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COMPARATIVE ANALGESIC ACTIVITY OF METHANOLIC EXTRACTS OF LEAF AND STEM EXTRACT OF *VITEX NEGUNDO* LINN

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ABSTRACT

The current investigation explores the analgesic activity of *Vitex negundo (VN)* Linn (Verbenaceae), known as Nirgundi which is an important medicinal plant with variety of phytoconstituents having significant pharmacological activities. Oral administration of Nirgundi leaves and stem extracts (250 mg/kg) exhibited a significant reduction in acetic acid induced writhing response and methanol extract of stem showed better (74.6) activity as compared to leaves (62.8*) and control. In Hot-plate method, (VS) stem fraction (4.4) showed better activity as compared to leaf (4.0) (VL). Analgesic effect of stem extract (7.3) was more as compared to the leaves (5.7) in tail flick method. In Randall Sellitto test, leaf extract showed better activity (390) as compared to stem (340) and after 2 hours all the extracts showed decrease in analgesic activity. The results concluded that stem extract of Nirgudi possess therapeutic potential in analgesia as compared to the leaf extract.

Keywords- *Vitex negundo* (VN), Writhing response, Tail Flick Method, anti-analgesic activity

INTRODUCTION-

Vitex negundo Linn. belonging to family Verbenaceae is commonly known as Nirgudi and its flowered variety is known as Sinduvaara, whereas its blue flowered variety is known as Nirgundi or Sephaali. The *Vitex negundo* (VN) leaves contain iridoid glycosides such as Negundoside and flavonoids such as Vitexin, which is a flavanol glycoside besides casticin and the glycosides- a-D-glucoside of tetrahydroxy monomethoxy flavones and luteolin-7-glucoside. Two pentacyclic triterpenoids, ursolic acid and betulinic acid along with β-sitosterol, p-hydroxybenzoic acid and aliphatic alcohol, have been isolated from leaves. Stigmasterol and hentriacontane are present in dried powder of roots. The seeds contain triterpenes, p-hydroxybenzoic acid and glucose **[1-3]**. The water extract of the leaves exhibited antihistaminic, anti-inflammatory, anti-analgesic **[4-6**] and antioxidant activity **[7]**. Different extract of leaves and bark of Nirgudi exhibited significant antibacterial activity **[8]**. Negundoside isolated from leaves of VN exhibited hepatoprotective activity against carbon tetrachloride using human liver cells **[9-11]**. Literature and research report the anti-implantation activity of VN leaves **[12]**. VN also showed laxative activity **[13]** and attenuate catractogenesis and calpain activation in selenite induced cataract **[14]**. The petroleum ether extract of VN served as a potent larvicidal agent and acted as a promising repellant against various adult vector mosquitoes **[15]**. It has been also found that VN extract reduced oxidative stress **[16]**. VN leaves methanol extract showed significant anti-arthritic activity in Complete Freund's adjuvant induced paw edema in rats **[17]** and also exhibited significant anticonvulsant activity **[18]**. The VN root extract showed activity against snake venom **[19]** and also exhibited immunomodulatory potential **[20]**.

MATERIALS AND METHODS

Collection and authentication of Plant

The plant material of VN was collected from Shiroor, 60 Kms from Pune and the voucher specimen (08-123) was kept at (Agharkar Research Institute) ARI departmental herbarium. The plant material was cleaned, dried in the shade and powdered to 40 mesh and stored in an airtight container at 25ºC.

Extraction of Plant

VN leaves and stem were washed and dried at 55 ºC in an air dryer for 48 h. Dried plant material was powdered separately with a Wiley mill (model-4276 M, Thomas, Scientific, USA) to pass through a 20-mesh

sieve and stored in a sealed plastic bag. About 500 mg of dried leaf and stem powder were taken in a 5 ml volumetric flask, mixed with 5ml of methanol and vortexed for two minutes followed by sonication (32 MHz, Roop telesonic, India) at room temperature for 5 min. The process was repeated thrice for complete extraction. After sonication, individual methanolic extracts were combined and evaporated to dryness in vacuo. Dried extract was obtained as 12.2 g.

Animals

Albino rats (100-150g) of either sex maintained in standard conditions for temperature, relative humidity, light/day cycle and fed with normal feed and water ad libitum were used for evaluating analgesic activities.

Preparation of suspension of drug extract

The methanol extract of stem and leaves at a dose 100 mg suspended in 2% solution of gum Acacia in water for oral administration.

Evaluation of analgesic activity

Acetic acid induced writhing response

Animals were divided into 4 groups of six animals in each group. Vehicle, Indomethacin (10 mg / Kg) and test solution (100 mg/Kg) were administered orally 30 min before the experiment and 0.1 ml per 10 g of 0.7% acetic acid saline was then injected i.p. 10 min after the injection. The number of writhing during the following 20 min was counted **[21]**. The percent inhibition (%analgesic activity) was calculated by

% inhibition = $N-N^t/N \times 100$ where,

N= Average number of stretching of control per group

N^t = Average number of stretching of test per group

Hot plate method

The device consisted of a water bath in which a metallic cylinder was placed. Animals were divided into four groups of six animals in each group. The temperature of the cylinder was set at 55±0.5˚C **[22]**. Each rat (six per group) acted as its control before the treatment; the reaction time of each rat (licking of the forepaw or jumping response) was don at 0- and 10-min interval. The average of the two readings was obtained as the initial reaction time. The reaction time following the administration of the extract (100 mg/kg p.o.), indomethacin (10 mg/kg) and distill water (10 ml/kg) was measured at 0.5-6 hr after a latency period of 30 min.

Tail flick method

Animals are divided into four groups of six animals in each group. This involved immersing extreme 3 cm of rat's tail in water bath containing water at a temperature of 55 ± 0.5˚C. Within a few minutes, the rats reacted by withdrawing the tail. The reaction

time was recorded with a stop watch. Each animal served as its control at 0- and 10-min interval. The average of the two values was the initial reaction time. The test groups were given the extract **(100 mg/kg),** ibuprofen **(400 mg/kg)** and distilled water **(100 ml/kg).** The reaction time for the test group was taken at interval 6 hrs after a latency period of 30 min followed by the administration of the extract and drugs **[23]**.

Randall Selitto method

Assessment of pain consisted of measurement of the threshold stimulus for reaction (escape or paw withdrawal) using a weight (maximum limit of 500 gm) applied to the pads of hind paws. Before the weight was applied on the paws, inflammation was induced with carrageenan. The threshold of pain sensation was measured after drug administration at 0, 1, 2, 3, 4, 5 and 6 hrs. **[24]**.

RESULTS AND DISCUSSIONS

The extracts (250 mg/kg) administered orally, significantly inhibited acetic acid induced writhing in rats. Their writhing response are related to increase in peritoneal level of prostaglandins and leukotrienes. The result strongly suggests that the mechanism of action of extracts may be linked to lipoxygenase and /or cyclooxygenase. Tail immersion model of analgesic assessment is best reserved for evaluating compounds having centrally acting analgesic activity.

Acetic acid induced writhing response

The VN extract significantly reduced acetic acid induced writhing response. Among them, methanol extract of stem showed better (74.6) as compared to leaves (62.8*) and control. Both extracts significantly (P< 0.05) reduced writhing response **(Table 1)**.

Hot plate method

The both extracts at a dose 250 mg/Kg showed significant (P<0.05) peripheral analgesic activity as compared to control (1.5). Among all samples, stem fraction (4.4) showed better activity as compared to leaf (4.0). Results were shown in **(Table 2)**.

Tail flick method

A significant reduction of painful sensation due to tail immersion in warm water was observed following oral administration of the extracts at a dose 250 mg/kg. The effect was noticed after a latency period of 1 hr. The analgesic effect of stem extract (7.3) was more as compared to the leaves (5.7). The both extracts significantly (P< 0.05) reduced pain as compared to control (1.5). The effect was most pronounced after 1 hr of drug treatment **(Table 3)**.

Randall selitto method

The both extracts significantly (p< 0.05) reduced pain as compared to control (**147.7**)

at 2 hr of drug treatment. Leaf extract showed better activity (**390**) as compared to stem (**340**) and after 2 hrs all the extracts showed decrease in analgesic activity **(Table 4)**.

Table 1: Effects of *Vitex negundo* and its fractions on writhing response

Drug	Response	% Inhibition
Control	51.2	0
VL	19	62.8
VS	13	74.6
Indomethacin	28.7 ± 1.96**	43.94

All values are expressed as mean ± S.E.M. n=6

Table 2: Effects of *Vitex negundo* on Hot Plate response

Drug	Response		
	0 hr	1 hr	2 hr
Control	1.4	1.9	1.7
VL	4.9	4.7	4.0
VS	3.4	5.1	4.4
Indomethacin	27.1	27.3	26.1

Table 3: Effects of *Vitex negundo* on Tail Flick response

Drug	Response		
	30 min	1 hr	2 hr
Control	1.4	1.6	1.5
VL	5.3	5.7	5.3
VS	4.7	7.3	6.3
Indomethacin	24.5	24.1	24.9

Table 4: Randell Selitto readings of *Vitex negundo*

Group	Force (Newton)					
	0	1	2	3	4	6
Control	146.1	148.0	147.7	151.1	142.3	152.2
VL	266.6	376.6	390.0	213.3	210.6	190.3
VS	260.0	316.6	340.0	320.0	310.0	290.0
Indomethacin	280	395	410	380	350	330

All values are expressed as mean ± SEM. The data obtained were analyzed statistically using Instat 2 (graph pad software). Statistical significance was calculated using one - way ANOVA followed by Dunnet comparison test. P values 0.05 were considered significant

CONCLUSION

In the present study a comparative Analgesic evaluation of leaf and stem extract of *Vitex negundo* was done and results showed that stem extracts showed better Analgesic activity than leaf extracts of *Vitex negundo*.

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