Research Article

Antipyretic activity of *Vitex negundo* Linn Leaves extracts

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ABSTRACT

The present study intended to investigate the antipyretic activity of leaf extracts of *Vitex negundo* linn Plant by using yeast induced pyrexia model in Wistar Albino rats. Acute toxicity study and phytochemical analysis was carried out using well established protocols and methods. The phytochemical analysis of leaves revealed the presence of steroids, triterpenoids, alkaloids, flavonoids, tannins and iridiod glycosides. The data obtained indicate that the Petroleum ether and Methanolic extracts of a leaves of plant *Vitex negundo* linn, at dose of 300 mg/kg body weight per oral route (P.O) showed the significant reduction in yeast provoked elevated temperature. The antipyretic effects of the extracts were compared with standard drug paracetamol.

Keywords: Antipyretic, Paracetamol, *Vitex negundo* linn, Yeast.

INTRODUCTION

Pyrexia or fever is caused as a secondary impact of infection, tissue damage, inflammation, graft rejection, malignancy or other diseased states. It is the body's natural defense to create an environment where infectious agent or damaged tissue cannot survive. Normally the infected or damaged tissue initiates the enhanced formation of pro-inflammatory mediators (cytokines like interleukin 1β, α, β and TNF-α), which increases the synthesis of prostaglandin E2 (PgE2) near proptic hypothalamus area and there by triggering the hypothalamus to elevate the body temperature.[1] Most of the antipyretic drugs inhibit COX-2 expression to reduce the elevated body temperature by inhibiting PgE2 biosynthesis. Moreover, these synthetic agents irreversibly inhibit COX-2 with high selectivity but are toxic to the hepatic cells, glomeruli, cotex of brain and heart muscles, where as the natural COX-2 inhibitors have lower selectivity with fever side effects.[2] A natural antipyretic agents with reduced or no toxicity is therefore essential.

Vitex negundo linn (Family - Verbenaceae) is a large, aromatic shrub or small tree of about 3m in height with quadrangular branches, leaves are opposite, exstipulate, long petioled and digitately 3–5 foliate, all leaflets with petiolules, the middle one longer, flowers bluish purple in panicules up to 30 cm long, fruits globose or ovoid or obovoid, four seeded drupe, black when ripe.[3] The leaves are aromatic, tonic and vermifuge. A decoction of leaves is given with the addition of long pepper in catarrhal fever with heaviness of head and dullness of hearing. A pillow stuffed with the leaves is placed under the head for relief of headache. The juice of leaves is said to have the property of removing foetid discharges and worms from ulcers. The leaves are discutient and are useful in dispersing swellings of joints from acute rheumatism and of the testes from suppressed gonorrhea.[4] A furanoeremophilane have been isolated from the

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roots of *Vitex negundo* linn.[5] Nishindaside a novel iridoid glycoside[6] and volatile constituents have been reported from the leaves of this plant.[7] The chloroform extract of defatted seeds of *Vitex negundo* linn yielded four triterpenoids and exhibited anti-inflammatory activity .[8] Traditionally the leaves of this plant are used in fever, to give scientific background an attempt is made to assess the efficacy of *Vitex negundo* linn leaves for its antipyretic activity in the present study.

MATERIALS AND METHODS

Plant material

The leaves of *Vitex negundo* linn was collected from the local areas of Hubli and Dharwad. It was identified and authenticated by Dr. B.D. Huddar, HOD, Department of Botany, Shri Kadasiddheshwar Arts College and H.S. Kotambari science institute, Vidyanagar, Hubli, Karnataka. A voucher specimen has been deposited in the department of Pharmacognosy, KLES's College of Pharmacy, vidyanagar, Hubli, Karnataka, for future reference. Leaves were dried under shade, coarsely powdered and stored in air tight container.

Preparation of the extracts

105.00 grams of the shade dried coarsely powdered leaves of *Vitex negundo* linn were successively extracted with soxhlet apparatus by using solvents like petroleum ether (40–60) and methanol. The solvents were concentrated under reduced pressure using rotaevaporator and semisolid mass was obtained which is then dried in desiccators over sodium sulphate. The petroleum ether and methanolic extracts were stored in a refrigerator and weighed quantity of an extracts was suspended in 1% tween 80 and used for the experiment.

Phytochemical screening

Preliminary phytochemical investigations were carried out for the extracts as per the literature.[9–10] The petroleum ether extract showed the presence of Steroids and triterpenoids by Salkowaski and Libermann burchards tests, Methanolic extract revealed the presence of alkaloids and it was confirmed by Mayers, Dragendrofs, Wagner and Hagers test, Carbohydrates by Molisch's test, Flavonoids by Shinoda, Iridoid glycosides by Wieffering field test and Tannins by Ferric chloride test.

Animals used

For antipyretic activity adult Albino rats (Wistar strain) of either sex weighing 180–200 g were used in 4 groups comprising of 6 animals in each group and for acute toxicity studies Albino mice (Wistar strain) either sex weighing 20–30 g were used in 5 groups comprising 6 animals in each group. The animals were maintained under suitable nutritional and environmental conditions throughout the experiment and the experiment was initiated after approval of Institutional animal ethical committee (KLESCOPH/IAEC.Clear/2007–2008)

Acute toxicity study

Acute toxicity of both the extract was evaluated on Albino mice according to OECD guideline no-420 fixed dose method.[11] Animals were kept in polypropylene cages and fasted for 24 h with water ad libitum, maintained at an ambient temperature of $25 \pm 2^{\circ}$C, Animals were then administered by oral route with petroleum ether extract and methanolic extract (50–5000 mg/kg body weight) suspended in 2% gum acacia solution (vehicle). Control group received only vehicle. Animals were observed for clinical signs and mortality continuously for the initial 4 h and intermittetently for next 6 h and then again 24 h and 48 h after dosing the parameters observed and recorded were sedation, hyperactivity, grooming, loss of righting reflex, respiratory rate and convulsion. LD_{50} was found to be 4500 mg/kg body weight and1/15th of the lethal dose was taken as screening dose (300 mg/kg body weight).

Antipyretic activity
Yeast induced pyrexia model.

Adult Albino rats (Wistar strain) of either sex weighing 120–180 g were used. The animals were maintained under suitable nutritional and environmental conditions throughout the experiment. The animals were maintained under standard laboratory condition for an acclimatization period of seven days prior to performing the experiments. Rats of either sex were divided into four groups, comprising six animals in each group for this experiment. The normal body temperature of each rat was measured rectally at 1h interval on a thermometer and recorded. Fever was induced by injecting the yeast suspension by subcutaneous route of administration in hind limbs of the rats. Rats were then returned to their housing cages. After 19 h of yeast injection, the petroleum ether extract of *Vitex negundo* linn and methanolic extract of *Vitex negundo* linn was administered orally at dose of 300 mg/kg body weight to two groups of animals respectively. Normal saline was administered orally to the control group of animals and the last group of animals received the standard drug paracetamol (150 mg/kg) orally. Rats were restrained for recording of their rectal temperatures at 1h just before petroleum ether extract of *Vitex negundo* linn (PEVN) or methanolic extract of *Vitex negundo* linn (MEVN) or normal saline or paracetamol administration and again at 1h interval up to 23 h after yeast injection.[12]

Statistical analysis

Statistical significance was analyzed using one way ANOVA.

The results were expressed as Mean ±SE. *p*-values were calculated versus control groups. $P < 0.001$ implies significance.

RESULTS

The % yield of petroleum ether extract was found to be 3.01 (3.17 g) which was brownish yellow in color having characteristic odor where as methanol extracts yield was found to be 14.24 (14.96 g) which was greenish black in color having characteristic odor. The LD_{50} of both extracts are found to be 4.5 g/kg body weight, Both MEVN and PEVN at a dose of 300 mg/kg body weight has showed significant antipyretic activity as compared to standard drug paracetamol.

DISCUSSION

Fever may be due to infection or one of the sequels of tissue damage, inflammation, graft rejection, or other disease conditions. Antipyretic are the agents which

Table1: Effects of petroleum ether and methanolic extracts of *Vitex negundo* linn in yeast induced pyrexia in rats.

	Initial temp. (°C)	Temp. at 19 h after yeast Inj. (°C)	Temp. at different h after treatment (°C)			
Treatment	0 h	19 h	20 h	21 h	22 h	23 h
Control (5 ml/kg)	37.1±0.10	39.13±0.10	39.26±0.09	39.45±0.08	39.5±0.08	39.6±0.05
Paracetamol (150 mg/kg)	36.98±0.10	39.2±0.22	37.48±0.15[a]	37.31±0.15[a]	37.21±0.14[a]	37.1±0.14[a]
Petroleum ether extract (300 mg/kg)	37.18±0.0	39.25±0.07	38.38±0.13[a]	38.23±0.14[a]	38.01±0.16[a]	37.75±0.15[a]
Methanolic extract (300 mg/kg)	37.28±0.12	39.4±0.14	38.56±0.15[b]	38.21±0.16[b]	38.06±0.17[b]	37.9±0.16[b]

Values represent mean +\– S.E.M.

[a] $P < 0.0001$, [b] $P < 0.01$ highly significant as compared to control values at corresponding hour.

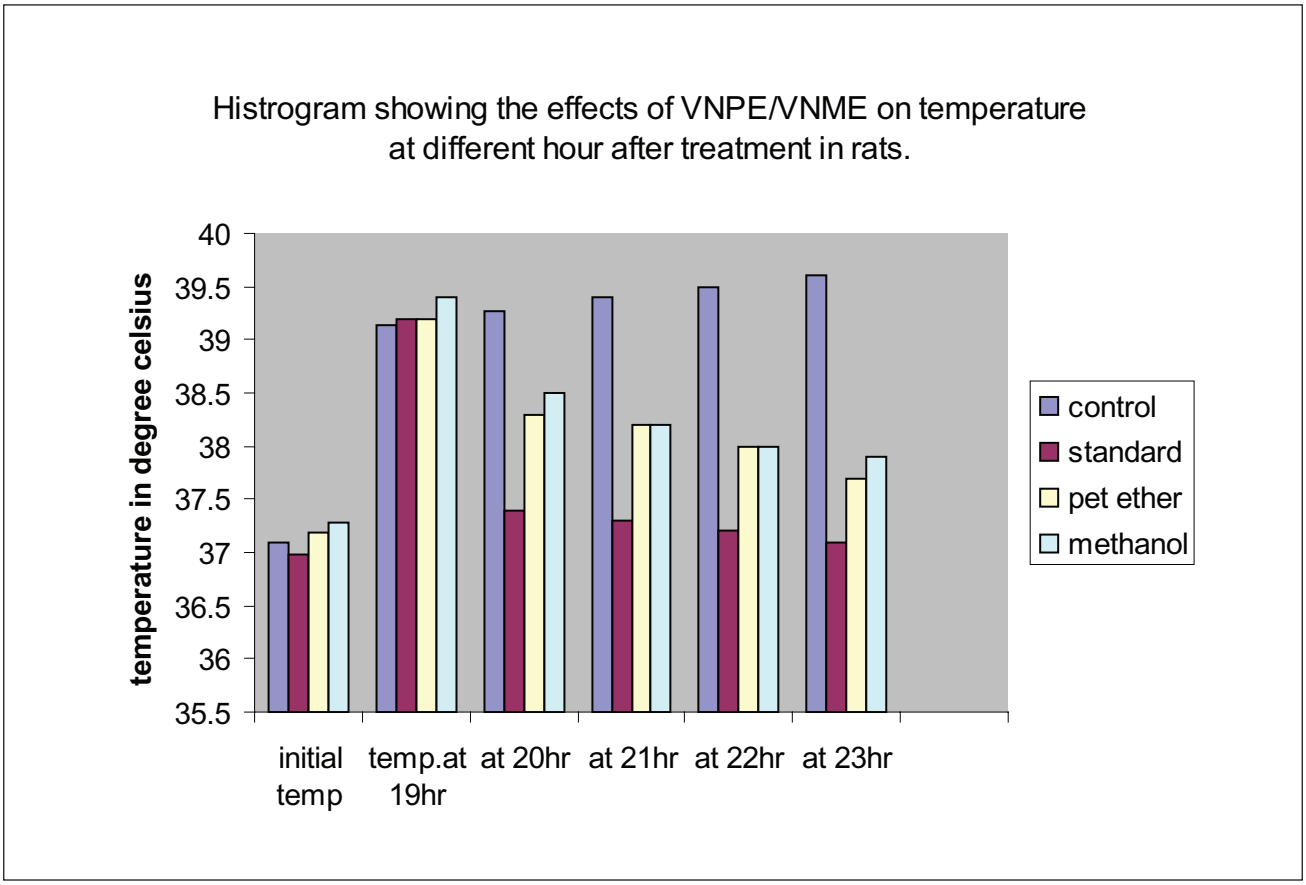

Figure 1: Histogram showing the effects of petroleum ether and methanolic extracts of *Vitex negundo* linn on temperature at different hours after treatment in rats.

VNPE-*Vitex negundo* linn petroleum ether extract.

VNME-*Vitex negundo* linn methanolic extract.

reduce the elevated body temperature.[13] Normal body temperature is regulated by a center in the hypothalamus that ensures a balance between heat loss and heat production. Fever occurs when there is a disturbance of this hypothalamic thermostat, which leads to the set point of body temperature being raised. Non steroidal anti-inflammatory drugs (NSAIDS) reset the thermostat. Once there has been return to the normal set point, the temperature regulating mechanism (dilatation of superficial blood vessels, sweating etc) then operate to reduce temperature were as normal temperature is not affected by NSAIDS.[14] Yeast induced fever is called pathogenic fever. Its etiology includes production of prostaglandins, which set the thermoregulatory center at a lower temperature.[15] When the body temperature becomes high, the temperature regulatory system which is governed by a nervous feed back mechanism dilates the blood vessels and increase in sweating to reduce the temperature. When the body temperature becomes low, hypothalamus protects the internal temperature by vasoconstriction. High fever often increases faster disease progression by increase tissue catabolism, dehydration and existing complaints as found in Human Immuno deficiency Virus (HIV).[16] Due to the increasing frequency of intake of NSAIDS and their reported common side effects, there is need to focus on the scientific exploration of potential herbal drugs which are having fewer side effects. Here the plant *Vitex negundo* linn being an indigenous drug used by different communities for long period and is tried on experimental animals to assess the efficacy of the drug. In this experiment yeast induced pyrexia model in Wistar Albino rats has been used to evaluate the possible antipyretic action of plant extracts. It was noted that the extracts has antipyretic activity. The extract considerably reduces the febrile response in rats.

The effect PEVN and MEVN of leaves of *Vitex negundo* linn on yeast induced hyperpyrexia are given in Table 1. The PEVN at the dose of 300 mg/kg body weight showed significant ($P < 0.0001$) antipyretic activity at 20, 22 and 23 h of yeast administration as compare to control however, MEVN at a dose of 300 mg/kg body weight has exhibited significant ($P < 0.01$) antipyretic activity at 21 h of yeast administration.

The phytochemical investigation of PEVN indicated the presence of steroids and triterpenoids, where as MEVN revealed the presence of alkaloids, carbohydrates, flavanoids, irridoid glycosides and tannins. With the observed significant antipyretic activity in experimental animals by the PEVN it can be correlates with the presence of steroids and triterpenoids, as they are well known antipyretic phytoconstituents.

CONCLUSION

On the basis of the present study, it can be concluded that the reduction of temperature has been observed with in successive hours after administration of petroleum ether and methanolic extracts of a plant *Vitex negundo* linn. Both petroleum ether extract and methanolic extract showed significant antipyretic activity. However, detailed phytochemical investigation of the leaves of the plant is worthwhile to pinpoint the activity and to elucidate the structure of bioactive principles responsible for antipyretic activity. This is to be further studied for the exact mechanism of action. Thus the present pharmacological evidence provides support for the folklore claim as an antipyretic agent.

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