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# Investigation of Anti-Inflammatory Activity of Aqueous and Ethanolic Extract *Diplocyclos Palmatus* (L.) Jeffry. in Experimental Animals

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## Abstract
*Diplocyclos palmatus* (L.) Jeffry. (H: Shivlingi; Family: Cucurbitaceae) had been widely used for its reported biological activities in indigenous system of medicine. The present investigation was carried out to access the effect of aqueous and ethanolic extract of stem and leaves 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 ($p < 0.01$).

**Keywords**
*Diplocyclos palmatus* (l.) Jeffry., Stem, Leaves, Anti-inflammatory activity

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## INTRODUCTION
Inflammation is a local response of living mammalian tissues to the injury. It is a body defense reaction in order to eliminate or limit the spread of injurious agents. There are various components to an inflammatory reaction that can contribute to the associated symptoms and tissue injury. Oedema formation, leukocyte infiltration and granuloma formation represent such components of inflammation[1]. The wild plants have been a source of food and medicine from the dawn of human civilization. These plants are more economic and often more acceptable during the days of famine and scarcity. The tribal mostly uses wild plants as food and vegetable. The natives have also sold these plants in local village markets[112]. Some of these are rich in nutrients and also consumed by urban people. The study of wild edible plants is important not only identifies the potential source, which could be utilized as alternative food but to select promising type of domestication.[2] Plants represent still a large untapped source of structurally novel compounds that might serve as lead for the development of novel drugs[6]. Screening of the plants for their biological activity is done on the basis of either their chemotaxonomic investigation or ethno botanical 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.[3] *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.[4] 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 aqueous and ethanolic extract stem and leaves 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 2012 from the various local sites of Rewa, M.P. and identified & authenticated by Dr. S. N. Dwivedi, 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/156.

### Preparation of Extract

### Successive Extraction of Plant Material[5]

Sample were shattered and screened with 40 meshes. The shade dried coarsely powdered stem and leaves of *D. palmatus* (250gms) were loaded in Soxhlet apparatus and was extracted with petroleum ether (60-62°C), Chloroform, ethanol and water 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.

### Pharmacological Screening[6-8]

### 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

### 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 10 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 others group were treated with different doses of *D. palmatus* aqueous and ethanolic extracts.

### Anti-inflammatory Screening

The aqueous and ethanolic 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,

$$\% \text{ Inhibition of paw edema} = (V_C - V_T / V_C)\ 100$$

**$V_C$ and $V_T$ 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 aqueous and ethanolic 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 4.50% at 1h, 9.8 % at 3 h and 31.47% at 5h. The inhibitions elicited by the aqueous and ethanolic extract were comparable to that of diclofenac and the results were presented in Table 1.

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

| S/No. | Group | Change in paw edema volume (%) | | |
|---|---|---|---|---|
| | | 1h | 3h | 5h |
| 1. | Carrageenan Control | 1.11 ± 0.06 | 3.06 ± 0.05 | 3.94 ± 0.07 |
| 2. | Diclofenac 10 mg/kg | 1.06 ± 0.01 | 2.76±0.06** | 2.70 ± 0.06*** |
| 3. | AEDPS (100 mg/kg) | 2.11± 0.02$^{+}$ | 3.56± 0.08$^{+}$ | 3.54± 0.10*** |
| 4. | AEDPS (200 mg/kg) | 1.90± 0.12$^{+}$ | 3.47± 0.01$^{+}$ | 3.44± 0.04*** |
| 5. | EEDPS (100 mg/kg) | 2.02± 0.08$^{+}$ | 3.23± 0.11$^{+}$ | 3.20± 0.05** |
| 6. | EEDPS (200 mg/kg) | 1.94± 0.10$^{+}$ | 3.08± 0.13$^{+}$ | 3.02± 0.07*** |
| 7. | AEDPL (100 mg/kg) | 1.47± 0.19$^{+}$ | 3.01± 0.04* | 2.99± 0.08*** |
| 8. | AEDPL (200 mg/kg) | 1.37± 0.04$^{+}$ | 2.98± 0.09** | 2.95± 0.09** |
| 9. | EEDPL (100 mg/kg) | 1.09± 0.03** | 2.84± 0.02** | 2.83± 0.11*** |
| 10. | EEDPL (200 mg/kg) | 1.08± 0.01*** | 2.81± 0.07** | 2.79± 0.03** |

**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 carrageenan control *P<0.05, **P<0.01, ***P<0.001, +NS**

**Table 2: Effect of *Diplocyclos palmatus* (L) Jeffrey on inhibition of right hind paws edema on carrageenan induced inflammation in rats**

| S/No. | Group | % Inhibition at | | |
|---|---|---|---|---|
| | | 1h | 3h | 5h |
| 1. | Carrageenan control | - | - | - |
| 2. | Diclofenac 10 mg/kg | 4.50 | 9.80 | 31.47 |
| 3. | AEDPS (100 mg/kg) | -90.09 | -16.33 | 10.15 |
| 4. | AEDPS (200 mg/kg) | -71.17 | -13.39 | 12.69 |
| 5. | EEDPS (100 mg/kg) | -81.98 | -5.55 | 18.78 |
| 6. | EEDPS (200 mg/kg) | -74.77 | -0.65 | 23.35 |
| 7. | AEDPL (100 mg/kg) | -32.43 | 1.63 | 24.11 |
| 8. | AEDPL (200 mg/kg) | -23.42 | 2.61 | 24.11 |
| 9. | EEDPL (100 mg/kg) | 1.80 | 7.14 | 28.17 |
| 10. | EEDPL (200 mg/kg) | 97.29 | 8.16 | 29.18 |

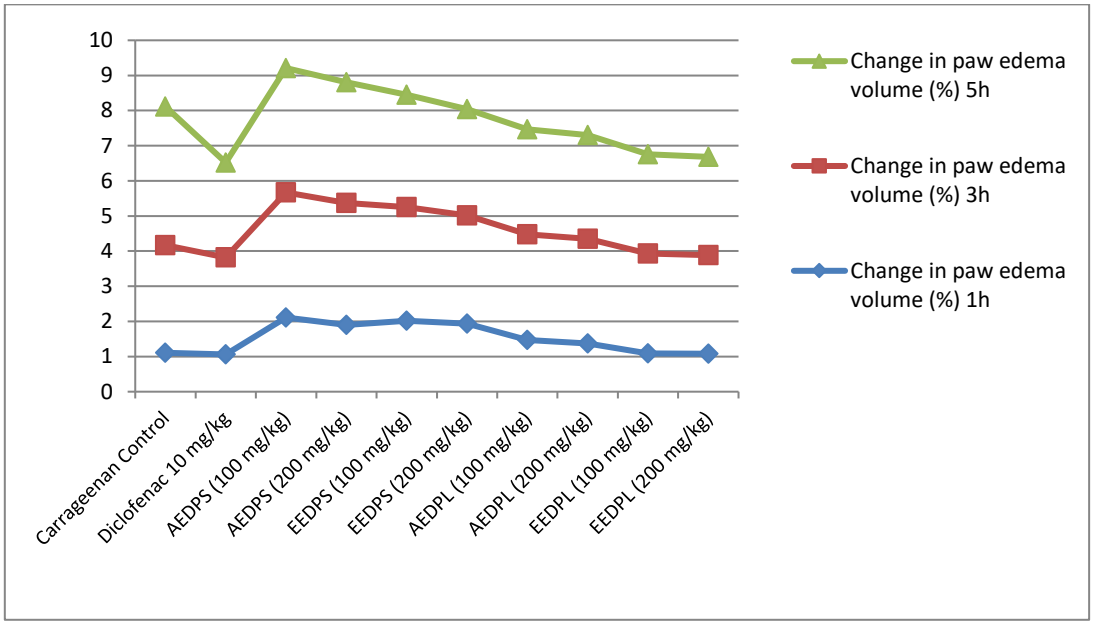


**Fig. 1: Change in Paw edema volume of *Diplocyclos palmatus* (L.) Jeffrey on carrageenan induced inflammation in rats**

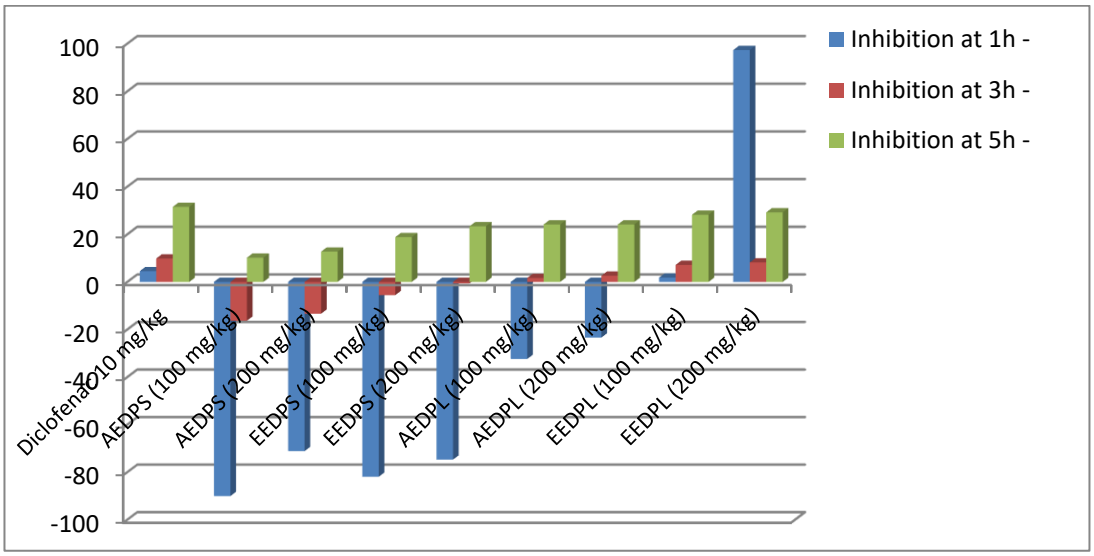


**Fig. 2: Percent Inhibition of *Diplocyclos palmatus* (L.) Jeffrey on carrageenan induced inflammation in rats**

## CONCLUSION

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 ($p < 0.01$). It was concluded in the present investigation that ethanolic extract of leaves showed maximum activity as compared to other extract.

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