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TO EVALUATE THE ANALGESIC ACTIVITY OF VITEX NEGUNDO LINN.EXTRACT ON ALBINO MICE (SWISS STRAIN) MICE

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ABSTRACT
Present study is carried out to evaluate the analgesic activity of given drug Vitex nigundo Linn on mice. The fresh leaves of Vitex nigundo Linn. have been suggested to possess analgesic activity possibly mediated via prostaglandin (PG) synthesis inhibition Because it contain alkaloids, glycosides, flavonoids sterols, resin and tannins as per phytochemical evaluation of extract The Hot plate test used in this experiment to study the antinociceptive activity of ethanolic leaf extract of Vitex-negundo (VN) (500 mg/kg, p.o) on mice. The effect was compared with Diclofenac sodium (25 mg/kg, i.p) in Hot plate test & standard &control respectively. Vitex nigundo Linn has a mechanism of central analgesic action. The test drug showed significant analgesic activity in dose dependent manner in the experimental models. Our observations suggest Vitex nigundo Linn that possesses both central peripheral analgesic activities .It may prove to be a useful as analgesic drug.
KEY WORDS: *Antinociceptive, Vitex negundo, Hot plate method, Diclofenac sodium.*

INTRODUCTION

Herbs are different in several respects from the type of purified therapeutic agents we have become accustomed to call drugs in the last half of the twentieth century. In the first place, they are more dilute than the concentrated chemicals that are familiar to us in the form of aspirin tablets or tetracycline capsules. Now a day's peoples are using herbal plant because it does not show any side effect compare to drug. The whole Plant of Vitex negundo Linn has been investigated for its pharmacological activities i.e. Anti-inflammatory(10,11) Anticonvulsant, Antitumor, CNS Activity, Analgesic activities etc. For present study Vitex negundo Linn (Verbenaceae), possess many medicinal properties& Chemical constituent alkaloids allopathic, flavonoids, sterols, resin and tannins. Leaves of Vitex negundo Linn has been investigated for its analgesic activity including its mechanism of action (14) The fresh leaves of Vitex negundo Linn have been suggested to possess analgesic activity possibly mediated via prostaglandin (PG) synthesis inhibition, antihistaminic, membrane stabilizing and antioxidant activities. Therefore the following study was preformed to evaluate scientifically the analgesic activity of Vitex negundo Linn (VN) by Hot plate method on albino Mice.(10, 11, 14)

PLANT PROFILE

Common name: Huang Ping, Nirgudi,Dabtan, Dangla, Kamalan, Limo-limo, Sagarai ,Turagay, Agno-casto.

Biological Source Nirgudi consists of seeds, roots as well as fresh leaves [whole plant] of plant known as *Vitex negundo Linn.(***Family:** *Verbenaceae)*
The leaves of nirgundi contain fragrant, volatile oil and resins. The fruits contain resine, astringent organic acids, alkaloids and a pigment. The plant nirgundi also contains alkaloids, glycosides, flavonoids, reducing sugars, sterols, resin and tannins. From seeds-n-triacontane, n-hentriacontane, n-pentatriacontane, n-nonacosane, sitosterol, p-hydrobenzoic acid and 5- oxyisophthalic acid isolated. Also, 3, 4- dihydroxybenzoec acid is isolated from seeds. Vanillic acid, p-hydroxybenzoic acid and luteolin are isolated from bark.From essential oil pinene, limonene



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Uses

1. Seed - occasionally used as a condiment, it is a pepper substitute.
2. Leaves of Nirgudi are astringent, febrifuge, sedative, tonic and vermifuge
3. The plant is said to be a malarial preventative and is also used in the treatment of bacterial dysentery - extracts of the leaves have shown bactericidal and antitumor activity.
4. The root is thought to be tonic, febrifuge, and expectorant, and the fruit to be nervine, cephalic, and emmenagogue.

3.MATERIAL AND METHOD

3.1 PLANT MATERIAL

Leaves of Vitex nigundo linn. Was collected from wagholi ,(pune) district of Maharashtra in December 2008 and authenticated in BSI(Botanical Survey Of India)

By Botanist Prof.P.G.Diwakar (**Voucher no. -** VNNRSP1/2008).The leaves of plant was clean with water and dried in the shade until a constant weight was obtained Dried leaves were coarsely powdered material passed through 120 mesh to remove fine powder. The coarse powder was used for extraction

PREPARATION OF EXTRACT

The powder obtained was subjected to successive soxhlet extraction with the solvents with increasing order of polarity i.e. Ethanol (95%). Extraction were carry out for 72-80 hrs by standard Soxhlet apparatus method and the extracts were evaporate to determine the percentage yield and Phytochemical screening of plant.

EXTRACTIVE VALUE

Name of the Plant	Parts Used	Method of Extraction	Yield in percentage (ethanol)
Vitex nigundo Linn.	Dried Leaves	Soxhlet Extraction	11.5 %

Table. 1 Extractive value of *Vitex nigundo* Linn.

ANIMAL

Male Albino mice (Swiss strain) weighing 25-30g were be house under standard laboratory condition, in a group of five each. The animals were having free access to food and water at liabdum as per norms ethical committee and CPCSEA norms of the institute approved the protocol of the study.

STANDARD DRUG

Diclofenac sodium.

3.2 ACUTE TOXICITIE STUDY

LD_5

The lethal dose has been found of selected plant extract of *Vitex nigundo* for the selected analgesic activity on animal rat .There is 90% range of dose between 4000-5000 mg/kg body weight.

ED_{50}

As per above LD_{50} there is selection of effective dose is calculated this both dose 500mg/Kg is LD_{50}&ED_{50} as per reported respectively.

3.3 METHOD

1. Hot plate method
2. Acetic acid induced writhing in mice
3. Tail flick test in albino mice [29, 30]

EDDY'S HOT PLATE METHOD

Procedure

1) Swiss strain albino mice weighed (25-30 g) Selected for this method &total number of animal taken is 18.
2) Divided in to 3 groups each consists of 6 animals.
3) **Group -1** considered as Normal control group (Distilled water)
4) **Group-2** received standard drug serves as Standard group (Diclofenac sodium dose:25mg/kg)

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5) **Group-3** received ethanol extract of *Vitex negundo* Linn.as per body weight respectively serve as Test group (*Vitex negundo* Linn extract dose: 500 mg/kg)
6) Take the basal reaction time by observing hind paw licking or jump response (whichever appear first)in animals when placed on the hot plate maintained at constant temperature of about 55˚c (preferably ,55 ±1˚c).
7) A normally animal show such response in 6.8 seconds cut off period is of 15 seconds is observed to avoid damage to the paws.
8) Injection of analgesic drug given to the animal and note the reaction time of animal on the hot plate at 0, 30, 60, 90,120 minute .
9) After the drug administration as the reaction time increases with drug 15 second is taken as maximum analgesia.
10) Then animals are removed from the hot plate to avoid injury to the paws.
11) Then take average basal reaction time was calculated using one way ANOVA.
12) Then this average basal reaction time of test drug is compare with standard drug &find whether test drug show analgesic effect as compare to standard [16, 17,18,19,20].

Fig 1: - Eddy's Hot Plate Method

4.RESULT

4.1 PLANT

1) Extractive value: 11.5%
2) Phytochemical study of *Vitex negundo* Linn. Leaves contain alkaloids, glycosides, flavonoids sterols, resin and tannins to show analgesic activity.

Sr. no.	Constituents	Tests	Ethanol Extract
1.	β Sterols	Libermann's sterol test	+
		Salkowski's test	+
2.	Alkaloids	Dragendorff Test	+
		Mayer's Test	+
5.	Phenolic Compounds	Extract + $Fecl_3$	+
6.	Test for Tannins	Gelatin test	+
		$Fecl_3$ test	+
7.	Organic Acids	Test for Fats and oils	+
		Mallic acid test	+
		Citric Acid test	+
		Test for Chloride	+
9.	Glycosides	Baljet test	+
10.	Flavonoids	Shinoda's test	+

Table no. 2 Phytochemical study *Vitex negundo* Linn

4.2 ACUTE TOXICITIE STUDY

The lethal dose (LD_{50}) has been found in the selected extract of plant *vitex negundo* Linn on the selected albino mice. We get the range between 4000-5000 mg/kg body weight.
As per above lethal dose (LD_{50}) lower dose there is a selection of effective dose calculated 500mg/kg orally.

4.3 ANALGESIC ACTIVITY

An ethanolic extract *vitex negundo* Linn leaves showed significant analgesic activity. The result was significant at $p< 0.05$ for Eddy's Hot Plate Method.

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SR. NO	DRUGDOSE (in group of animal)	REACTION TIME (Sec) TIME AFTER DRUG ADMINISTRATION.				
		0 (min)	30 (min)	60 (min)	90 (min)	120 (min)
1	CONTROL	4.8±0.68	6.16±0.59	6.5±0.42	7.16±0.47	7.5±0.57
2	STANDARD	4.83±0.70	9.83±0.56	10.66±0.29	10.86±0.60	11±0.57
3	TEST	4.90±0.55	7.5±0.76	8±0.57	8.83±0.54*	9.83±056*

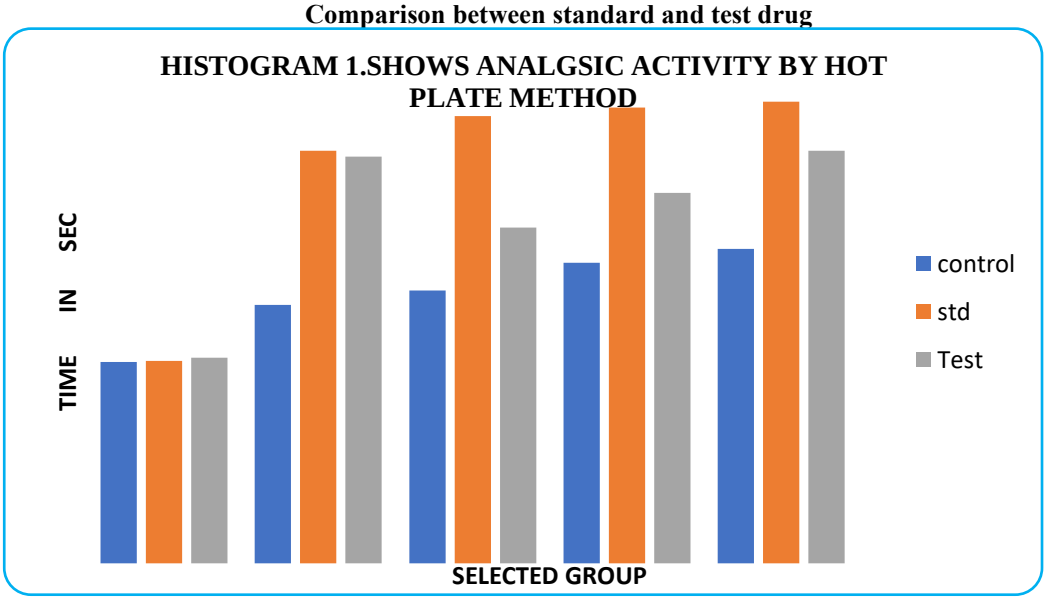
Table no.03 Comparison between standard drug (Diclofenac sodium) and test drug (ethanolic extract of Vitex negundo Linn) for analgesic activity.

N= 6, value are expressed by Mean ± SEM for 6 animals P value: *p<0.05as compared to respective control. Data was analyzed by one way ANOVA TEST.

CONTROL-Distilled water

STANDARD- Diclofenac sodium (dose:25mg/kg)
TEST- *Vitex negundo* Linn extract dose: (500 mg/kg)

Histogram Shows Analgesic Activity by Eddy's Hot Plate Method.
Comparison between standard and test drug



Histogram 1: Effect of Standard Control and Test Drug.

The analgesic activity of an ethanolic extract of bark of Vitex negundo Linn is comparable with standard drug Diclofenac. It is computed in Histogram-1 which shows the effectiveness and pharmacological rationale for the use of Vitex nigundo Linn as an analgesic drug.

DOSES OF ANIMAL

CONTROL-Distilled water

STANDARD- Diclofenac sodium (dose:25mg/kg)
TEST- *Vitex negundo* Linn extract dose: (500 mg/kg)

5.CONCLUSION

From this study, it can be concluded that ethanolic extract of *Vitex negundo* Linn possesses marked analgesic activity. *Vitex negundo* Linn may have both central and peripheral analgesic action. *Vitex negundo* Linn also possesses the analgesic and anti-inflammatory action of *Vitex negundo* Linn can be attributed to its flavonoid contents, which are known to act through inhibition of prostaglandin biosynthesis. In our study, we are trying to explore the antinociceptive action of Vitex negundo Linn. May be

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useful in relieving both the visceral and integumental pain. Because of its chemical content like flavonoids, glycosides, alkaloids sterols etc. Require to show analgesic activity. Further study is necessary to evaluate the active principle responsible for the analgesic activity and clear mechanism of action involved.

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