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Research Article

ANTI-INFLAMMATORY ACTIVITY OF *NEURACANTHUS SPHAEROSTACHYUS DALZ.* LEAVES

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ABSTRACT

Objective: *Neuracanthus sphaerostachyus* has been traditionally used to treat skin diseases, cough, and asthma. Lack of sufficient scientific evidence indicating the utility of this plant in the treatment of inflammation prompted us to investigate the anti-inflammatory activity of the plant in different experimental screening methods.

Methods: *In vitro* and *in vivo* anti-inflammatory activity of the methanolic and aqueous extracts of *N. sphaerostachyus* (MENS and AENS) leaves at doses of 125, 250, and 500 mg/kg was evaluated with albumin denaturation and carrageenan-induced paw edema in rats and acetic acid-induced increased vascular permeability in mice.

Results: Methanolic and aqueous extract significantly inhibited protein denaturation as well as edema induced by carrageenan and vascular permeability in mice dose dependently. Aspirin (0.1 mg/ml), indomethacin (10 mg/kg), and dexamethasone (5 mg/kg) were used as a standard control.

Conclusion: It is concluded that MENS and AENS leaves exhibited significant anti-inflammatory activity.

Keywords: *Neuracanthus sphaerostachyus*, Inflammation, Paw edema, Albumin denaturation, Vascular permeability.

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INTRODUCTION

Inflammation is the response to harmful stimuli which includes irritants, various pathogens, or damaged body cells from vascular tissues and it serves as a complex biological response. Tissue exhibits a protective attempt to remove the injurious stimuli as well as to initiate the healing process. Wounds and infections due to microorganisms would never heal without inflammation, and tissue may compromise its own survival. Inflammation may progress to atherosclerosis and rheumatoid arthritis in many instances. Current treatment shows availability of a wide range of medicines such as opioids and nonsteroidal anti-inflammatory drugs with certain limitations while being used. The ancient traditional system can be opted to minimize the side effects of allopathic drugs. Herbal medicines are the backbone of about 75–80% of the world, especially in developing countries. It has better access to primary health care, due to good acceptability, better compatibility with the human body, and lesser side effects. While focusing on the biological activities of plants during the past decade, it shows the presence of plenty of compounds with anti-inflammatory potential [1,2].

Neuracanthus sphaerostachyus Dalz. is known as Pincushion plant due to its floral structure and commonly known as Putliyo (Hindi), Golgonda (Marathi), and Ganthera–Gandharo (Gujarati). It is native to Indian regions and widely distributed in the Western Ghats (Goa), Deccan, and throughout the Gujarat [3]. This plant is traditionally used in different areas of the Western Ghats. The mixture of ash of the whole plant with jaggery or honey is used for 2–3 times a day orally to cure a cough and asthma [4]. Root paste is applied to ringworm. *N. sphaerostachyus* shows the presence of vanillic acid, syringic acid, melilotic acid, and 6-OH luteolin [5].

The scientific literature survey reveals no report on the pharmacological investigation of *N. sphaerostachyus* leaves prompted us to evaluate the acute anti-inflammatory property of leaf extracts.

MATERIALS AND METHODS

Collection and authentication of plant

N. sphaerostachyus Dalz. leaves were collected from Girnar forest region of Junagadh, Gujarat. Plant material was authenticated by the National Institute of Science Communication and Information Resources (NISCAIR)-Council of Scientific and Industrial Research, New Delhi (NISCAIR/RHMD/Consult/2016/2987-14).

Extraction of plant material

Extractive values of crude drugs were used to determine the number of active constituents extracted with solvents from a given amount of medicinal plant material. The successive extraction was carried out in Soxhlet apparatus with a known quantity of powder in different organic solvents such as hexane, chloroform, methanol, and then water. After exhaustive extraction, the solvent was filtered and concentrated under reduced pressure at 50–55°C [6].

Chemicals

Carrageenan, indomethacin, and acetic acid were procured from Chemdyes Corporation, Rajkot. Dexamethasone and aspirin were obtained from Restech Pharmaceuticals, Ahmedabad.

Animals

Female Wistar rats (150–200 g) and Swiss albino mice (25–30 g) were used for the study. The animals were kept in polypropylene cages and maintained at a temperature of 26±2°C. Animals were fed with diet provided by Pranav Agro Industries Ltd., Sangli. All the animal experiments were conducted in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) (Reg. No. 1846/PO/RE/s/16/CPCSEA), a guide for the care and use of laboratory animals. The animals were acclimatized for 10 days under standard husbandry conditions as relative humidity 45–55% and 12 h light and dark cycle [7].

Acute toxicity study

Female Wistar rats of 150–200 g and Swiss albino mice of 25–30 g body weight were selected to find the acute toxicity study of methanolic and aqueous extracts of *N. sphaerostachyus* (MENS and AENS) leaves. The dose of 2000 mg/kg was selected on the basis of up and down procedure as per the Organization for Economic Co-operation and Development Guideline No. 425. All animals were observed for 24 h to detect autonomic or behavioral changes in responses to the extracts. Then, the mortality in each group was observed for 14 days [8].The MENS and AENS were found to be nontoxic at a dose of 1500 mg/kg, orally. Hence, LD cutoff value of methanolic and aqueous extract was fixed as 1500 mg/kg. Therefore, $1/10^{th}$, $1/6^{th}$, and $1/3^{rd}$ of the LD_{50} cutoff value that was approximately 150, 250, and 500 mg/kg were selected as screening dose for anti-inflammatory activity.

ASSESSMENT OF *IN VITRO* ANTI-INFLAMMATORY ACTIVITY

Albumin denaturation technique

Albumin denaturation technique was performed to evaluate the anti-inflammatory activity of crude extracts. Freshly prepared plant extracts with different concentrations were added to eight test tubes followed by 1% egg albumin (1 ml). For positive control (tube 1), 0.1 mg/ml aspirin was added. To the negative control tube (tube 2), 1 ml of ethanol was added. Test tubes 3–8 were prefilled with different concentrations of MENS and AENS. The pH of the reaction mixtures was adjusted (7.4±0.2) using a small amount of phosphate buffer. The samples were kept for incubation at 37°C for 20 min. The turbidity of samples was measured spectrophotometrically at 660 nm. The experiment was performed in triplicate [9]. The percentage inhibition of protein denaturation was calculated as follows:

$$\% \text{ Inhibition} = (\text{Abs control} - \text{Abs sample}) \times 100 \div \text{Abs control}$$

ASSESSMENT OF *IN VIVO* ANTI-INFLAMMATORY ACTIVITY

Carrageenan-induced paw edema

Female Wistar rats (150–200 g) were selected and divided into eight groups of six each. The extracts were used as a suspension in 0.5% v/v Tween 80 in normal saline (0.9%) and administered orally. Group I served as positive control and received carrageenan (0.1 ml), and Group II received indomethacin (10 mg/kg, oral) and served as standard control. Groups III, IV, and V received 150, 250, and 500 mg/kg, oral doses of MENS, respectively. Groups VI, VII, and VIII received 150, 250, and 500 mg/kg doses of AENS, respectively. After 30 min of test drug administration, 0.1% of carrageenan (0.1 ml) was administered into the sub-plantar tissue of the right hind paw. After the duration of 30 min, 1 h, 2 h, 3 h, and 4 h, the paw volume was measured by the digital plethysmograph [10,11]. Percentage inhibition was calculated using the following formula:

$$\% \text{ Inhibition} = (1 - D \div C) \times 100$$

Where, D - the percentage difference in increased paw volume after the test drugs administration and C - the percentage difference of increased volume in the positive control group.

Acetic acid-induced vascular permeability

Swiss albino mice (female) of 25–30 g were selected and divided into eight groups of six each. The extracts were used as a suspension in 0.5% v/v Tween 80 in normal saline (0.9%) and administered orally. Group I received the only saline and served as positive control. Group II received standard drug dexamethasone (0.5 mg/kg). Groups III, IV, and V received 150, 250, and 500 mg/kg, oral doses of MENS, respectively. Groups VI, VII, and VIII received 150, 250, and 500 mg/kg doses of AENS, respectively. 1 h after treatments, animal received an intravenous injection of 2% Evans blue solution (w/v) in 0.9% of saline. 10 min later, each animal received intraperitoneally 0.4 ml of 0.5% acetic acid solution [12]. After 20 min, the dye that leaked into the peritoneal cavity was collected by lavaging with 10 ml distilled water transferred to a 10 ml volumetric flask. To each flask, 0.1 ml of 0.1 N sodium hydroxide solution was added and the volume made up to the mark with distilled water followed by measurement of absorbance at 610 nm [13]. Plasma exudation level induced by acetic acid in the treated group was compared with the control group.

Statistical analysis

All values are presented as mean±SEM of six animals. Differences between means were assessed by one-way analysis of variance (ANOVA) followed by Dunnett's test. $p<0.05$ was considered to be statistically significant.

RESULTS

Albumin denaturation technique

MENS was significantly effective in inhibiting heat-induced albumin denaturation at 500 µg/ml dose with 76.29% inhibition. Standard anti-inflammatory drug aspirin showed the maximum inhibition with 88.29% and compared with positive control (Table 1).

Carrageenan-induced paw edema

The effects of extracts of MENS and AENS on paw edema induced by carrageenan are shown in Table 2. The methanolic and aqueous extract showed a maximum anti-inflammatory effect of 500 mg/kg dose (Table 2). The anti-inflammatory effect of the MENS was more potent and significant after 4 h, compared to AENS.

Acetic acid-induced vascular permeability

Results of the study revealed that MENS and dexamethasone significantly inhibited acetic acid-induced vascular permeability in mice. MENS at the doses of 125, 250, and 500 mg/kg showed 55%, 58%, and 73%, respectively, inhibits vascular permeability (Table 3). Dexamethasone (0.5 mg/kg) showed a 79% inhibitory activity when results were compared with positive control.

DISCUSSION

Proteins lose their parent structure under the influences of stress or compounds such as strong acids or bases of concentrated inorganic salt and organic solvent or due to the application of heat. Most proteins may lose their biological activity when undergoes denaturation. Most cases of inflammation involve denaturation. The ability of plant extract to inhibit the protein denaturation was studied to establish the mechanism of the anti-inflammatory activity [14].

Carrageenan-induced hind paw edema is the standard experimental model to demonstrate acute inflammation. Carrageenan-induced inflammation involves the release of the mediators such as serotonin and histamine (0–2 h), kinins (3 h), and prostaglandin (4 h). The methanolic extract and indomethacin showed significant inhibition of the edema. It was observed that MENS was capable of inhibiting edema induced by carrageenan more significantly as compared to AENS [15].

Increased vascular permeability results into the exudation of fluids like plasma protein and due to the alterations into the normal nomenclature of endothelial cells; it may lead to contract and separate endothelial

Table 1: Effect of MENS and AENS on heat-induced protein denaturation

Test tube	Treatment (µg/ml)	Absorbance	% Inhibition
I	Positive control	0.814±0.0017	-
II	Standard control	0.095±0.0031***	88.29
III	MENS 150	0.427±0.0061*	47.58
IV	MENS 250	0.347±0.0020**	57.37
V	MENS 500	0.193±0.0030***	76.29
VI	AENS 150	0.463±0.0046*	43.12
VII	AENS 250	0.392±0.0030*	51.84
VIII	AENS 500	0.243±0.0046**	70.15

Values are expressed as mean±SEM. n=3, *p<0.05, **p<0.01, ***p<0.001 versus positive control (ethanol). Data were analyzed using one-way ANOVA followed by Dunnett's test. ANOVA: Analysis of variance, MENS: Methanolic extract of *Neuracanthus sphaerostachyus*, AENS: Aqueous extract of *Neuracanthus sphaerostachyus*

Table 2: Effect of MENS and AENS on carrageenan-induced paw edema

S. No	Treatment	Dose (mg/kg)	Paw volume at different time intervals (h)				
			0.5	1	2	3	4
1	Positive control	0	0.24±0.015	0.37±0.040	0.39±0.015	0.48±0.020	0.51±0.005
2	Standard control (indomethacin)	10	0.15±0.005***	0.18±0.005***	0.18±0.01***	0.15±0.01***	0.12±0.0057***
3	MENS	150	0.20±0.015**	0.21±0.005**	0.21±0.005**	0.20±0.005**	0.20±0.005**
4	MENS	250	0.19±0.005**	0.21±0.005**	0.19±0.005**	0.18±0.005**	0.18±0.011**
5	MENS	500	0.19±0.005***	0.18±0.005***	0.18±0.005***	0.18±0.005***	0.16±0.005***
6	AENS	150	0.22±0.005*	0.22±0.005*	0.21±0.005*	0.21±0.0057*	0.21±0.011*
7	AENS	250	0.20±0.005**	0.21±0.005**	0.21±0.005**	0.20±0.005**	0.19±0.005**
8	AENS	500	0.19±0.005***	0.21±0.005***	0.21±0.005***	0.20±0.005***	0.19±0.005***

Values are expressed as mean±SEM. n=6 animals in a group; *p<0.05, **p<0.01, ***p<0.001 versus positive control. Data were analyzed using one-way ANOVA followed by Dunnett's test. ANOVA: Analysis of variance, MENS: Methanolic extract of *Neuracanthus sphaerostachyus*, AENS: Aqueous extract of *Neuracanthus sphaerostachyus*

Table 3: Effect of MENS and AENS on acetic acid-induced vascular permeability

S. No	Dose (mg/kg)	Drug	Evans blue concentration (μg/ml)	% Inhibition
I	-	Positive control	1.26±0.015	-
II	0.5	Standard control (dexamethasone)	0.258±0.007***	79
III	150	MENS	0.570±0.015*	55
IV	250	MENS	0.524±0.013*	58
V	500	MENS	0.343±0.004**	73
VI	150	AENS	0.616±0.005*	51
VII	250	AENS	0.587±0.070*	53
VII	500	AENS	0.478±0.007**	62

Values were expressed as mean±SEM. *p<0.05, **p<0.01, ***p<0.001 versus positive control. Data were analyzed using one-way ANOVA followed by Dunnett's test. ANOVA: Analysis of variance, MENS: Methanolic extract of *Neuracanthus sphaerostachyus*, AENS: Aqueous extract of *Neuracanthus sphaerostachyus*

cells altering permeability to plasma proteins and fluid. Plasma exudation being a part of increased vascular permeability plays a vital role in the progression of inflammation with release of many a kind of reactive oxygen species which may lead to diseases such as cancer, rheumatoid arthritis, and atherosclerosis [16,17]. Chemical (acetic acid)-induced vascular permeability exhibits an immediate reaction that is continued over 24 h and its inhibition suggests that the MENS and AENS extract effectively reduces the vascular permeability in dose-dependent manner with maximum inhibition at larger dose.

CONCLUSION

The present study establishes acute toxicity study, *in vitro* and *in vivo* anti-inflammatory screening for MENS and AENS. The anti-inflammatory activity of *Neuracanthus sphaerostachyus* might be because of the presence of flavonoid (6-OH Luteolin) and other various potential phytoconstituents like phenolic compounds/tannins, steroids and, triterpenoids. Methanolic extract shows significant anti-inflammatory activity at a dose of 500 mg/kg. Further investigations are required to extrapolate active component of the extract and to establish the mechanism of action.

AUTHORS' CONTRIBUTIONS

All the authors have contributed equally.

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CONFLICTS OF INTEREST

The authors declared that they have no conflicts of interest.

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