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Evaluation of Anti-inflammatory Activity of Naravelia Zeylanica Linn. Leaves Extract

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Abstract

Naravelia Zeylanica Linn. commonly known as Vatnisini is traditionally used for several medicinal purposes in India. The present study assessed anti-inflammatory activity of its leaf. Dried and crushed leaves of *Naravelia Zeylanica*were extracted with ethanol. The ethanol extract (EENZ) at the doses of 250 mg/kg and 500 mg/kg body weight was subjected for evaluation of anti-inflammatory activity in experimental animal models.Acute anti-inflammatory activity was evaluated by Carrageenan, Histamine and Serotonon-induced paw oedema in Wister albino rats. Diclofenac sod.were employed as reference drug for anti-inflammatory study. In the present study, the ethanol extract of the leaves of *Naravelia Zeylanica*demonstrated significant acute anti-inflammatory activity in the tested models.

Key words: *Naravelia Zeylanica Linn.*, Anti-inflammatory, Pain

Introduction

Inflammation is a normal protective response to tissue injury caused by physical trauma, noxious chemical or microbial agents. It is the body response to inactivate or destroy the invading organisms, to remove irritant and set the stage for tissue repair. It is triggered by the release of chemical mediators from injured tissue and migrating cells (Bhitre et al., 2008). Drugs from plant origin are used in India for treatment of many diseases in traditional system of medicine. Naravaliazeylanica DC (Ranunculaceae) is a climbing shrub with tuberous roots; wiry stem strong tendrils, leaves 3-foliate, opposite, terminal leaflet modified into a 3 branched tendril, leaflets ovate-lanceolate, serrate or crenate, prominently nerved; flowers yellow, fragrant, in axillary and terminal penicles, sepals downy, petals linear-clavate, elongate; fruits aggregate of achenes, ending in twisted feathery tales. The plant is available rich all around south India (Raja et al., 2008). Plant is reported to contain mainly alkaloids,flavonoids, saponins and tannins.Methanolic extract of leaves of*Naravelia zeylanica*berberine is present,sterols are also present in the ethanolic extract of leaves and stem (RajaNaika et al., 2002). The whole plant is traditionally used in vitiated vata, pitta, inflammation, skin diseases, arthritis, headache, colic, wounds and ulcers. Leaf paste is consumed to treat Chest pain. The vines when crushed give a pungent odour which is inhaled to cure cold, all type of headaches including migraine. Root and stem paste is applied externally for psoriasis, itches and skin allergies. The traditional medi- cine practioners using the leaf and stem juices for treating intestinalworms, psoriasis & dermatitis (Udayan et al., 2006).The literature survey reveals no reports on the anti-inflammatory activity ofthe leaves extracts of Naravelia Zeylanica. This prompted us to investigate the anti-inflammatory activity of Naravelia Zeylanica leaves extract.

Materials and Methods

Plant material

The leaves ofof Naravelia Zeylanica were collected from Udupi, Karnataka, during July. It was authenticated by Dr.GopalakrishnaBhat, Department of Botany, PoornaPrajna College, Udupi, Karnataka, India. A voucher specimen has been maintained in our lab for future reference.

Preparation of Extract

The leaves of *Naravelia zeylanica* will be collected fromUdupi, Karnataka. Fresh leaves of *N.zeylanica*were shade dried, powdered mechanically & stored in airtight containers until the extraction. The

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powder extracted with 95% ethanol in Soxhlet extractor for 30h. Extract was concentrated under reduced pressure using rotary flash evaporator to a syrupy consistency. Then it was dried in the dessicator (68% yield) (AshokaShenoy et al., 2009).

Animal Used: Wistar strain rats of either sex (150-180 g) of either sex were procured from Indian Institute of Sciences. They are maintained under standard conditions (temperature 22 ± 2°C, relative humidity 50±5% and 12 h light/dark cycle).The animals were housed in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to standard pellet diet and water *ad libitum*. The Institutional Animal Ethics Committee approved the experimental protocol(ref no. 367001/C/CPCACA). All the animals received humane care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the "National Academy of Sciences" and published by the "National Institute of Health".

Evaluation of anti-inflammatory activity

Carrageenan induced rat paw oedema

The rats were divided into four groups containing six rats in each group. 0.1 ml of 1.0% carrageenan in normal saline (0.9% w/v NaCl) was injected to the sub plantar region of right hind paw. The EENZ was administered to the rats 1 h before carrageenan injection. Different groups were treated as follows:

Group I: Carrageenan (0.1 ml of 1.0%carrageenan/rat to the sub plantar region).
Group II: Carrageenan + Diclofenac sod.(10 mg/kg b. w., p. o.)
Group II and IV: Carrageenan + EENZ (250 mg/kg and 500 mg/kg b. w., p. o.respectively).
The paw volume was measured initially and at 0.5, 1, 3 and 5 h after carrageenan injection, using Plethysmograph, inflammation was calculated for comparison (Majumder et al.,2000; Biswas et al., 2009).

Histamine induced rat paw oedema

The rats were divided into four groups containing six rats in each group. 0.1 ml of 1.0% histamine sulphate in normal saline (0.9% w/v NaCl) was injected to the sub-plantar region of right hind paw. The EENZ was administered to the rats 1 h before histamine injection. Different groups were treated as follows:

Group I: Histamine (0.1 ml of 1.0% histamine/rat to the sub plantar region).
Group II: Histamine + Diclofenac sod. (10 mg/kg b. w., p. o.)
Group III and IV: Histamine + EENZ (250 mg/kg and 500 mg/kg b.w., p.o. respectively).

The paw volume was measured initially and at 0.5, 1, 3 and 5 h after histamine injection, using Plethysmograph, inflammation was calculated for comparison (Majumder et al.,2000; Biswas et al., 2009).

Serotonin induced rat paw oedema

The rats were divided into four groups containing six rats in each group. 0.1 ml of 1.0% serotonin in normal saline (0.9% w/v NaCl) was injected to the sub plantar region of right hind paw. The EENZ was administered to the rats 1 h before serotonin injection. Different groups were treated as follows:

Group I: Serotonin (0.1 ml of 1.0% serotonin/rat to the sub plantar region).
Group II: Serotonin + Diclofenac sod. (10 mg/kg b. w., p. o.)

Group III and IV: Srotonin + EENZ (250 mg/kg and 500 mg/kg b.w., p.o. respectively).
The paw volume was measured initially and at 0.5, 1, 3 and 5 h after serotonin injection, using Plethysmograph, inflammation was calculated for comparison (Majumder *et al.,* 2000; Biswas *et al.,* 2009).

Cotton pellet induced granuloma

This is sub-acute model for inflammatory study. This method was adopted from D'Arcy (1960), which was carried out by using sterilized cotton pellet implantation method in rats. Under light ether anaesthesia, using blunted forceps subcutaneous tunnel was made and sterilized cotton pellets (10 ± 1 mg) were implanted in the axilla and groin region of the rat. Different groups were treated as follows:

Group-I: Vehicle control received 1% Tween-20 (dose: 10 ml/kg).
Group-II: Animals treated with Diclofenac sod (dose: 10 mg/kg).

Group-III and IV: Animals treated with + EENZ (250 mg/kg and 500 mg/kg b.w., p.o. respectively). After recovering from anesthesia, animals were treated orally with vehicle control, diclofenac sod. and various doses of herbal formulation for consecutive 7 days, once per day. They were sacrificed on day 8th by cervical dislocation and the pellets were removed, freed from extraneous tissue and dried at 60^0 C for 24 hrs. The

percentage inhibition of the dry weight of the granuloma were calculated and compared (D'Arcy et al., 1960).

Statistical Analysis

All data were expressed as the mean ± SEM. The results were analyzed for statistical significance (P<0.05) by One-way (ANOVA) followed by Dunnett's test using computerized Graph Pad InStat version 3.05, Graph pad software, U.S.A.

Result

The present study evaluated the anti-inflammatory activity of ethanolic extract from *N. zeylanica* leaves in experimental rodent models. The results carrageenan induced rat paw oedema shows 56. 26% inhibition over control is more or less same with that tested medicine. The results are presented in Tables 1-4.

Table 1. Anti-inflammatory effect of EENZ on carrageenan induced rat paw oedema

Sr.no	Treatment	Dose mg/kg p.o.	0.5h	1h	3h	5h	% Inhibition
1	Control		0.21±0.020	0.41±0.018	0.43±0.012	0.47±0.015	-
2	Diclofenac sod.	10	0.17±0.012	0.19±0.014*	0.11±0.020**	0.07±0.015**	65.81
3	EENZ	250	0.19±0.017	0.31±0.009	0.27±0.014*	0.18±0.015*	37.23
4	EENZ	500	0.17±0.008	0.22±0.010*	0.19±0.012*	0.11±0.013**	56.26

Values are mean ± SEM (n = 6). *$p < 0.05$, **$p < 0.001$ when compared to control

Table 2. Anti-inflammatory effect of EENZ on Histamine induced rat paw oedema

Sr no	Treatment	Dose mg/kg p.o.	0.5h	1h	3h	5h	% inhibition
1	Control		0.35±0.020	0.31±0.014	0.27±0.02	0.33±0.020	-
2	Doclofenac sod.	10	0.10±0.015*	0.13±0.006*	0.09±0.00**	0.06±0.001**	71.31
3	EENZ	250	0.21±0.012	0.24±0.012	0.19±0.008	0.15±0.010*	39.22
4	EENZ	500	0.13±0.018*	0.18±0.014*	0.13±0.008*	0.09±0.002**	59.23

Values are mean ± SEM (n = 6). *$p < 0.05$, **$p < 0.001$ when compared to control

Table 3. Anti-inflammatory effect of EENZ on Serotonin induced rat paw oedema

Sr no	Treatment	Dose	0.5h	1h	3h	5h	%inhibition
1	Control		0.26±0.02	0.29±0.015	0.35±0.020	0.38±0.011	-
2	Diclofenac sod.	10	0.18±0.018	0.15±0.015*	0.10±0.014**	0.08±0.001**	63.42
3	EENZ	250	0.24±0.020	0.22±0.005	0.19±0.013*	0.16±0.020*	38.31
4	EENZ	500	0.20±0.016	0.17±0.014	0.13±0.012*	0.11±0.011**	54.19

Values are mean ± SEM (n = 6). *$p < 0.05$, **$p < 0.001$ when compared to control

Table4. Anti-inflammatory effect of EENZ onCotton pellet induced granuloma

Treatment	Dose mg/kg	Mean Wet Weight of Pellet (mg)	Percentage inhibition	Mean Dry Weight of Pellet (mg)	Percentage inhibition
Control		198.68 ±10.47	-	45.60 ±2.04	-
Diclofenac sod.	10	95.30 ±3.46 *	52.03	20.88 ±0.77 *	54.21
EENZ	250	157.68 ±8.18	20.64	37.40 ±1.59	17.98
EENZ	500	115.15 ±7.43 *	42.43	25.63 ±1.53 *	44.26

Values are mean ± SEM (n = 6). *$p < 0.05$ when compared to control

Discussion

In Indian system of medicine, certain herbs are claimed to provide relief of pain and inflammation. The claimed therapeutic reputation has to be verified in a scientific manner. In the present study one such drug *N. Zeylanica* was taken for the study. The leaves extracts of *N.Zeylanica*possess significant anti-inflammatory effect in the anti-inflammatory model of inflammation in rats. The carrageenan, Histamine and serotonin-induced hind paw oedema a model in rats are known to be the acute inflammatory model sensitive to cyclooxygenase (COX) inhibitors and has been used to evaluate the effect of nonsteroidal anti-inflammatory agents (NSAID), which primarily inhibit the cyclooxygenase involved in prostaglandin (PG) synthesis. In case of the time course of oedema development in these paw oedema models in rats are generally two phases are found. The first phase, which occurs between 0 to 2.5 h of injection of the phlogistic agent, has been attributed to the release of histamine or serotonin. The edema volume reaches to its maximum approximately 3 h post treatment and then begin to decline. The second phase of inflammatory reaction which is measured at 3h is caused by the release of bradykinin, protease, prostaglandin and lysosome. Therefore, it can be inferred that the inhibitory effect of the extract on the carrageenan, Histamine and Serotonin induced inflammation could be due to the inhibition of enzyme cyclooxygenase leading to inhibition of prostaglandin synthesis (Alcatraz and Jimenez,1998). Thus, the results of the present study demonstrates that the ethanolic extract obtained from leaves of *Naravelia Zeylanica* exhibited acute anti-inflammatory activity in the tested models which was found to be the most effective at higher concentrations employed. The cotton pellet granuloma method, has been widely employed to assess the transudative, exudative and proliferative components of chronic inflammation. The fluid absorbed by the pellet greatly influences the wet weight of the granuloma (Della et al., 1986). The results indicate that extract of *N. neylanica*has anti-transudative and anti-proliferative activity. However, a more extensive study is necessary to determine the exact mechanism of action of the extract and its active compound.

Conclusion

It is concluded that Ethanolic extract of leaves of *Naravelia Zeylanica*possess significant anti-inflammatory activity against all experimental models in rats. This may be due to the presence of reported active Phytoconstituents& their influence on the prostaglandins pathway. Further research, to isolate anti-inflammatory principle and exact mechanism involved, is needed.

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