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Investigation of Anti-inflammatory activity of *Hemedesmus indicus* L. on Carrageenan induced paw oedema in rats

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ABSTRACT

Hemidesmus indicus L. (Family: Apocynaceae), is commonly referred as Indian sarsaparilla, Anantamool or Nannari, commonly available perennial climbing plant 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 aqueous and ethanolic extract of leaves and stem of *Hemidesmus indicus* 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 200 and 400 mg/kg b.w. exhibited dose dependent and significant anti-inflammatory activity in ($p < 0.05$). Hence, present investigation established pharmacological evidences to support the folklore claim that *Hemidesmus indicus* is used as anti-inflammatory agent.

Keywords: *Hemidesmus indicus (H. indicus)*, Leaves, Stem, Anti-inflammatory activity

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INTRODUCTION

Inflammation is a local response of living tissues to the injury and is a body defense reaction in order to eliminate or limit the spread of injurious agents. There are several 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. Oedema formation in the paw is the result of a synergism between various inflammatory mediators that increase vascular permeability and/or the mediators that increase blood flow[1-2]. Carrageenan-induced paw oedema is widely used for determining the acute phase of inflammation. Histamine, 5-hydroxytryptamine and bradykinin are the first detectable mediators in the early phase of carrageenan-induced inflammation whereas prostaglandins are detectable in the late phase of inflammation[3-4]. Many medicines of plant origin had been used since long time without any adverse effects. For centuries people have been trying to alleviate and treat disease with different plant extracts and formulations[5]. Screening of the plants for their biological activity is done on the basis of either their chemotaxonomic investigation for a particular disease[6].

Hemidesmus indicus L. (Family: Apocynaceae), is commonly referred as Indian sarsaparilla, Anantamool or Nannari, commonly available perennial climbing plant. It is used as main ingredient in the preparation of the cool and refreshing drink Nannari sharbat. Its native is India, also found in south tropical Asian countries such as Sri Lanka & Pakistan. The plant is used by the various tribal communities of India in the treatment of various disease and disorders[7]; 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 leaves and stem of *Hemidesmus indicus*.

MATERIAL AND METHODS

Selection, collection and authentication of plant/plant material

The crude drug of *Hemidesmus indicus* L. leaves and stem was obtained from local area of Indore, M.P. and authenticated by Dr. S.N.Dwivedi, Professor and Head, Department of Botany, Janata PG College, A.P.S. University, Rewa, M.P. (Voucher No. J/BO T/L-251).

Preparation of Extract (Leaves & Stem)

Sample were shattered and screened with 40 meshes. The shade dried coarsely powdered leaves & stem of *Hemidesmus indicus* L. (250gms) was loaded in Soxhlet apparatus and was extracted with 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.[8]

Animals

Adult rats of Wister strains of 150-200 grams have been obtained from local market of Indore. The Institutional Animal Ethical Committee approved the experimental protocols (PBRI/IAEC/PN-17047a). The animals have been placed in a controlled room, with normal room temperature 25 ± 3^0C and humidity 35 - 50 %. Normal rat feeds and water *ad li betum* have been provided at regular interval of time. Animals have been housed in polypropylene cages. The animals have been allowed to acclimatize to laboratory conditions prior to experimental procedures.

Acute Toxicity Studies of Extracts

Acute oral toxicity studies have been conducted separately followed by using OECD guideline 423. The method used defined doses of 5, 50, 300, 2000 mg/kg *p.o.* body weight. Results were allowed substance rank and classify according to the Globally Harmonized System (GHS) for classification of chemicals which causes acute toxicity. From LD_{50} determination, $1/10^{th}$ of the dose was focused as the medial for pharmacological screening. Since all animals were alive & no toxicity and no significant changes in the body weight between the control and treated group were demonstrated at doses up to 2000 mg.[9]

Experimental design

Group	Category	Drug administered
1.	Normal Control	Normal saline or no any drug
2.	Positive control	standard drug as per activity
3.	Test 1 200 mg/kg	*H. indicus stem extract (ethanol)*
4.	Test 2 400 mg/kg	*H. indicus stem extract (ethanol)*
5.	Test 3 200 mg/kg	*H. indicus stem extract (Aqueous)*
6.	Test 4 400 mg/kg	*H. indicus stem extract (Aqueous)*
7.	Test 5 200 mg/kg	*H. indicus leaves extract (ethanol)*
8.	Test 6 400 mg/kg	*H. indicus leaves extract (ethanol)*
9.	Test 7 200 mg/kg	*H. indicus leaves extract (Aqueous)*
10.	Test 8 400 mg/kg	*H. indicus leaves extract (Aqueous)*

Anti-inflammatory activity (Carrageenan induced paw oedema)

The adult wistar Albino rats (150-200 gm) were divided into 10 groups and each group has 06 rats. Inflammation induced by injecting 0.1ml of 1% carrageenin into the subplanter tissue of the hind paw of either one side. All group administered drug as per schedule design and phenylbutazone (100 mg/kg) *i.p* was used as standard drug. It should be given 30 minutes prior to the carrageenin injection. The paw volume has been measured before and 03 hours after carrageenin administration by the volume displacement of water-mercury column using a plethysmometer.[10-11]

Statistical analysis

Results were tabulated and the data was expressed as mean ± SEM. The difference between experimental group were determined using one way analysis of variance (ANOVA) followed by Dunnet test. $P \leq 0.05$ was considered significant.

RESULTS AND CONCLUSION

The aqueous and ethanolic extracts of leaves and stem of plant of *H. indicus* L. were screened for acute toxicity study by OECD guideline no. 423 for determination of LD_{50}. This result indicates 200 mg/kg dose has been considered as effective dose (ED_{50}), for *H. indicus.*

Table 1: LD_{50} & ED_{50} of *H. indicus* L.

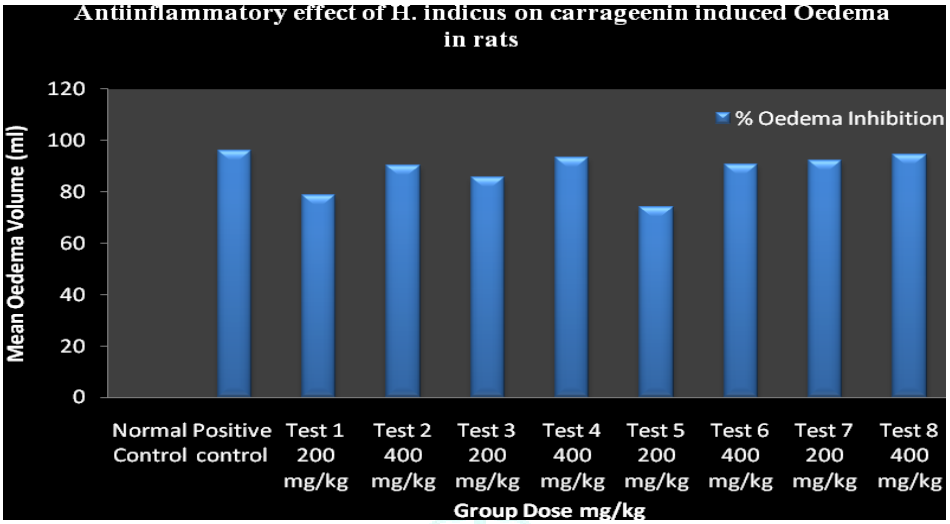
Plant Name	LD_{50}	ED_{50}
***H. indicus* L.**	2000 mg/kg	200 mg/kg

The aqueous and ethanolic extract of *H. indicus* L. leaves and stem were evaluated for anti-inflammatory activity in animal models and the results are summarized in Table 2. The result obtained indicates that the extract found to have significant ($P < 0.05$) anti inflammatory activity in rats. The aqueous leaves extract at the test doses 400 mg/kg b.w. reduced the oedema induced by carrageenan by 96.32% and was found to be maximum as compared to standard drug which showed 96.09% of inhibition.

Table 2: Anti-inflammatory effect of *H. indicus* on carrageenin induced oedema in rats

Group Dose mg/kg *(p.o.)*	Treatment (mg/kg)	Mean Oedema Volume (ml)	% Oedema inhibition
Normal Control	Carrageenin (0.1ml / paw, *s.c.)*	1.41 ± 0.025	--
Positive control	Carrageenin 0.1ml + Phenylbutazone 100 mg/kg *p.o.*	0.055 ± 0.010**	96.09
Test 1 200 mg/kg	Carrageenin 0.1ml + 5% Stem extract *(ethanol)*	0.300 ± 0.022*	79.00
Test 2 400 mg/kg	Carrageenin 0.1ml + 10% Stem extract *(ethanol)*	0.140 ± 0.045*	90.07
Test 3 200 mg/kg	Carrageenin 0.1ml + 5% Stem extract *(Aqueous)*	0.202 ± 0.032**	85.67
Test 4 400 mg/kg	Carrageenin 0.1ml + 10% Stem extract *(Aqueous)*	0.096 ± 0.022**	93.19
Test 5 200 mg/kg	Carrageenin 0.1ml + 5% Leaves extract *(ethanol)*	0.365 ± 0.025*	74.11
Test 6 400 mg/kg	Carrageenin 0.1ml + 10% Leaves extract *(ethanol)*	0.130 ± 0.036*	91.00
Test 7 200 mg/kg	Carrageenin 0.1ml + 5% Leaves extract *(Aqueous)*	0.112 ± 0.020**	92.05
Test 8 400 mg/kg	Carrageenin 0.1ml + 10% Leaves extract *(Aqueous)*	0.080 ± 0.023**	94.32

All values are mean ± SEM, n=6, *P<0.05 indicates significant and **P<0.001 is more significant when compared with control.



Groups Dose [mg/kg (p.o.)]	Abbreviation	Treatment (mg/kg)
Normo Control	NC	Saline
Positive Control	PC	Paracetamol (100 mg/kg)
Test 1 200 mg/kg	T1	Stem extract *(ethanol)*
Test 2 400 mg/kg	T2	Stem extract *(ethanol)*
Test 3 200 mg/kg	T3	Stem extract *(Aqueous)*
Test 4 400 mg/kg	T4	Stem extract *(Aqueous)*
Test 5 200 mg/kg	T5	Leaves extract *(ethanol)*
Test 6 400 mg/kg	T6	Leaves extract *(ethanol)*
Test 7 200 mg/kg	T7	Leaves extract *(Aqueous)*
Test 8 400 mg/kg	T8	Leaves extract *(Aqueous)*

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