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Research Article

PHARMACOLOGICAL SCREENING OF LEAF EXTRACTS OF ETHNOMEDICINAL PLANT, *VITEX ALTISSIMA* (VERBENACEAE) FOR ITS TRADITIONAL CLAIMS

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ABSTRACT

Objective: *Vitex altissima* (Verbenaceae) has been extensively used in folk medicine for the treatment of pain and associated ailments. Hence, the present study was intended to evaluate total methanolic and aqueous leaf extracts of *Vitex altissima* for analgesic, anti-inflammatory and antipyretic activities. Methods: The analgesic activity was studied by tail immersion model, while the anti-inflammatory activity by carrageenan induced paw oedema model and the antipyretic activity was studied by brewer's yeast-induced pyrexia model. Acute toxicity study and preliminary phytochemical screening were also studied to evaluate the toxicity and chemical profile of the both extracts respectively. Results: No toxicity profile was observed in rats after oral administration of the both extracts at the dose of 5g/kg body weight. The different extracts at a dose of 200 mg/kg and 400 mg/kg body weight *p.o* produced a significant ($p<0.05$) dosage dependent inhibition of pain and oedema in analgesic and anti-inflammatory models respectively. Both extract also showed significant and dosage dependent inhibition of temperature elevation compared with the standard drug paracetamol (150 mg/kg/ body weight). Phytochemical screening of the plant extract revealed the presence of tannins, alkaloids, flavonoids and saponins, coumarins and terpenoids. Conclusion: These results indicate that leaf extracts of *Vitex altissima* possesses potent analgesic, anti-inflammatory and antipyretic effects and thus pharmacologically justifying its folkloric use in the management of pain and related ailments.

Keywords: Acute toxicity, Flavonoids, Inflammation, Prostaglandin, *Vitex altissima*.

INTRODUCTION

Herbal medicines are assumed to be of great importance in the primary health care of individual and communities [1]. The World Health Organization has estimated that 80% of the population of developing countries still relies on traditional medicines, mostly plant drugs, for the primary health care needs. The high degree of efficacy and safety with herbal medicines make them more acceptable compared to other therapeutic invention [2]. Plant-based traditional knowledge has become a recognized tool in search for new sources of drugs and neutraceuticals [3, 4].

Pain, inflammation and fever are some of the most common manifestations of many diseases afflicting millions of people worldwide [5]. Non-steroidal anti-inflammatory drugs (NSAIDs) and glucorticoids, opiods, etc. currently available in market to treat pain and related ailments explaining the side effects of gastrointestinal ulcer and renal dysfunction [6, 7]. In this contest, the research in medicinal plants with claimed folkloric use should therefore be viewed as a fruitful and logical research strategy in the search for new drugs with low toxicity profile.

The plant *Vitex altissima* is used to treat stomatitis, cardiac diseases, anorexia, blindness, leprosy and worm infestation [8]. Stem bark is taken to treat ephemeral fever [9], snake bite [10], rheumatic swellings and chest pains [11]. Leaves are taken for wounds [12, 13], skin allergies [14], snake and scorpion bites [15] and rheumatism [16]. Heart-wood, leaves and bark contain the flavonoid–vitexin [17]. Sridhar et al [18] isolated and characterized several new iridoids from the ethyl acetate extractives of the leaves of *Vitex altissima*. Further investigation on the ethyl acetate extracts of the plant has led to the isolation of a new lignan, named altissinone and a new flavonoid, 2''-O-p hydroxybenzoylorientin, along with nine known triterpene acids and two flavonoids. Wound healing [19], antibacterial [20, 21] and larvicidal activity [22] have been reported earlier. Sridhar et al [23] studied anti-inflammatory of different solvent fractions of leaves. Yet, to support the traditional claims, pharmacological analysis of crude extract with different dosage is needed. Hence the present work is aimed to study the anti-inflammatory activity along with analgesic and antipyretic activity of crude total aqueous and methanolic extract of *Vitex altissima*.

MATERIALS AND METHODS

Plant material

The fresh leaves of *Vitex altissima* was collected from their natural habitat at Grizzled Giant Squirrel Wildlife Sanctuary, Virudhunagar district, Tamil Nadu, India in January 2012 and identified by referring the local flora [24]. The specimen (No: VHNSN 487/2012/TN) was deposited in the Department of Botany, V.H.N.Senthikumara Nadar College, Virdhunagar for future reference. The plant leaves were washed and rinsed with tap water to remove all the dirt and unwanted particles prior to the drying process. Then the plant parts were kept under shad and dried for 3-4 weeks at room temperature (27 ± 1° C). The leaves then pulverized into a coarse dry powder (<1 mm from our observation) with a mechanical grinder and passed through 60# sieve and stored in airtight container.

The dried powder material was defatted with petroleum ether (60-80°c) then successively extracted with methanol and double distilled water separately using Soxhlet extractor. The methanolic and aqueous extracts were dried under reduced pressure using a rotary vacuum evaporator. The aqueous extract was spray dried further to remove trace of solvent. The extracts were kept in refrigerator (4°C) for future use. Immediately before use, the extract was dissolved in normal saline at concentrations required to produce doses of 200 and 400 mg/kg and administered before subjecting animals to the respective assays.

Phytochemical screening

Freshly prepared *Vitex altissima* extracts were subjected to preliminary phytochemical screening tests for the detection of various phytoconstituents using conventional protocol [25, 26].

Pharmacological screening

Drugs

Chemicals used in the experiments all are analytical grade were purchased from Sigma-Aldrich, U.S.A. All preparations were freshly made in distilled water prior to the experiments.

Experimental animals

Animals

Healthy adult cross-bred *Wistar* albino rats (weighing 110-170 g) were used throughout the experiment. Animals were procured from the animal house of Sankaralingam Bhuvaneshwari College of Pharmacy, Sivakasi, Tamil Nadu, India. Albino rats of either sex was kept under standard environmental conditions (12:12 hour light/dark cycle at 25 ± 2ºC and relative humidity of 45-55%) in sanitized polypropylene cages. Standard animal feed and drinking water were provided *ad libitum* throughout the experimental period. The animals were acclimatized to laboratory conditions one week prior to the initiation of experimental work to minimize if any of non-specific stress. Institutional Animal Ethical Committee IAEC (SBCP/ 2012-2013/CPCSEA/IAEC-III/07) has approved the experimental protocol and care of animals was taken as per the guidelines of CPCSEA, Department of Animal Welfare, Government of India.

Acute toxicity study

Acute oral toxicity study was performed as per revised OECD (Organization for Economic Cooperation and Development) guidelines No. 425 [27]. *Wistar* rats (n = 3) of either sex selected by random sampling technique were used for acute toxicity study. The animals were kept fasting for overnight providing only water, after which a single high dose, 2000 mg/kg of methanolic and aqueous extracts of leaves of *Vitex altissima* suspended in normal saline was administered orally. After a single administration, a sign of toxicity and behavior was observed each hour up to the 24 h. If this higher dose caused mortality, then one lesser dose of that higher dose (1000, 800, 400 200, 100 or 50 mg/kg of b.w. consecutively) was administered to the next group of animals and observed for sign of toxicity and behavior. The same procedure was followed until to find out the highest non-lethal dose in which no mortality was observed.

Study of analgesic activity by tail immersion model

Tail immersion test allows the study of drugs with spinal analgesic activity by evaluating the time, in seconds (s), that the animal takes to remove the tail from the local impact of a painful thermal stimulus. The methanolic and aqueous extract of *Vitex altissima* was studied for analgesic activity by tail immersion test [28]. The *Wistar* albino rats (90-160 g) of either sex were divided into six groups containing four animals in each. The rats were fasted for 12 h prior to induction of analgesia. Group 1 was treated as negative control and received 10ml of normal saline. Group 2 served as positive control which received Tramadol 5 mg/kg/b.w. suspended in 1% DMSO which served as standard analgesic agent. Group 3 and 4 were treated with 200 mg/kg/ml (low dose) and 400 mg/kg/ml (high dose) of methanolic extract of *Vitex altissima* leaves suspended in DMSO respectively. The 5 and 6 groups were treated with 200 mg/kg/ml (low dose) and 400 mg/kg/ml (high dose) of aqueous extract of *Vitex altissima* leaves respectively. The animal was kept in vertical position to hang the tail. The distal part of the tails of the animals (3 cm) was immersed in hot water maintained at 55.0 ± 0.5 °C. Within a few minutes, the rats reacted by withdrawing the tail. The time in seconds to withdraw the tail out of water was taken as the reaction time (Ta). The reaction time was recorded by a stopwatch. After each determination the tail was carefully dried by a cloth. The first reading (0 min) was taken immediately after administration of the test drugs and subsequently taken at time 30, 60, 90 and 120 min. The first reading is discarded and the reaction time was taken as a mean of the next two readings. The cut-off time, i.e. time of no response was put upto 15 sec. [29] to prevent tissue damage, while Tb was consider the reaction time of control group. Percentage analgesic activity was calculated as per the formula shown below [30]:

$$\text{Percentage of analgesic activity} = \frac{Ta - Tb}{Tb} \times 100$$

Study of anti-inflammatory experiment by carrageenin-induced paw oedema model

The antiinflammatory activity the methanolic and aqueous extract of leaves of *Vitex altissima* was studied using carrageenin-induced paw oedema (acute inflammation) method in rats [31]. The animals were divided into six groups of four animals each and were fasted for a period of 24 hours prior to the study. Group 1 was treated as negative control and received 10ml of normal saline. Group 2 served as positive control which received NSAID indomethacin 10 mg/kg/ml suspended in 1% DMSO. Group 3 and 4 were treated with 200 mg/kg/ml (low dose) and 400 mg/kg/ml (high dose) of methanolic extract of *Vitex altissima* leaves suspended in DMSO respectively. The 5 and 6 groups were treated with 200 mg/kg/ml (low dose) and 400 mg/kg/ml (high dose) of aqueous extract of *Vitex altissima* leaves respectively. Thirty minutes later, 0.1ml of freshly prepared 1% carrageenin suspension (Phlogistic agent) in saline was injected into the sub-planter surface of right hind paw of each of the rats in all the groups under mild ether anaesthesia using a 25 G needle and 1 mL syringe. Measurement of paw size was done by wrapping a piece of cotton thread round the treated paw of each rat and measuring the linear circumference on a meter rule [32, 33]. Increase in the linear paw diameter was taken as an index of the paw volume which was measured on a linear scale immediately before injecting the carrageenan and at hourly intervals thereafter up to 4 h. The volume of oedema was expressed for each animal as the difference in the diameter of the rat paw before and after injection of the carrageenan. Percentage of inhibitory activity was calculated at 1, 2, 3 and 4 h after carrageenan treatment (representing the peaks of oedema size), using the following formula [34]:

$$\text{Percentage of inhibition} = \frac{\{(Ct\text{-}Co)\ \text{control} - (Ct\text{-}Co)\ \text{treated}\}}{(Ct\text{-}Co)\ \text{control}} \times 100$$

Where Ct is paw size after a specific time interval in hours after carrageenin injection and Co is paw size before carrageenin injection.

Study of antipyretic activity by brewer's yeast induced pyrexia model

The antipyretic activity of the methanolic and aqueous extract of leaves of *Vitex altissima* were evaluated using brewer's yeast-induced pyrexia in rats as described by Loux et al [35]. Rats were fasted overnight with water *ad libitum* before the experiments. Twenty-four rats were randomly divided into six groups (n=4). Group 1 was treated as positive control and received 10 ml/kg/b.w of normal saline. Group 2 served as negative control which received paracetamol 150 mg/kg/b.w suspended in 1% DMSO. Group 3 and 4 were treated with 200 mg/kg/ml (sub maximal dose) and 400 mg/kg/ml (maximal dose) of methanolic extract of *Vitex altissima* leaves suspended in DMSO respectively. The 5 and 6 groups were treated with 200 mg/kg/ml (submaximal dose) and 400 mg/kg/ml (maximal dose) of aqueous extract of *Vitex altissima* leaves respectively. The normal body temperature (pre-treatment temperature) of each rat was recorded before starting of the experiments. The fever was induced by administering 10 ml/kg of 20% w/v aqueous suspension of brewer's yeast in normal saline subcutaneously into the animal's dorsum region. All groups were fasted overnight but allowed free accesses to drinking water and after 24 h rectal temperature of each rat was recorded. The induction of pyrexia was confirmed by rise in temperature more than 0.5°C, while animals showed rise in temperature less than 0.5°C were excluded from experiment [36]. All the drugs were administered by orally. Rectal temperature was determined by digital thermometer (Model No: 461 R) at 1, 2, 3 and 4 hrs after test extract/reference drug administration [35].

The percent of reduction in pyrexia was calculated by the following formula.

$$\text{Percent of reduction} = \frac{B - C_n}{B - A} \times 100$$

Where, B represents temperature after pyrexia induction; Cn temperature after 1, 2, 3, 4 and 5 h and A, normal body temperature.

Statistical analysis

The data are expressed as the means ± S.E.M. and statistical significance was determined using one-way analysis of variance (ANOVA) followed by post hoc Dunnett's t-test for multiple comparisons. A probability level of less than 5% ($P < 0.05$) was considered significant.

RESULTS AND DISCUSSION

Phytochemical screening

The phytochemical screening of methanolic and aqueous extract of *Vitex altissima* demonstrated the presence of flavonoids, saponins, tannins, coumarin, terpenoids and alkaloids (Table 1), which are suggested to act synergistically to exert the observed pharmacological activity [37, 38]. The fact that strong synergism of several constituents in the crude drug may prove more potent and effective than any single purified compound, is always overlooked. Moreover, this may help to nullify the toxic effects (if any) of individual constituents.

Table1: Result of chemical group tests of the aqueous and methanol extract of *Vitex altissima.*

Extract	Flavonoids	Coumarin	Saponin	Alkaloids	Tannin	Terpenoids
AE	+	+	+	+	+	-
ME	+	+	-	+	-	+

AE: Aqueous extract; ME: Methanol extract

Acute toxicity study

In view of the increasing popular consumption and prolonged use of medicinal plants as alternative therapy, it is necessary to ensure that the plants are indeed safe for human consumption [39, 40]. The methanolic and aqueous extracts of *Vitex altissima* plant did not produce any significant toxic symptoms or mortality at single dose (2000 mg/kg b.w.) and hence the drug was considered broad nontoxic and safe for further pharmacological screening. So $1/10^{th}$ and $1/5^{th}$ (200mg and 400mg) of extracts were selected for experiments as sub maximal and maximal dose respectively.

Analgesic activity

The methanolic and aqueous extract showed significant and dosage-dependent analgesic activities from 30 minutes onwards. The aqueous extract (400 mg/kg/b.w.) showed significant activity than methanolic extract compared with control (Table 2). At 90 minutes aqueous extract (400 mg/kg/b.w.) showed higher percentage of inhibition compared to standard drug. The tail-immersion method is very effective for evaluating drugs possessing analgesic properties act via centrally mediated antinociceptive responses [41, 42, 43].

Thermal injuries precipitate an increase in vascular permeability, proteolysis, systemic inflammatory response and release of chemical mediators which are followed by persistent pain. It is known that several chemical mediators, i.e., bradykinin and prostaglandin, produce pain in thermal injury and that μ, δ, and κ opiod receptor agonists mediate potent antinociceptive activity in animals subjected to thermal injury [44]. The μ receptor has generally been regarded as the receptor type associated with pain relief and has been shown to be potent in regulating thermal pain [45]. Activation of μ opioid subtype leads to spinal analgesia and commonly through constipation adverse effect [46]. The probable antinociceptive action of *Vitex altissima* against thermal stimulus is via the opioid receptor. This finding was further supported by the claims made by Janssen et al [28] who stated that the nociception experience is short lasting and it is well accepted that agonist of μ-opioid receptors produced antinociception in acute pain model. Therefore, it is believed that substances that are effective in tail immersion test exert their effects predominantly through μ-opioid receptors. Because thermal nociceptive tests are more specific to opioid μ receptors but non-thermal tests are specific to opioid κ receptors [47].

In general, several mechanisms of action could be used to explain the observed antinociceptive activity of the aqueous and methanolic leaves extract of *Vitex altissima*. The ability to inhibit/reverse the centrally synthesized prostaglandins or COX (cyclooxygenase) [48] could be one of the possible mechanisms that contribute to the central antinociceptive activities of the aqueous and methanolic leaves extract of *Vitex altissima* seen in the present study. So it can be assumed that cyclooxygenase (COX) inhibitory activity of the aqueous and methanolic leaves extract of *Vitex altissima* may reduce the production of free arachidonic acid from phospholipid or may inhibit the enzyme system responsible for the synthesis of prostaglandins and ultimately relive pain-sensation.

Table 2: Effect of the various extracts of *Vitex altissima* leaves on tail immersion in 55±1°C hot water in rats.

Group	Dose (mg/kg)	Reaction time after drug Administration (Sec.)			
		15 minutes	30 minutes	60 minutes	90 minutes
Control (Saline)		2.00±0.00 (-)	2.00±0.41 (-)	1.75±0.25 (-)	2.00±0.00 (-)
Tramadol	5	1.75±0.25 (-)	2.75±0.48 (37.50)	4.25±0.25** (142.50)	4.50±0.29* (125.00)
Methanol extract	200	2.25±0.25 (12.50)	1.25±0.25 (-)	2.50±0.50 (42.86)	3.00±0.70 (50.0)
Methanol extract	400	2.25±0.25 (12.50)	1.50±0.29 (-)	2.50±0.50 (42.86)	3.00±0.41 (50.0)
Aqueous extract	200	3.75±0.25 (87.50)	2.50±0.29 (25.00)	2.75±0.75 (57.14)	3.75±0.48 (87.50)
Aqueous extract	400	1.50±0.28 (-)	1.50±0.29 (-)	2.00±0.41 (14.29)	4.00±0.71* (100.00)
One-way ANOVA	F	11.25	03.11	03.41	03.220
	P	P<0.01	P<0.05	P<0.05	P<0.05

n=6 in each group. Values Mean±S.E.M *p<0.05, ** p<0.01, Percentage of analgesic activity are noted in the parenthesis

Anti-inflammatory activity

Aqueous and methanolic extract of *Vitex altissima* produced significant and dosage-dependent activity from second hour onwards (Table 3). At 4^{th} hour aqueous extract (200 mg/kg/b.w.) produced significant results when compared to control. Considering about the percentage of inhibition, the aqueous extract (200 mg/kg/b.w.) at 4^{th} hour reduced the volume of the edema induced by the carrageenan (61.49%) comparable with standard drug indomethacin (64.93%).

According to Di Rosa et al [49] and Vinegar et al [50], the development of carrageenan-induced edema is biphasic. The early phase is predominately a non-phagocytic edema occurs within one hour of carrageenan administration and is attributed to the release of cytoplasmic enzymes, mediators such as histamine, serotonin (particularly 5-hydroxytryptamine (5-HT)) and platelet activating

factor (PAF) from the mast cell. The late phase (>1.0 h) is associated with the increased production of inducible cyclooxygenase (COX) and thereby increases the synthesis of prostaglandins mediated by bradykinin, leukotrienes and polymorphonuclear cells in the inflammatory area [51, 52, 53]. In this experiment, rats pre-treated with *Vitex altissima* leaves extract showed a significant oedema inhibitory response at 2 h following carrageenan injection. This result suggests that *Vitex altissima* extract may act by suppressing the later phase of the inflammatory process by the inhibition cyclooxygenase, the enzyme involved in the formation of prostaglandins or neutrophils mobilization [54]. Similarly inhibition of COX activity is supported based on claims that the carrageenan induced inflammation model is selective to COX-2 inhibitors [55, 56] and is more effectively controlled with arachidonate cyclooxygenase, but not arachidonate lipooxygenase, inhibitors [57, 58 59].

Table 3: Effect of the various extract of *Vitex altissima* leaves on carrageenan-induced paw edema in rats.

Group	Dose (mg/kg)	Paw volume measured in mm			
		1st hour	2nd hour	3rd hour	4th hour
Control (Saline)		1.35±0.186 (-)	4.55±0.117 (-)	6.67±0.319 (-)	6.70±0.211 (-)
Indomethacin	10	1.21±0.351 (10.37)	0.97±0.501** (78.68)	1.34±0.340** (79.91)	1.35±0.387** (64.93)
Methanol extract	200	1.14±0.075 (15.56)	4.41±0.118 (03.08)	6.32±0.220 (05.25)	4.83±1.390 (27.91)
Methanol extract	400	1.21±0.284 (10.37)	3.00±0.468 (34.07)	4.24±0.790* (36.43)	3.97±0.680 (40.75)
Aqueous extract	200	1.14±0.307 (15.56)	2.75±0.93 (39.56)	4.09±0.810* (38.68)	2.58±1.005* (61.49)
Aqueous extract	400	0.887±0.287 (34.30)	4.11±0.343 (09.67)	5.75±0.470 (13.79)	3.84±1.270 (42.69)
One-way ANOVA	F	00.337	07.414	13.12	03.863
	P	P=0.884	P<0.01	P<0.001	P<0.05

n = 4 in each group. Values Mean ± S.E.M *P<0.05 ** P<0.01, Percentage of reduction are noted in the parenthesis

Our preparation of *Vitex altissima* extract is differed somewhat from that of Sridhar *et al* [23]. They used fractioned methanolic extract in a single concentration for investigation and found that methanolic fraction did not possessed inhibitory activity against COX-1 and COX-2 enzymes. In our results, the crude total methanolic extract at higher concentration showed significant anti-inflammatory activity. These results are not reconcilable; it is possible that there are some processes of extraction that differ from those of Sridhar *et al* [23] and/or the active compound(s) contained in the fractionated methanolic extract tested *in vivo* is not high enough to show the activity and/ or some compound may counteract that of activity and some active compound(s) preferably contained in the crude methanolic extract.

Antipyretic study

The effects of methanolic and aqueous extract of leaves of plant *Vitex altissima* on brewer's yeast induced pyrexia in rats are depicted (Table 4). Both the methanolic and aqueous extract produced significant (P<0.05) and dosage dependent activity from second hour onwards. Methanolic extract at 200 mg/kg/b.w. and aqueous extract at 400 mg/kg/b.w. produced highly significant activity at second and third hour respectively. Aqueous extract at fourth hour produced higher percentage of inhibition (183.33%) than standard drug paracetamol (133.33%).

The yeast induced fever is a well-established model for assessing antipyretic effect and it has been used in a number of studies [60]. Yeast-induced pyrexia is called pathogenic fever and its etiology could be the production of prostaglandins [61] in central nervous system. Baker's yeast binds to an immunological protein called Lipopolysaccharide-Binding Protein (LBP). The binding results in the synthesis and release of various endogenous cytokine factors such as interleukin 1 (IL-1), interleukin 6 (IL-6) and the tumor necrosis factor-alpha which in turn activate the arachidonic acid pathway and ultimately results in the synthesis and release of prostaglandin E2 (PGE2) in the surroundings of the hypothalamic thermoregulator centers [62]. PGE2 is the ultimate mediator of the febrile response induced by the baker's yeast [48, 63, 64]. It slows the rate of firing of warm sensitive neurons and results in increased body temperature. The set-point temperature of the body will remain elevated until PGE2 is no longer present [65, 66]. It may therefore be plausible to conclude that inhibition/reversion of the synthesis of prostaglandins or COX is the possible mechanisms that contribute to antipyretic activities of the methanolic and aqueous extract of *Vitex altissima*.

Table 4: Effect of the various extracts of *Vitex altissima* leaves on brewer's yeast induced pyrexia in rats.

Group	Dose (mg/kg)	Pretemperature (°C)	Temp. after induced pyrexia (°C)	Temperature after drug administration (°C)			
				1st hour	2nd hour	3rd hour	4th hour
I	Saline (10ml/kg)	36.9±0.560	37.5±0.504	38.5±0.155 (-)	38.5±0.210 (-)	38.4±0.108 (-)	37.9±0.108 (-)
II	Paracetamol (150mg/kg)	37.0±0.488	37.6±0.500	37.3±0.504* (50.00)	37.3±0.345** (50.00)	37.3±0.500* (50.00)	36.8±0.308** (133.33)
III	Methanol (200mg/kg)	37.2±0.149	37.8±0.125	37.5±0.173 (50.00)	37.4±0.147** (66.67)	37.6±0.111 (33.33)	37.1±0.212* (116.67)
IV	Methanol (400mg/kg)	37.2±0.307	37.8±0.296	37.4±0.221* (66.67)	37.5±0.188* (50.00)	37.5±0.047 (50.00)	37.5±0.040 (50.00)
V	Aqueous (200mg/kg)	37.7±0.188	38.3±0.201	38.0±0.149 (83.83)	37.8±0.166 (83.83)	37.9±0.320 (66.67)	37.2±0.239 (183.33)
VI	Aqueous (400mg/kg)	36.9±0.187	37.5±0.240	37.5±0.240 (-)	37.5±0.143* (-)	36.9±0.249** (100.00)	37.3±0.201 (33.33)
One-way ANOVA			F	02.68	03.90	03.52	03.32
			P	P<0.10	P<0.05	P<0.05	P<0.05

n=6 in each group. Values mean ± S.E.M *P<0.05, ** P<0.01, Percentage of reduction are noted in the parenthesis

Flavonoids are predominant inhibitors of either cyclooxygenase or lipooxygenase [67]. Flavonoids are also known to have ability to target prostoglandins which are involved in the acute inflammation, pyrexia and pain perception [67, 68, 69] and/or inhibit the enzyme responsible for prostaglandin synthesis [70, 71]. Therefore, it is possible that the presence of flavonoids in the aqueous and methanolic extract of *Vitex altissima* may be responsible for the analgesic, anti-inflammatory and antipyretic effect of selected medicinal plants.

Generally, plants showing the antipyretic activity also possess analgesic and anti-inflammatory activity [72, 73]. In our studies, the plant extract shows significant antipyretic activity, it may be attributed by its analgesic and anti-inflammatory activity.

CONCLUSION

Therefore, the plant extract of *Vitex altissima* possesses a significant analgesic anti-inflammatory and antipyretic effect in rats. These results therefore support the traditional use of this plant in pain and related conditions. However, further studies are necessary to examine underlying mechanisms of antipyretic activities and to isolate the active compound (s) responsible for these pharmacological activities.

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