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Study of Multifaceted Effect of Vitexnegundo Leaves Aqueous Extract and Decoction for Pain Relief in Experimental Models

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Pain is the warning signal of underlying pathology. Analgesic drugs are used as per the severity of pain. These drugs have their own adverse drug reactions, sometimes requiring the discontinuation of treatment. Perception and reaction to the pain are two important facets of pain. Reaction in the form of fear,restlessness, spasm of the muscles etc. are seen. Effectiveness of Vitexnegundoin pain have been studied, we planned to evaluate the associated components of it. To evaluate, the Analgesic, Anxiolytic action and Muscle relaxant action of Vitexnegundo leaves aqueous extract (VNLE) and Vitexnegundo dried leaves decoction (VNDLD). Freshly prepared VNLE and VNDLD are the two preparations of Vitexnegundo used to evaluate the analgesic, anxiolytic and skeletal muscle relaxant activity. Wistar rats and mice of either sex were divided into four groups, control, positive control, VNLE&VNDLD. Standard drug pentazocine & diazepam were used for analgesic & skeletal muscle relaxant property respectively. Rats were exposed to hot plate for Analgesic activity, elevated plus maze (EPM) for anxiolytic effect and mice to rotarod apparatus for checking skeletal muscle relaxation after the drug treatment. Analysis was done with graph pad prism 6. Pain threshold was significantly increased at ($p<0.001$) Pentazocine and ($p<0.01$) in Vitexnigundo juice as observed from increase in reaction time. VNLE and VNDLD significantly showed positive effect on the parameters of the EPM. This observation was comparable with the standard drug diazepam. Time spent on the rota rod was significantly reduced in all drug treated mice. Conclusion:Analgesic and antianxiety activity of Vitexnegundo is confirmed.Fresh juice VNLE was more effective than the VNDLD. It also possesses skeletal muscle relaxant action, enhancing the pain relief.

Keywords: Anxiety; Decoction; Pain Threshold; Rota Rod; Reaction Time; Skeletal Muscle Relaxant.

Pain is an unpleasant and unwanted sensation. Pain may be somatic, visceral, and neuropathic, referred or psychogenic in nature[1]. Whatever is the origin of pain, it has many components like-peripheral (tissue damage), central (perception of pain), emotional (anxiety), and muscle spasm (induced by sprains and arthritis) and oxidative stress may also be present. It is an important symptom of many medical conditions which is guiding the diagnosis of the condition and it should be relieved on priority basis.

There are many analgesic drugs available, like NSAIDs and opioids. But apart from the side effects like gastric irritation and dependence, they do not act on all components of pain mentioned above[2]. For treating arthritis in clinical practice,

along with analgesic and anti-inflammatory drugs, benzodiazepines (BZDs) are added for central muscle relaxation. Anxiolytic action of BZDs also helps in alleviating pain.

Many traditional plants are used in the treatment of pain since ages. They are claimed to have no side effects. One of such plants is *Vitex negundo Linn*, commonly known as Nirgundi (a large aromatic shrub), and is distributed throughout India[3]. In Ayurveda it is commonly used in arthritis. Its anti-inflammatory and analgesic properties are reported in papers[4]. This study was planned to evaluate anxiolytic and muscle relaxant action of VNLE & VNDLD, which would be helpful in the treatment of arthritis, but not conclusively evaluated.

MATERIAL AND METHODS

Animals for the experiment

24 Albino rats of either sex, weighing 150-200gm. and 24 mice of either sex, weighing 50-60 gm. were obtained from central animal house (Regd. no.258/PO/ReBi/S/2000/CPCSEA), BVDUMC, Pune. Housing was done as 3 animals per cage as per CPCSEA. Strictly controlled temperature and a 12 hr light and dark cycle. Animals were given rodent diet of Pranav agro 40g per animal per day and aqua water was given *ad libitum.* Animal handling was done by trained persons following Good Laboratory Practice (GLP).

Study was started after obtaining the ethics committee approval BVDUMC/060/2018/001/001

Preparation of *Vitex negundo* leaves extract (VNLE)

Fresh leaves of *Vitex negundo* were collected from local area and authenticated in college of Ayurved, BVDTU, Pune. Fresh juice was prepared in the mixer. It was filtered through the muslin cloth and used to study the various activity. Dose of the juice -0.3 ml/200gm[5, 6,7]

Preparation of *Vitex negundo dried leaves decoction* (VNDLD)

Vitex negundo (VN) leaves powder was procured from the authentic Ayurved vender. The decoction was prepared by boiling 20 grams of coarse powder of leaves of *Vitex negundo* in 250 ml water till it gets reduces to half to its original volume. The decoction was filtered through the muslin cloth and used to study the various activity. Dose of the decoction - 40mg/200gm rat [8]

Method to evaluate analgesic action of *Vitex negundo Linn* in Rats

Standard Drug- Pentazocine (1mg/kg, i.p) for central analgesic activity.

Pentazocine (30mg/ml) was diluted in 9ml distilled water to make it 3mg/ml.

Groups: Wistar Rats were divided into 4 groups, 6 rats per group

Group I - Vehicle control (Distilled water orally)

Group II- VNLE-0.3ml/200mg rat (orally)

Group III-VNDLD-40 mg/200mg rat (orally)

Group IV- Inj.Pentazocine (1 mg/kg, intraperitoneally)

Testing of centrally acting analgesic drugs are done with hot plate method. To compare this activity centrally acting analgesic Pentazocine was used as standard drug, but it is not effective orally so it was given by intraperitoneal route.

Hot plate method

Temperature of the hot plate instrument was maintained at 55 ± 10^0c .[9] The animals from all the groups were kept on the hot plate one by one and the baseline reading was taken. Parameter assessed was licking of paw or jumping response, whichever was appeared first was recorded as 0 min. Rat was placed on hot plate for maximum 15 sec., rats were removed from hot plate to avoid damage to the paws.

Drug treatment was given as per groups. The response observed is known as reaction time which was measured in seconds was recorded at different time intervals like 30, 60, and 120 min. after the treatment.[10] The reaction time for all the rats was recorded.

Method to evaluate Anxiolytic action of *Vitex negundo Linn* in Rats

Standard drug: Diazepam was used as a standard drug. It was purchased from local chemist. One ampoule contains 2ml (10 mg/2 ml). 1ml of Diazepam was diluted with 9ml of distilled water.[11] 1 ml contained 0.5mg Diazepam. Dose-Diazepam 1 mg/kg.

Group I-Vehicle control (orally)

Group II- VNLE-0.3ml/200mg rat (orally)

Group III-VNDLD-40 mg/200mg rat (orally)

Group IV-Diazepam 1 mg/kg. (orally) [12]

Elevated plus Maze method [13]

This is a widely used method to test anxiety and related behavior of rodents. The apparatus consists of four arms, 2closed and 2 open arms. Closed arm 50 cm×40cm×10 cm & open arm of 50 cm ×10 cm. From central platform the arms are extended up to 10 cm ×10cm and the plus maze is elevated to a height of 50 cm from the ground. Reliable results can be obtained in a short period of time.The position of the rat on the EPM was fixed, rat was placed in the centre of the maze, facing the open arm opposite to the experimenter.

During the 5 min test following observations were recorded-

1. Preference of the rat for its first entry into the open or closed arms
2. The total number of entries in open or closed arms
3. The time spent in the open and enclosed arms.

An entry was defined as all 4 paws crossing the line into that arm. All the precaution were taken to maintain the constant experimental atmosphere avoiding external stimuli. Baseline readings were taken. Then rats were treated as per the group and test was carried out1 hr after dosing.

Method to evaluate Muscle relaxant action of *Vitex negundo Linn* in mice

Standard drug: Diazepam was used as a standard drug.[14] It was purchased from local chemist. Animals 24 mice of either sex were taken. Diazepam ampoule (10 mg/2 ml) was purchased from local market. Dilution was done same as above. Dose- Diazepam 1 mg/kg.

Group I-Vehicle control. (orally)

Group II-*Vitex negundo* (VNLE)-0.3ml/200mg rat. (orally)

Group III-*Vitex negundo* (VNDLD)-40 mg/200mg rat. (orally)

Group IV-Diazepam 1 mg/kg. (orally) [12]

Rotarod apparatus method[15]

Evaluation of muscle relaxation was carried out using rotarod. Rotarod is the instrument which has a horizontal metal rod coated with rubber, 3cm in diameter, was put at a rotation of 15 rpm. The metal rod is about 50 cm above the surface to prevent the animal from jumping off the roller. There are 4 compartments in the instrument where 4 mice are kept at a time. The mice were placed on the revolving rod. The time spent on the rotating rod was observed. When the animal gets exhausted, fall on the platform. The time taken by each rat to fall on the platform was taken as an index of muscle relaxation.

Statistical Analysis

Data expressed as mean±SEM. One-way ANOVA is used for comparison between the groups, followed by post-hoc Dennett's test. $p<0.05$ was considered statistically significant.

RESULTS

Hot Plate test

Reaction time was measured in the test and control animals

At 0 min (Baseline) reaction time was almost similar between the test and control animals

At 30 min reaction time was increased in the Pentazocine treated animals but was not significant

At 60 min increase in reaction time was observed in all drug treated animals. Significantly high in ($p<0.001$) in Pentazocine, ($p<0.05$) in VNLE

At 120 min increase in the reaction time was statistically significantly more ($p<0.001$) in

Table 1. Effect of VNLE & VNDLD on number of open arm entry on Elevated Plus Maze experiment

	Open arm entry Pre test	Open arm entry Post test	Time Spent Pretreat in open arm	Time Spent Post treat in open arm	Time Spent Post treat in closed arm
Control	2.16± 0.16	2.66±0.33	82.33±3.96	84.00±5.82	216.0±5.82
Diazepam	2.33±0.21	6.33±0.49***	76.50±4.89	205.33±11.17***	94.66±11.17
VNLE	2.16± 0.16	5.83±0.47**	75.66±3.48	201.33±12.04***	98.66±12.04
VNDLD	2.66±0.66	4.66±0.42*	78.50±5.27	163.50±8.51***	136.5±8.51

All values are expressed as mean ± SEM (n=6); *$p<0.05$, **$p<0.01$ &***$p<0.001$ when they were compared to control. One way ANOVA, Dunnett's Multiple Comparison post hoc tests.

Pentazocine, ($p<0.01$) in VNLE group and ($p<0.05$) in VNDLD group in comparison with control group

Elevated Plus Maze experiment

The entries in open arms in pre-test seen to be similar in all groups.

In the post test after the drug administration, there was significant increase in number of entries in the open arm in diazepam treated (***$p<0.001$) group and VNLE(**$p<0.01$) and VNDLD (*$p<0.05$) treated animals on EPM.

Increased in duration in the open arms (***$p<0.001$) was observed in all drug treated groups after the drug treatment in comparison with control.

Rota Rod test

Percentage decrease in activity on the rota rod was measured in the animals treated with the diazepam and nirgundi two formulations. It was observed that diazepam treated animals showed least activity on the rota rod ($p<0.001$), Nirgundi treated animals also showed decreased in the duration on the rota rod with VNLE ($p<0.01$) & VNDLD ($p<0.05$)

DISCUSSION

Vitex Nigundu is studied for various activities like antiseptic, astringent, analgesic [16],anti-inflammatory, antioxidant [17] , antifungal [18] and antipyretic [19].Different parts of the plants are also evaluated like seeds and leaves for anti-inflammatory action [20,21] betulinic acid and ursolic acid biological active substances isolated from vitex nigundo also have been studied.[22]Almost all the parts like leaves, roots, bark, fruits, flowers and seeds are used traditionally for medicinal purpose.[23] Anticonvulsant activity of Vitex-negundo particularly against PTZ induced convulsion was promising and found to be effective as an adjuvant with Valproaic acid in the treatment of petit mal epilepsy. [24] In most of the studies alcoholic and aqueous extract of Vitex-negundo has been evaluated.

Pain and anxiety are the intercalated things, both increases the severity of each other. Anxiety is associated with increased muscle tone

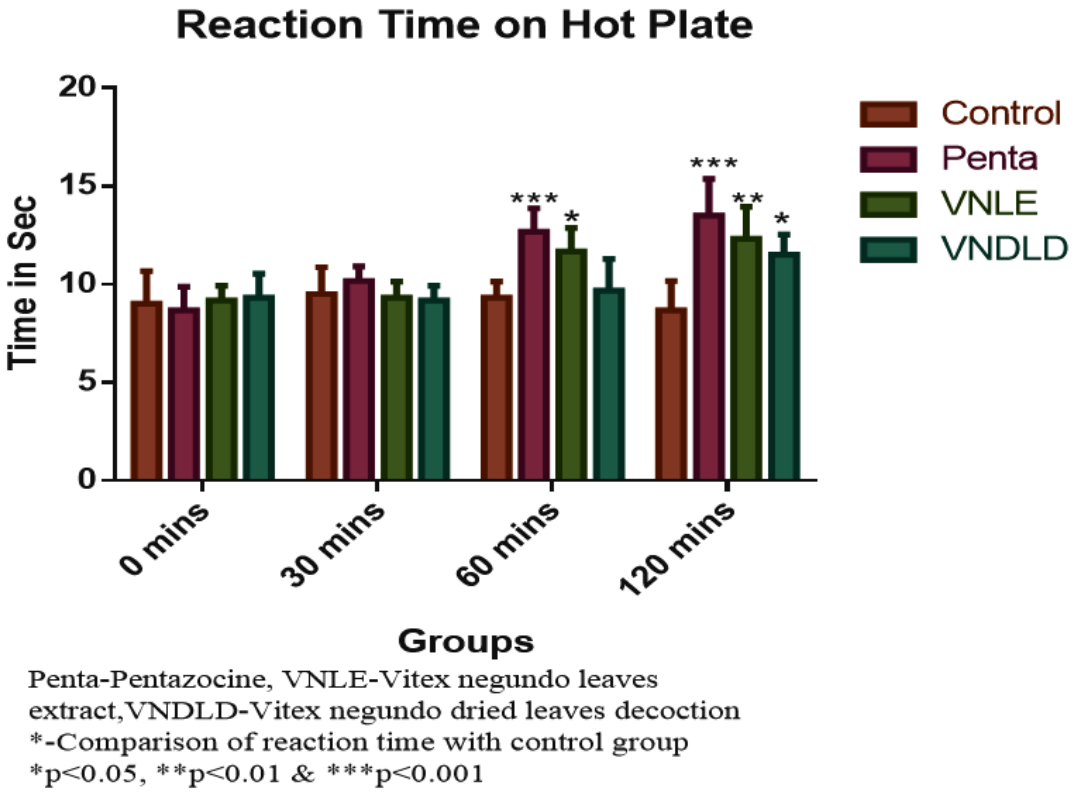
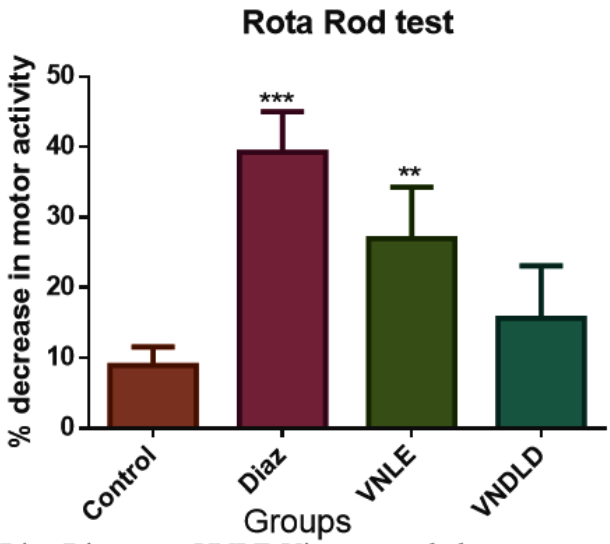


Fig. 1. Effect of VNLE & VNDLD on reaction time on Hot Plate



Diaz-Diazepam, VNLE-Vitex negundo leaves extract ,VNDLD- Vitex negundo dried leaves decoction

Values are expressed as Mean ±SEM.***p<0.001, **p<0.01

Fig. 2. Effect of VNLE & VNDLD on time spent on Rota rod

along with the other symptoms such as restlessness, easily fatigability, difficulty in concentration, irritability and sleep disturbance.[25] Therefore, in the present study we evaluated all three components like analgesic activity, anxiolytic and skeletal muscle relaxant property of VNLE and VNDLD.

For the analgesic activity hot plate method which is the most commonly used method to test the centrally acting analgesic drugs was used. [26] Pentazocin was compared with the test drug for analgesic activity. VNLE showed maximum analgesic activity which was comparable to the pentazocine. Action of Nirgundi started after the 60 min of administration and remained till 120 min. Results are consistent with the findings of M.G. Dharmasiri *et al.* (2003), analgesic action of the VNLE in Wistar rats using hot plate, tail flick and formalin tests. He compared the analgesic activity with aspirin. [27] P. S. Mishra *et al.(2014*) evaluated that water extract of Vitex negundo for analgesic and anti-inflammatory activity. Both of these authors used the aqueous extract of the Vitex nigundo, definitely the fresh juice has significantly more analgesic activity than the extract. This could be because of the antioxidant and the GABA mimetic effect of Vitex nigundo. [28]

Antianxiety drugs act through the mechanism like modifying GABA ergic & serotonergic activity. Diazepam which is commonly used antianxiety agent acts through increasing GABA ergic transmission with additional muscle relaxant property, whereas Buspirone acts through the serotonergic system, it acts on 5-HT1A receptors are which inhibitory auto-receptors are and binding to the receptor inhibits the release of 5HT. Diazepam, a standard anxiolytic used clinically for treatment of anxiety.

Natural tendency of the rodents is to stay in the dark places. The Elevated Plus Maze is usually performed to evaluate anxiety generated behaviour. In this experiment, as per natural tendency rats avoid the entry in open arms, increase in duration in the close arms, and a low count in rearing indicates anxious behaviour.[29] In this experiment, it was observed that the VNLE showed the open arm entries and duration in open arms are more and thus we can say that it has anxiolytic effects.

Rotarod experiment is commonly *used* to show the action of the drug on motor coordination, balance and motor learning in mice.[30] The time gap in the falling time from the rotating rod between the

control and animals which were given the test drug was taken as an index of skeletal muscle relaxation. Time spent on the rod is significantly reduced in diazepam and Vitex nigundo group indicating the muscle power is reduced in this group. Maximum muscle relaxation was observed with VNLE. The skeletal muscle relaxation with the VNLE was similar to the Diazepam, which acts as a centrally acting skeletal muscle relaxant.

CONCLUSION

The analgesic, antianxiety and skeletal muscle relaxation activity of the two preparations of Vitex nigundo was tested. Results showed very promising effects of VNLE and VNDLD on all three activities may be contributing to the pain relief in various elements. VNLE was significantly more effective than the VNDLD. Further studies are planned to evaluate the detailed mechanism of action.

Sources of funding

No

Conflict of interest

None.

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