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Original Research Article

Peripheral and central analgesic activity evaluation of ethanolic extract of *Vitex Negundo* flowers in experimental animals

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ABSTRACT

Background: *Vitex negundo* Linn (Family: Verbenaceae), locally known as 'Nirgundi' an important medicinal plant is a woody, aromatic shrub growing to a small tree. It commonly bears tri- or penta-foliate leaves on quadrangular branches, which give rise to bluish-purple coloured flowers in branched tomentose cymes. It has been claimed to possess analgesic activity apart from many medicinal properties. The aim of the present study was to evaluate both the peripheral and central analgesic activity of ethanolic extract of *Vitex negundo* flowers (EEVNF) in experimental animals.
Methods: Acute toxicity test was done following the Organization of Economic Cooperation and Development guidelines. EEVNF (100mg/kg, 200mg/kg, and 400 mg/kg body weight [b.w.] p.o) was evaluated for peripheral analgesic activity by the acetic acid (0.7%) induced writhing test and central analgesic activity by the tail flick method respectively using aspirin (100mg/kg b.w. and 300mg/kg b.w.) as the standard drug.
Results: EEVNF significantly decreased the number of writhing in writhing test at all the doses ($p<0.001$) and increased the reaction time in tail-flick method ($p<0.001$) at all the doses when compared to control. EEVNF in the dosage of 400mg/kg b.w. produced analgesic effects which was comparable with that of the standard drug aspirin at dose 100mg/kg b.w in writhing test and produced greater analgesic activity than that of standard drug aspirin at dose 300mg/kg b.w in tail flick method.
Conclusions: EEVNF has significant peripheral and central analgesic activity.

Keywords: Analgesic, Pain, Tail flick, *Vitex negundo*, Writhing

INTRODUCTION

Pain has been officially defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage. It is always a warning signal and primarily protective in nature but often causes a lot of discomfort and lead to many adverse effects.[1] Pain perception involves two components, the nociceptive component and affective component. Based on a clinical diagnosis of pain it is classified as somatic pain, visceral pain, referred pain, psychogenic/functional pain and neuropathic pain. Pain is a disabling accompaniment of many medical conditions and pain control is one of the most important therapeutic priorities.[2] Common drugs for pain relief such as aspirin and morphine have been widely used in recent decades. In most instances, these analgesic drugs, particularly opioids and nonsteroidal anti-inflammatory drugs (NSAIDs), can only relieve 50% of the pain in about 30% of patients. In addition, many of these drugs cause serious side effects. Studies have shown that opiates cause physical dependency, tolerance, and addiction while NSAIDs usually cause gastrointestinal disorders.[3] On the contrary many medicines of plant origin have been used since ages without any side effects. It is therefore essential that efforts should be made to introduce new medicinal plants to develop more effective analgesics

without any adverse effects. One among them is *Vitex negundo* Linn belongs to the family Verbenaceae.

Vitex negundo Linn (Family: Verbenaceae) is a woody, aromatic shrub growing to a small tree. Some common names are in Hindi nirgundi and in Sanskrit as sindhuvara. It commonly bears tri- or pentafoliate leaves on quadrangular branches, which give rise to bluish-purple colored flowers in branched tomentose cymes. It thrives in humid places or along water courses in wastelands and mixed open forests. It is found throughout the greater part of India at warmer zones and ascending to an altitude of 15,00m in outer Western Himalayas.[4]

All parts of the plant from root to fruit possess a multitude of phytochemical secondary metabolites which impart an unprecedented variety of medicinal uses to the plant.[5,6] *Vitex negundo* has been investigated for antipyretic, analgesic, anti-inflammatory, anticonvulsant, hepatoprotective and bronchial relaxant.[7-13] Very few studies have been done to evaluate its analgesic activity and no study was done on analgesic activity of flowers of *Vitex negundo*. Therefore, the present study was undertaken to investigate analgesic activity of ethanolic extract of flowers of *Vitex negundo*.

METHODS

Plant material

Fresh flower powder of *Vitex negundo* Linn was brought from AGHP Enterprises, Chennai, India in the month of June 2016 which was authenticated by Dr. D. Aravind, Assistant Professor, Botany, National Institute of Siddha, Chennai. The specimen (Voucher number: SNMC/Pharma 009), is preserved for reference in the herbarium of department of Pharmacology, S. Nijalingappa Medical College, Bagalkot, Karnataka, India.

Drugs and chemicals

Aspirin, 0.7% acetic acid and normal saline were used in this study.

Instruments

Analgesiometer, Soxhlet apparatus, digital weighing balance, stopwatch, feeding tube, insulin syringe, mouth gags, tuberculin syringe, Ryle's tube, beaker, glass jar, glass rod.

Preparation of plant extract

The material was extracted with 80% ethanol using soxhlet extraction apparatus and it was evaporated to dry at 60°C. Flower powder (20g) of *Vitex negundo* yeilded 4g of crude extract. The solid residues were stored in airtight container and preserved in the refrigerator at -20°C.[14] From this stock, fresh preparations were obtained whenever required.

Experimental animals

All the animals were procured from the Central Animal house, S. Nijalingappa Medical College, Bagalkot, India. Swiss albino mice of either gender weighing 25-30g and Albino Wistar rats of either gender weighing 150-250g were selected for the experiment. Pregnant animals, animals with an infection, animals with injuries, deformities were excluded from the study.

Prior to and during study, all the animals were maintained under standard animal house conditions at 12:12 hours dark: light cycle, at temp 25±2°C, humidity 35-60% and other micro and macro environment conditions as suggested by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

All animals were housed in a polypropylene cage covered with a stainless-steel wire mesh and a paddy husk bed, with adequate provision for feed and water. All the animals were maintained on standard laboratory diet (VRK Nutritionals, Pune) and water was provided ad libitum. The study was started after getting the Institutional Animal Ethics Committee approval (IAEC/ S. Nijalingappa Medical College, Bagalkot, India, Reg No.829/AC/04/CPCSEA).

Phytochemical screening

The freshly prepared extract of the flowers of *Vitex negundo* was subjected to phytochemical screening tests for the detection of various constituents.[15]

Acute toxicity study

For acute toxicity study 15 healthy swiss albino mice of either gender weighing 25-30g were selected. The animals were treated with increasing doses of EEVNF. The toxicity studies were conducted according to the Organization for Economic co-operation and development (OECD) 423 guidelines.[16] All the treated animals were observed for any abnormal or toxic manifestations and mortality.

Evaluation of analgesic activity

Peripheral analgesic activity

Acetic acid induced writhing test: Following 12 hours fasting 30 healthy Swiss albino mice of 25-30 g were randomly divided into five groups of six animals each. Group I received 0.5ml of normal saline (control group), Group II received 100mg/kg of aspirin (standard group) Group III, IV, V received EEVNF in doses of 100mg/kg, 200mg/kg and 400mg/kg b.w (test groups).[17] All the drugs were given orally. After 1 hr all the animals were given 10 ml/kg of 0.7% v/v acetic acid injection intraperitoneally (i.p.). Number of writhings were counted between 5 and 20 mins after acetic acid injection.[18]

Percentage inhibition was calculated using the following formula

% inhibition=Wc−Wt/Wc*100 Where, Wc=Number of writhings in the control group, Wt=Number of writhings in test group.

Central analgesic activity

Tail flick method

The test was carried out in healthy Albino Wistar rats. 30 animals weighing 150-250g were randomly divided into five groups of six animals each after 12 hrs fasting. Group I received 0.5 ml of normal saline (control group), Group II received 300 mg/kg of aspirin (standard group), and Groups III, IV, V received EEVNF in doses of 100mg/kg, 200mg/kg and 400mg/kg b.w (test groups).[19] All the drugs were given orally. After ½ hour, 1 hour, 2 hours, 3 hours the tail flick response was carried out and the reaction time was measured by placing the distal 1/3rd of the tail about 1 cm from the radiant heat source. The time taken by the animal to withdraw the tail was taken as the reaction time. Cut off time was kept as 20-30 sec. The animals showing reaction time of >20-30 were excluded from the study.[20]

Statistical analysis

The stastical data were presented as Mean±SEM and results were analysed using One-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests. For all the tests 'p' value of 0.05 or less was considered as statistical significance.

RESULTS

Phytochemical screening

Phytochemical screening of EEVNF showed that the crude extract contained tannins, alkaloids, terpenoids, flavonoids, sterols, phenolic compounds and proteins.

Acute oral toxicity study

No adverse effect or mortality was detected in Swiss albino mice at 2g/kg of EEVNF. All the animals were alive, healthy and active during the observational period of 14 days. So, the LD 50 was considered as >2000mg/kg.

Acetic acid induced writhing test for evaluating peripheral analgesic activity

Table 1 shows the results of the peripheral analgesic activity of EEVNF by acetic acid induced writhing test in albino mice. In the present study the test drug at all the doses of (100mg/kg, 200mg/kg, 400mg/kg b.w) of EEVNF and standard drug showed significant reduction in the number of writhings [31.67±1.14, 23.00±0.57,14.33±0.88 and 12.33±0.49 ($p<0.001$)] when compared to control (40.00±0.57). The test drug at the dose of, 100mg/kg, 200mg/kg and 400mg/kg produced 20.82%, 42.50% and 64.17% inhibition of writhings compared to control group. The standard drug aspirin showed 69.17% inhibition of writhings. The peripheral analgesic activity of test drug at 400mg/kg is comparable to that of the standard drug of aspirin at dose 100mg/kg b.w.

Table 1: Number of writhings and percentage inhibition of acetic acid induced writhing test.

Groups	M±SEM	Percentage of Inhibition (%)
Group I (Control)	40.00±0.57	-
Group II (Aspirin 300 mg/kg)	12.33±0.49***	69.17
Group III (EEVNF 100 mg/kg)	31.67±1.14***	20.82
Group IV (EEVNF 200 mg/kg)	23.00±0.57***	42.50
Group V (EEVNF 400 mg/kg)	14.33±0.88***	64.17
When compared with control; *p<0.05, **p<0.01, ***p<0.001. All the values are expressed as mean±Standard error of mean (n=06).		

Table 2: Mean reaction time (seconds) in tail-flick method.

Groups	0-hour M±SEM	½ hour M±SEM	1-hour M±SEM	2-hour M±SEM	3-hour M±SEM
Group I control	11.43±0.20	11.30±0.19	11.26±0.24	11.26±0.17	11.21±0.27
Group II standard aspirin (300 mg/kg)	10.66±0.51	11.46±0.42	13.84±0.59**	16.06±0.53***	18.92±0.48***
Group III EEVNF (100mg/kg)	11.50±0.49	12.58±0.60	13.06±0.49*	14.37±0.35***	14.74±0.64***
Group IV EEVNF (200mg/kg)	11.87±0.43	13.57±0.59**	15.63±0.51***	17.44±0.25***	17.72±0.31***
Group V EEVNF (400mg/kg)	11.76±0.53	13.97±0.43**	16.62±0.38***	18.51±0.25***	19.62±0.19***
When compared with control; *p<0.05, **p<0.01, ***p<0.001. All the values are expressed as, Mean±SEM (n=06), SEM=Standard error of mean.					

Tail flick test for evaluating central analgesic activity

Table 2 shows the central analgesic activity of the EEVNF by Tail-flick method in Albino Wistar rats. In the present study there was no significant difference between mean reaction time of different groups at 0 hour. Test groups showed increase in the mean reaction time significantly in doses of 200mg/kg, 400 mg/kg from 1hour, and in dose of 100mg/kg from 2nd hour onward.

The control group showed the mean reaction time of 11.21±0.27 sec at 3rd hour. Standard drug aspirin at the dose of 300mg/kg b.w body weight showed the mean reaction time of 18.92±0.48 sec at 3rd hour and test drug in the dose of 400 mg/kg showed the mean reaction time of 19.62±0.19 sec at 3rd hour. Test drug at dose of 400mg/kg b.w has greater analgesic activity when compared to that of the standard drug aspirin at dose of 300mg/kg b.w.

DISCUSSION

Vitex negundo L. (Verbenaceae) is a hardy plant, flourishing mainly in the Indian subcontinent. All parts of the plant, from root to fruit, possess a multitude of phytochemical secondary metabolites which impart an unprecedented variety of medicinal uses to the plant. The present study was undertaken to investigate peripheral and central analgesic activity of ethanolic extract of flowers of *Vitex negundo*. Previous studies have shown significant analgesic activity of EEVN leaves.[21]

This study is the first report regarding analgesic activity of EEVN flowers. Anti-nociceptive models like acetic acid induced writhing test and tail flick method were used to evaluate analgesic activity of EEVNF.

Peripheral analgesic activity of EEVNF was evaluated by using writhing test in mice according to the method of Koster et al.[22,23]Acetic acid-induced writhing reflex is a model of visceral pain which is highly useful for screening analgesic drugs and several chemicals such as phenyl quinone and acetic acid could induce writhing reflex in laboratory animals. Intraperitoneal injection of acetic acid produces pain through activation of chemosensitive nociceptors[20] or irritation of the visceral surface, which lead to the liberation of histamine, bradykinin, prostaglandins and serotonin.[24]

Also, it has been noted that the level of analgesia in acetic acid-induced models is indicated by the percent reduction in the number of abdominal constrictions. Intraperitoneal injection of 0.7% glacial acetic acid produced abdominal writhing in this experiment. The extracts derived from flowers of *Vitex negundo* Linn. exhibited significant analgesic activity in swiss albino mice by inhibiting acetic acid induced writhing. Therefore, EEVNF might be inhibiting synthesis or release of these endogenous substances.

Even though writhing test is very sensitive, it may give false positive results, so tail flick method was conducted to confirm and study the analgesic property in EEVNF.

Central analgesic activity was evaluated by using the tail flick method which is considered to be a spinal reflex induced by heat according to Schumacher et al, and Wolff et al, but could also involve higher neural structures (central analgesic activity).[22,25,26] Pain is centrally modulated via a number of complex processes including opiate, dopaminergic descending noradrenergic and serotonergic systems.[27]

The significant increase in pain threshold produced by EEVNF at 100mg/kg, 200mg/kg, 400mg/kg b.w and aspirin 300mg/kg b.w in radiant heat tail flick model may be via central mechanisms involving these receptor systems or via peripheral mechanisms involved in the inhibition of prostaglandins, leukotrienes and other endogenous substances that are key mediators in pain.

The results of the present study have shown that EEVNF produced significant analgesic activity against chemical and thermal models of nociception in mice and rats. Phytochemical analysis of EEVNF showed presence of tannins, alkaloids, terpenoids, flavonoids, sterols, phenolic compounds and proteins.

It is suggested that some flavonoids block both cyclooxygenase and lipoxygenase pathway of the arachidonate cascade at high concentration, while at low concentration only lipoxygenase pathway is blocked.[28] Flavonoids produces analgesic action by opioid like action.[29]Furthermore, there are few reports on the role of tannins in analgesic activity.[30] Previous studies suggested that alkaloids also involve in analgesic action through non-narcotic action.[31,32] In the present study flavonoids, tannins and alkaloids might be attributed to the peripheral and central analgesic activities.

CONCLUSION

In conclusion ethanolic extract of *Vitex negundo* flowers possess both peripheral and central analgesic activity. Further pharmacological analysis of the extract is needed to isolate and characterize the active ingredient responsible for its analgesic effect. The plant can be recommended for the further studies to isolate the active ingredients.

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