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# ANALGESIC ACTIVITY OF METHANOLIC BARK EXTRACT OF *VITEX NEGUNDO Linn*

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**ABSTRACT**

The present study was undertaken to assess the analgesic effect of methanolic extract of *Vitex negundo* bark in albino rats. The analgesic action in acute pain models was studied by tail flick method and hot plate method. The methanolic extract of *Vitex negundo* bark was screened for phytochemical analysis and it's revealed the presence of all components. The adult Sprague- Dawley rats were divided into four groups of six each and maintained under ideal laboratory conditions. Group I was taken as control and group II treated with the standard drug diclofenac sodium (9mg/kg), the methanolic extract of *Vitex negundo* bark 200mg/kg and 300mg/kg were fed to group III, IV. It is observed that the both *Vitex negundo* bark shows considerable analgesic effect in acute pain models which is less than the effect of Diclofenac group. The higher dose groups of *Vitex negundo* bark extract (300mg/kg) was revealed more activity than their corresponding lower dose.

**Keywords:** Vavili, Nirgundi, viridiflora

## INTRODUCTION

Plant *Vitex negundo* Linn. (Family: Verbenaceae) is a large aromatic shrub commonly known as '*Sindhuvara'*; *Vavili*; in Telugu ("Nirgundi" in Hindi). It is an erect 2-5m in height, found throughout the tropical, semi-tropical and warm temperature regions of India. It is also abundant along the bank of rivers in moist places and near the deciduous forests and the rural areas of the country. It is widely planted as a hedge plant along the road, between the fields and usually not browsed by cattle. All the parts of the plant are used as medicine but the leaves and roots are more important and sold as drugs. Its leaves are reported to possess medicinal [1], anti inflammatory [2-5], pesticidal [6] and antibacterial properties. Previous studies on the steam barks of *Vitex negundo* have resulted in the isolation of many terpenes, sterols, phenolic compounds, flavanoids, alkaloids, organic acids, glucosides, and anthocyanines. Most of them had reported to have antifeedant, antibacterial activities, antiarthritic activity and anti-inflammatory activity[10].The roots are used in rheumatism, dyspepsia, dysentery, piles and considered as tonic, febrifuge, expectorant, antihelmintic, antiulcer[9] and diuretic. The flowers are astringent and are employed in fever, diarrhoea and liver complaints. The dried fruits are vermifuge and the bark is used in toothache. Thus it was thought worthwhile to carryout evaluation of analgesic activity of its bark extract.

## MATERIAL AND METHODS

**Plant Material*:*** The fresh bark of *Vitex negundo* Linn.(Family: Verbenaceae) was collected from the Village-kothapalli, Mandal: Peddapalli, a local area of the Distt: Karimnagar, state: Andhrapradesh, India in the month of January 2010. The plant was identified& authenticated by Head, Dep't. Of Botany and a specimen was kept for record at Osmania University of Hyderabad, Andhrapradesh. India. The

bark of the plant was separated from adulterants, shade dried and powered coarsely.

**Extraction:** About 400 gm of air dried coarse powdered bark was soaked with petroleum ether for 2 days. At the end of second day the powder was taken out and it was dried. After drying it was packed in 1000ml soxhlet apparatus and extracted by using methanol as solvent, till colour disappeared .The temperature was maintained at 55-65$^0$C.After that extract was concentrated by distillation and solvent was recovered. The final solution was evaporated to dryness. The colour, consistency and yield (16.75% w/v) of methanolic extract were noted.

**Animals:** Albino rats of either sex (weight 150- 200 g), procured from National Institute of Nutrition, Hyderabad, India were used for the present study. The study protocol was approved by the institutional ethical committee. Animals were housed in polypropylene cages (4 per cage) with dust free rice husk as a bedding material under laboratory conditions (under standard conditions in an animal house approved by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA)) with control environment of temperature 28±2$^0$C, humidity (60%±10%) and 12 light/dark cycle. They were fed *ad libitum* with rodents chow and given free access to drinking water. Before subjecting them to experimentation, the animals were given a week time to get acclimatized with laboratory conditions. The experimental protocol was approved by Institutional Animal Ethical Committee.

**Acute toxicity studies:** The procedure was followed by using OECD guide lines 423(Acute toxic class method) twelve animals (albino rats-150-200grs) were selected for studies. The acute toxic class method is a step wise procedure with three animals of single sex per step. Depending on the mortality and or moribund states of the animals, on average 2-4steps may be necessary to allow judgment on the acute toxicity of the test animals while allowing for acceptable data based scientific conclusion. The method uses defined doses (5, 50,300,2000mg/kg body weight) and the results allow a substance to be ranked and classified according to the globally harmonized system (GHS) for the classification of chemical which cause acute toxicity. Most of the crude extracts possess LD50 value more than 2000mg/kg of the body weight of animal used.

**Analgesic activity:** Analgesic activity of *Vitex negundo* bark methanolic extract was studied by eddy's hot plate and Radiant heat tail-flick method. The animals were divided into four groups of 6 animals each. And they were fasted overnight during the experiment free access to water.

Group I (control) received 5 ml/kg of water ( p.o.)
Group II served as standard and were injected Diclofenac sodium (9 mg/kg) intraperitonially.
Group III received *Vitex negundo* bark methanolic extract (200 mg/kg, p.o.)
Group IV received *Vitex negundo* bark methanolic extract (300 mg/kg, p.o.)

**Eddy's hot plate method:** Analgesic activity was performed by using Eddy's hot plate (Inco. India) maintained at a temperature of 55±1$^o$C. The basal reaction time of all animals towards thermal heat was recorded. The animals which showed paw licking or jump response within 6-8secT were selected for the study. The animals were individually placed on the hot plate maintained at 55°C, one hour after their respective treatments. The response time was noted as the time at which animals reacted to the pain stimulus either by paw licking or jump response, whichever appeared first. The cut off time for the reaction was 15 seconds [7].

**Evaluation:** The mean reaction time for each treated group was determined and compared with that obtained for each group before treatment. Percentage increase in reaction time (I %), was derived, using the formula I% = {(It – Io)/Io} × 100, Where It = reaction time at time, t, and Io = reaction time at time zero (0 h). The animals were subjected to the same test procedure at +30, +60, +90, and +120min after the administration of test/standard/control drug.

**Radiant heat tail-flick method:** The central analgesic activity was determined by radiant heat tail-flick model in rats[8]. The analgesic activity of the plant extract was studied by measuring drug induced changes in the sensitivity of the prescreened rats (the intensity of the light beam has been experimentally defined such that naive animals will withdraw their tails within 2 to 4 s) to heat stress applied to their tails by using analgesiometer.

Tail-flick latency was assessed by the analgesiometer. All drugs were given orally to the respective group rats as a suspension in gum acacia. The strength of the current passing through the naked nichrome wire was kept constant at 5 ampere. The distance between heat source and the tail was 1.5 cm and the application site of the heat on the tail was maintained within 2 cm, measured from the root of the tail. Cut-off reaction time was +10 s to avoid any tissue injury during the process.

**Evaluation:** The time taken by rats to withdraw (flick) the tail was taken as the reaction time. The animals were subjected to the same test procedure at +30, +60, +90, and +120min after the administration of test/standard/ control drug.

**Analysis of data:** All the values ware statistically analyzed by one-way analysis of variance (ANOVA) followed by Tukey- Kramer multiple comparison test. Comparison between control and drug treated groups were considered to be significant. All values are expressed as mean ± SEM.

## RESULTS

**Acute toxicity test:** The acute oral toxicity of the methanolic extract of *Vitex negundo* was carried out as per OECD423 guide lines (acute toxic class method).the acute toxicity studies revealed that LD50>2000mg/kg for the extracts. Hence the biological dose was fixed at200, 300mg/kg body weight for the extract.

**Analgesic activity in rats:**

***Hot plate method*:** In the hot-plate method, both the extract and Diclofenac sodium caused significant increase (P<0.001) in the reaction time. The increase in latency period at different time points significantly differed (P<0.01) compared to baseline values within the same drug treated groups. The percentage increase in the reaction time was dose-dependent and differed significantly among the groups of rats (P<0.001) receiving different dose levels of the extract and Diclofenac sodium. (Table: I).

The percentage increase in the reaction time caused by the extract and Diclofenac sodium was detectable and peaked, at +1 h and +30 min respectively but thereafter declined relatively at +2h after the administration of the extract or Diclofenac sodium At +2h after administration, Diclofenac sodium (P<0.001) than the extract.

At all the specified time intervals, the percentage increase in reaction time differed significantly (P<0.001) between the extract and Diclofenac sodium e, being greater for Diclofenac sodium. At the peak of activity, 200 mg/kg and 300 mg/kg extract caused 52.04% and 56.56% increase in the reaction time respectively whilst Diclofenac sodium gave 82.16% increment. Time to peak activity was different for the extract (+1 h) and Diclofenac sodium (+30 min).

**Tail flick method:** In the tail flick method, the increase in latency period at different time points significantly differed (P<0.01) compared to baseline values within the same drug treated groups. The extract and Diclofenac sodium caused significant increase (P<0.01) in the percentage reaction time whilst the control and lower dose of extract (200 mg/kg) caused no change. The percentage increase in reaction time was dose dependent. At all the specified time intervals, the percentage of tail flick elongation time differed significantly (P<0.001) between the extract and Diclofenac sodium at both the doses of plant extract, being greater for Diclofenac sodium. At the peak of activity, 200mg/kg and 300mg/kg extract showed 43.66% (P<0.001) and 46.76% (P<0.001) percentages of tail flick elongation time respectively, whilst Diclofenac sodium gave 80.08% (P< 0.001) elongation of tail flicking time **(**Table: II). Time to reach peak activity was same (+30 min) for the extract and Diclofenac sodium.

## DISCUSSION

The present study was undertaken to determine the analgesic activity of the methanolic extract from the bark of *Vitex negundo L.* The pharmacological and acute toxicity studies of methanolic extracts were performed as follows.OECD-423guidelines (acute toxic class method).No mortality or acute toxicity was observed (3days) up to 2000mg/kg body weight. The phyto constituents like flavonoids, tannins, alkaloids have been reported in several analgesic literatures as possible to produce analgesic effect. In the present study, the Diclofenac sodium (Group-II) and methanolic extract of *Vitex negundo* bark treated groups (Groups III, IV) showed a significant analgesic effect compared to that of control group (Group I), but the activity shown by the methanolic extract of *Vitex negundo* bark were less than to that of diclofenac treated group (Table: I& II). The methanolic extract of *Vitex negundo* bark at both dose levels exerts a similar reaction time suggesting an increase in the dose from200mg/kg to 300mg/kg body weight will have significant influence in the analgesic activity. A significant increase in the reaction time for acute pain models (tail flick and hot plate method) indicated the analgesic effect by methanolic extract of *Vitex negundo* bark and also elucidates the involvement of central mechanism in analgesic action. The flavonoids were reported to have analgesic activity by reduced availability of prostaglandins. Hence, the presence of flavonoids in the methanolic extract of *Vitex negundo* bark may also contribute for the analgesic activity.

## CONCLUSION

From the results of the present study it can be inferred that methanolic extract of *Vitex negundo*

bark is an effective analgesic agents. While comparing the *Vitex negundo* bark extract (200mg/kg), the 300 mg/kg body weight revealed higher effect.

**Table: I Analgesic effect methanolic bark extract of *Vitex negundo* by hot plate method**

| Group | Drug Treatment | Reaction Time (Sec) *(mean ± SEM)* Basal Reaction Time (Sec) | 30min | 60 min | 90min | 120 min |
|---|---|---|---|---|---|---|
| I | 5ml/kg of water | 4.56±0.03 | 5.183±0.03* (33.03) | 5.42±0.031* (18.84) | 6.067±0.012* (13.66) | 6.23±0.014* (36.60) |
| II | Diclofenac sodium (9mg/kg I.P.) | 4.334±0.030 | 7.890±0.05*a (82.16) | 7.25±0.04*a (67.40) | 7.02±0.015*a (61.80) | 6.81±0.011*a (57.20) |
| III | *Vitex negundo* (200 mg/kg, p.o.) | 5.052±0.016 | 5.360±0.01* (12.14) | 5.60±0.014* (17.16) | 6.083±0.008* (27.27) | 6.313±0.009* (32.08) |
| IV | *Vitex negundo* (300 mg/kg, p.o.) | 4.917±0.024 | 7.29±0.04*ab (44.28) | 7.68±0.033*ab (52.04) | 6.96±0.017*a (37.78) | 7.04±0.01*ab (39.43) |

*P<0.01 vs. Baseline value of the respective drug group, aP<0.001 vs Control, bP<0.001 vs Diclofenac sodium, (n=6/group), One-way ANOVA; SEM = Standard error of mean.

**Table: II Analgesic effect of methanolic bark extract of *Vitex negundo* by Tail Flick method**

| Group | Drug Treatment | Reaction Time (Sec) *(mean ± SEM)* Basal Reaction Time (Sec) | 30min | 60min | 90min | 120min |
|---|---|---|---|---|---|---|
| I | 5ml/kg of water | 4.25±0.015 | 4.37±0.015 (2.95) | 4.61±0.021* (8.39) | 4.79±0.006* (12.78) | 4.65±0.033* (9.49) |
| II | Diclofenac sodium (9mg/kg I.P.) | 4.39+0.006 | 7.02±0.012ab (46.76) | 6.66±0.020*ab (39.23 | 5.85±0.01* ab (22.43) | 5.55±0.013*ab (16.09) |
| III | *Vitex negundo* (200 mg/kg, p.o.) | 4.81±0.008 | 6.91±0.016*ab (43.66) | 6.34±0.015* ab (31.86) | 5.89±0.033*ab (22.36) | 5.46±0.007*ab (13.43) |
| IV | *Vitex negundo* (300 mg/kg, p.o.) | 4.78±0.006 | 4.44±0.020 (1.10) | 4.71±0.048* (7.20) | 4.86±0.009* (10.77) | 4.72±0.009* (7.58) |

*P<0.01 vs Baseline value of the respective drug group, aP<0.001 vs Control, bP<0.001 vs Diclofenac sodium, (n=6/ group), One-way ANOVA; SEM = Standard error of mean.

**REFERENCE**


1. Tandon VR. Medicinal uses and biological activities of Vitex negundo. Nat Prod Rad. 2005; 4: 162-5.
2. Telang RS, Chatterjee S, Varshneya C. Studies on analgesic and anti-inflammatory activities of Vitex negundo linn. Indian J Pharmacol 1999; 31: 363-6.
3. Jana U, Chattopadhyay RN, Shaw BP. Preliminary studies on antiinflammatory activity of Zingcher officinal Rosc, Vitex negundo Linn and Tinospora Cordifolia (wild) Miers in albino rats. Indian J Pharmacol. 1999; 31: 232-3.
4. Dharmasiri MG, Jayakody JR, Galhena G, Liyanage SS, Ratnasooriya WD. Anti-inflammatory and analgesic activities of mature fresh leaves of Vitex negundo. J.Ethnopharmacol. 2003; 87: 199-206.
5. N.Y Chowdhury, W Islam, M Khalequzzaman insecticidal activities of stem bark extracts from vitex negundo against tribolium castaneum (herbst). J. bio-sci. 2009; 17: 63-70.
6. S. K. Dutta, S. K. Panda. H. N. Thatoi. Antibacterial activity and phytochemical screening of leaf and bark extracts of Vitex negundo. Journal of Medicinal Plants Research. 2009; 3(4): 294-300.
7. Eddy NB, Leimbach DJ. Synthetic analgesics: II Dithienyl butenyl and Dithienyl butylamines (Retracted by Turner RA. Screening methods in Pharmacology I, 1st ed. New York. London Academic Press 1965; pp. 105-109.
8. D'Amour FE, Smith DL. A method for determining loss of pain sensation. J Pharmacol Exp Ther. 1941; 72: 74–79.
9. Zaware BB, Nirmal SA, Baheti DG, Patil AN, Mandal SC. Potential of Vitex negundo roots in the treatment of ulcerative colitis in mice. Pharm Biol. 2011; 18(8):874-8.
10. M.G. Dharmasiri, J.R.A.C. Jayakody, G. Galhena, S.S.P. Liyanage, W.D. Ratnasooriya Anti-inflammatory and analgesic activities of mature fresh leaves of Vitexnegundo. Journal of Ethnopharmacology.2003; 87:199–206.