the experiment. The experimental protocols were approved by Institutional Animal Ethics Committee after scrutinization.

Study Design

The animals were divided into 4 groups each containing six animals. Group I served as vehicle control (2% Tween 80), Group II served as standard (diclofenac, 10 mg/kg, p.o.) and Group III and IV group were treated with different doses of *D. palmatus* hydro-alcoholic extracts.

Anti-inflammatory Screening

The hydro-alcoholic extract of *D. palmatus* and standard drug diclofenac were administered in prescribed doses. Control received 0.1 ml of 1% carrageenan in 2% Tween 80. The administration of extract and drug was 30 min prior to injection of 0.1 ml of 1% carrageenan in the right hind paw subplatar of each rat. The paw volume was measured plesthysmometrically (model 7140, Ugo Basil, Italy). Prior to injection of carrageenan, the average volume of the right hind paw of each rat was calculated. At 1, 2, 3, 4, 5 and 6 hr after injection paw volume was measure. Reduction in the paw volume compared to the vehicle-treated control animals was considered as anti-inflammatory response. Then percentage of inhibition of edema was calculated for each group with respect to the control group as follows,

% Inhibition of paw edema = (VC-VT/VC) 100

VC and VT represent average paw volume of control and drug treated animals respectively

Statistical analysis

All the values ware statistically analyzed by one-way analysis of variance (ANOVA) followed Bonferroni's post hoc test. Comparison between control and drug treated groups were considered to be significant (*P<0.01). All values are expressed as mean ± SEM.

Results and Discussion

The hydro-alcoholic extract of *D. palmatus* were administered for 7 days before the injection of

carrageenan caused dose dependent inhibition of increase in paw edema from 1 h to 5 h. The inhibitory effect of the all the treatment were recorded with a dose of 100 and 200 mg/kg at 1h, 3h and 5h respectively. Diclofenac (10 mg/kg) were administered 1 h before the injection of carrageenan caused significant (P<0.001) inhibition of increase in paw edema at 5th h. The inhibitory effect of the diclofenac at 10 mg/kg was recorded. The results were presented in Table 1. Graph showed the % inhition of standard drug, diclofenac and different doses of hydro-alcoholic extract of *D. palmatus.*

Conclusion

From the results obtained it was concluded that HEEDPR at the dose of 200 mg/kg bw produced significant results when compared with standard drug.

Table 1: Effect of hydro-alcoholic extract of *Diplocyclos palmatus* (L.) Jeffrey root on inhibition of right hind paws edema on carrageenan induced inflammation in rats

Group	Change in paw edema volume (%)		
	1h	3h	5h
Control	1.19 ± 0.06	3.16 ± 0.05	3.84 ± 0.07
Diclofenac 10 mg/kg	1.14± 0.01	2.48±0.06**	2.68 ± 0.06***
HEDPR (100 mg/kg)	2.09± 0.02^{+}	3.44± 0.08^{+}	3.49± 0.10***
HEDPR (200 mg/kg)	1.86± 0.12^{+}	3.39± 0.01^{+}	3.38± 0.04***

Data are expressed as mean ± S.E.M.; n=6 rats per group. Two way ANOVA followed by Bonferroni's post hoc test when compared with control *P<0.05, **P<0.01, ***P<0.001, +NS

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