



Research Article

ISSN : 0975-7384  
CODEN(USA) : JCPRC5

## Evaluation of Antipyretic Activity of Alcoholic Extract of *Vitex negundo* Leaves In PGE<sub>1</sub> induced pyrexia model in Albino Rats.

Jayasree Tirumalasetty\*, Sheikh Ubedulla, Chandrasekhar. N, P.V. Kishan and Kavitha Rasamal

Department of Pharmacology, Mamata Medical College, Khammam-507002, Andhra Pradesh, India

### ABSTRACT

Evaluate antipyretic Activity of Alcoholic Extract of *vitex negundo* In PGE<sub>1</sub> induced pyrexia in Albino rats. *Vitex negundo* is generally known as *Negundo* in India. It is also known as the five-leaved chaste tree, is a large aromatic shrub with quadrangular, densely whitish, tomentose branch lets. It is widely used in folk medicine, particularly in South and Southeast Asia. It belongs to family Verbanaceae and is found throughout India. *Vitex negundo* has been used for various medicinal purposes in Ayurveda and Unani systems of medicine. The leaves and whole plant is used as an anti-inflammatory, antiseptic, antipyretic and diuretic. Antipyretic activity of leaves of *vitex negundo* is studied in brewer's yeast induced pyrexia models. Our study is to evaluate antipyretic activity of alcohol extract of *vitex negundo* in PGE<sub>1</sub> induced hyperpyrexia model in albino rats

**Key words-** Antipyretic activity, Aspirin, Digital Thermometer, Soxhelts apparatus, *Vitex negundo*,

### INTRODUCTION

*Vitex negundo* Linn. (Verbanaceae) [VN] commonly known as *Negundo*, has been used by several traditional systems of medicine including Ayurveda, Unani and Siddha for different ailments. In recent times, focus on plant research has increased all over the world and a large body of evidence has been collected to show immense potential of medicinal plants used in various traditional systems [1]. The leaves and whole plant is used as an anti-inflammatory, antiseptic, antipyretic and diuretic (2-8). The fresh aromatic leaves are reportedly useful in Rheumatism and to relieve pain [9]. Extensive studies were done on actions such as anti bacterial, antifungal, antioxidant, antinociceptive, Hepatoprotective and ant fertility [10-14]. Antipyretic action of *vitex negundo* was well studied by using yeast induced hyperpyrexia method [15]. ProstaglandinE<sub>1</sub> induced hyperpyrexia models are few in literature so the study has been done to elucidate antipyretic action of *vitex negundo* leaves on ProstaglandinE<sub>1</sub> induced hyperpyrexia model

### EXPERIMENTAL SECTION

The study was done during January 2012 to February 2012. The leaves of *Vitex negundo* was obtained from a vegetable garden near Khammam. The identification and authentication of the leaves was done at the department of Botany, Government Degree College. The leaves are shade dried and extraction was done with 95% ethanol by Soxhelts apparatus in department of Pharmacology. The extract was dried under vacuum, stored at room temperature and protected from direct sunlight

**Animals:** Swiss albino Rats aged 8-10 weeks of male sex weighing about 150–200g, were obtained from the central Animal house. The animals were fed pellet diet and water ad libitum and were maintained under standard conditions of temperature, humidity and light (12 hours light/12 hours dark cycle). The experiment complied with the guidelines for animal experimentation of our laboratory and was approved by the Institutional Animal Ethics Committee (IAEC). Registration number 285/CPCSEA. The guidelines for the investigation of experiments in conscious animals were followed in all tests.

**Acute toxicity:** Oral LD50 dose of VN leaf extract in rats is 7.58 g/kg, body weight [16]. Two dosages of AEVN of 2000mg/kg and 4000mg/kg were chosen for the evaluation of antipyretic activity in PGE<sub>1</sub> induced hyperpyrexia.

**Drugs and Chemicals:** Prostaglandin E<sub>1</sub> (Mesoprostal G.D. Searle & Company (now Pfizer) Aspirin (cipla limited) 150mg/kg, Normal saline (0.9% NaCl solution) were administered in the volume of 2 ml/kg. The extracts were suspended in distilled water and were administered orally (p. o.) in the volume of 2ml/kg of body weight in the doses of 2000mg and 8000mg/kg

#### Experimental design:

Group I – Control Rats (Normal saline 2ml/kg)

Group II – Standard (Aspirin 150 mg/kg)

Group III – AEVN 2000mg/kg

Group IV – AEVN 4000mg/kg

#### Evaluation of Antipyretic activity [17].

The antipyretic activity of the alcoholic extract of *Vitex negundo* Linn was screened by using Prostaglandin E<sub>1</sub> induced hyper pyrexia method. Albino rats of male sex weighing between 150-200 gm were selected and divided into 4 groups having six animals each. They were maintained at a constant temperature of 24-25 °C for 24 hrs. Animals were fasted over night. After noting the initial rectal temperature with digital thermometer, Pyrexia was induced by S.C injection of 100µg/kg of Prostaglandin E<sub>1</sub> (Mesoprostal). After 1 hour of injection of PGE<sub>1</sub> the animals having more than 0.5 to 1 degree Fahrenheit to the normal temperature were included in the study. The extract (AEVN) was suspended in distilled water and administered orally. Group 1 received 2 ml/kg of Normal saline. Group II received 150mg/kg Aspirin, Group III and Group IV received 2000 and 4000 mg/kg of alcoholic extract of *Vitex negundo* suspended in 2ml of distilled water. Rectal temperatures were noted at 30min interval up to 180mins.

#### Statistical analysis

The results were expressed as mean ± SEM. Statistical analysis was carried out by using ANOVA followed by Dunnett's multiple comparison tests using primer of windows McGraw –Hill software version 5.0.0.0 (2011). *P*-values < 0.05 were considered significant.

## RESULTS

**Table-1 Effect of Alcohol extract of Vitex Negundo Leaves on PGE<sub>1</sub> induced pyrexia in Albino rats**

Time in minutes	Temperature ( ° F)					
	0 hr	30min	60min	90min	120min	180min
Group I control Normal saline n=6	98.31 ±0.09	100.7 ±0.16	100.5 ±0.13	100.5 ±0.13	100.3 ±0.21	98.31 ±0.06
Group II Standard Aspirin(150)mg/kg n=6	98.37 ±0.06	95.49 ±0.33**	96.4 ±0.13**	96.55 ±0.14**	97.55 ±0.16**	97.87 ±0.09**
Group III Test I (AEVN) 2000mg/kg n=6	98.68 ±0.09	97.87 ±0.07	97.88 ±0.02**	97.38 ±0.16**	98.27 ±0.06**	98 ±0.02*
Group IV Test II(AEVN) 4000mg/kg n=6	98.59 ±0.05	97.35 ±0.11**	97.25 ±0.04**	97.1 ±0.077**	98.3 ±0.074*	98.28 ±0.05*

Each value presents the mean ± S.E.M. of six observations, \**P* < 0.05 Significant,  
\* \**P* < 0.000 Highly Significant. AEVN- Alcoholic extract of Vitex Negundo.

The Alcoholic extract of *Vitex Negundo* at a dose of 2000mg/kg body weight has shown significant ( $p < 0.05$ ) antipyretic activity, it has shown significant fall in body temperature up to 180mins following its administration. Whereas at a dose of 4000mg/kg shown highly significant ( $P < 0.000$ ) antipyretic activity. The response was compared to that of a standard antipyretic drug Aspirin. (Table 1).

## DISCUSSION

Fever also known as pyrexia may be due to infection, inflammation, or any tissue damage and diseases states. Pyrexia or fever arises as a secondary impact of infection, malignancy or other diseased states [18]. Antipyretics are the agents which reduce the elevated body temperature. Regulation of body temperature requires a delicate balance between Production and loss of heat in the hypothalamus centrally which regulates the set point at which body temperature is maintained. In fever this set point is elevated by the factors mentioned above. Antipyretic drugs reduce elevated body temperature and are known to act centrally on the temperature regulation center in the brain or peripherally through vasodilatation and heat dissipation. They reset the hypothalamic thermostat and rapidly reduce fever by promoting heat loss (sweating, cutaneous vasodilatation [19]. They also act by inhibiting the synthesis of prostaglandin  $E_2$  [20].

Several medicinal plants have been used as antipyretic agents traditionally. One of those medicinal plants that have been used is *Vitex Negundo*. *Vitex negundo* (Sambhalu) is an aromatic large shrub or small slender tree of about 3 meter in height with quadrangular branches. It is found in moist area, often on banks of rivers, throughout India, up to an altitude of 1500 meters. *Vitex negundo* are commonly found near bodies of water, recently disturbed land, grasslands, and mixed open forests [2]. *Vitex negundo* belongs to the genus *Vitex* of the subfamily Viticoideae. It is classified under the family Lamiaceae, but is sometimes placed under Verbanaceae. [22] Various medicinal properties are attributed to it particularly in the treatment of anti-inflammatory, fungal diseases, antioxidant and hepatoprotective disorders [23, 24]. It contains fragrant, volatile oil and resins with several reported phytochemicals *e.g.* nishindaside, negundoside (irridoid glycoside), and artemetin [25, 26]. Besides, several alkaloids, glycosides, flavonoids, reducing sugars, Beta sitosterols, C-glycoside, casticin, resin and tannins have also been reported [27].

The Constituents previously isolated from the plant include eight lignans [28] ( negundin A, negundin B, 6-hydroxy-4-( 4-hydroxy -3 methoxy)-3- hydroxyl methyl -7 methoxy -3,4 di hydro 2 naphthaldehyde, vitrofolal, (+) – iynioresinol, (+) – iynioresinol -3 $\alpha$ -0- $\beta$ -Dglucoside, (+)(-)(-) pinorecinol and (+) –diasyringaresinol, irridoid glycoside[29],(2-p-hydroxy benzyl mussaenosidic acid), flavonones[30] (5,3', di hydroxyl -7,8,4' trimethoxy flavonone and (5,3' dihydroxy -6,7,4' trimethoxy flavonone), flavone[31] (vitexicarpin),  $\beta$  –sitosterols [32] essential oils[33] ( $\alpha$  –pinene, linalool, terpinyl acetate, beta caryophyllene ), non diterpene [34] (vitedoin B), pentacyclic triterpenoids [35] (beutinilic acid, ursolic acid) and flavonoid glycoside [36] luteolin, agnuside, negundoside, iso-orientin).

The result show that alcoholic extract of *Vitex Negundo* possesses a significant antipyretic effect in PGE1 provoked elevation of body temperature in rats and its effect is compared to the Aspirin (standard drug).So, inhibition of prostaglandin synthesis Could be the possible mechanism of antipyretic action as that of Aspirin [37], also there are several mediators or multi-processes underlining the pathogenesis of fever. Inhibition of any of these mediators may bring about antipyresis [38]. From the above study we have concluded the antipyretic activity of the plant *Vitex Negundo* .However, to know the exact mechanism of action alcoholic extract of *Vitex Negundo* further study with purified fractions/ bioactive compounds are warranted. One study evaluated analgesic activity from the aqueous extract of fresh leaves of *Vitex negundo* in female Westar rats using hot plate, tail flick and formalin tests. The standard drug used in hot plate and tail flick was aspirin (100 mg/kg).[39].In our study also we have taken Aspirin as standard which has both analgesic and antipyretic action and the alcoholic extract of *Vitex Negundo* has shown equal antipyretic activity when compare to the standard drug Aspirin. Fresh leaves of VN have been suggested to possess anti-inflammatory and pain suppressing activities possibly mediated via prostaglandin (PG) synthesis inhibition, antihistaminic, membrane stabilizing and antioxidant activities [40]. Since prostaglandins are involved in swelling and are inhibited by flavonoids [41] it could be suggested that reduced availability of prostaglandins by flavonoids of AEVN might be responsible for its anti-inflammatory effect.

It is well known that most of the anti-inflammatory analgesic drugs possess antipyretic activity. AEVN revealed marked antipyretic activity in PGE1induced pyrexia in rats. In general, nonsteroidal anti-inflammatory drugs

produce their antipyretic action through inhibition of prostaglandin synthesis within the hypothalamus. [42].The antipyretic effect of the test drug may be due to presence of flavonoid compounds, as some flavonoid are predominant inhibitors of cyclooxygenase or lipoxygenase. As our study itself is on PGE1 induced pyrexia model which is involved in pathologically induced pyresis, we can conclude that Vitex Negundo extract reduce pyrexia by inhibiting prostaglandin synthesis in the same way as Aspirin.

### CONCLUSION

In this study we concluded that the alcoholic extract of Vitex Negundo has equal antipyretic activity when compare to the standard drug Aspirin. The antipyretic activities of Vitex Negundo Supports it use in the management of fever by traditional medicine practitioners.

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