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Antipyretic, Analgesic and Anti-Inflammatory Activity of *Vitex castofolia* Leaf N-Butanol Fraction

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Abstract

The current study was thus aimed at evaluating antipyretic, analgesic, and anti-inflammatory activities of the leaf methanolic extract of N-Butanol fraction of *Vitex Castofolia.* Pyrexia, analgesia and inflammation are associated with several pathological conditions.To assess antipyretic activity in yeast induced hyperthermia. Analgesic profile was ascertained in acetic acid induced writhing, hot plate and tail immersion test. Nevertheless, the anti-inflammatory activity was tested in carrageenan induced paw edema and histamine induced inflammatory tests. Rats were used at test doses of 50, 100 and 200mg/kg body weight intra peritoneally (i.p).In yeast induced pyrexia, leaf buanol fraction of *vitex castofolia* demonstrated dose dependently (78.23%) protection at 200mg/kg, similar to standard drug, paracetamol (90%) at 100mg/kg i.p. showed a dose dependent analgesia in various pain models i. e. acetic acid, hot plate and tail immersion having 78.90%, 69.96% and 68.58% protection respectively at 200mg/kg. However, the analgesic action of fraction was completely antagonized by the injection of naloxone like opiate antagonists. Similarly carrageenan and histamine induces inflammation was significantly antagonized by fraction, 66.30% and 60.80% respectively at 200mg/kg.It is concluded that butanol fraction has marked antipyretic, analgesic and anti-inflammatory activities in various animal models and this strongly supports the ethnopharmacological uses of *Vitex castofolia* as antipyretic, analgesic and anti-inflammatory plant.

Keywords

Vitex castofolia, yeast, paracetamol, carrageenan, naloxone Antipyretic, Analgesic, Anti-inflammatory, buanol.

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INTRODUCTION

Pain as an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage.1The role of medicine is to protect and reestablish health and to ease distress. Knowledge of pain is vital to both these goals. Fever has been defined as the elevation of core body temperature above normal; in normal adults, the average oral temperature is 37°C (98.6°F)2. Although host defense mechanism gets

increased by fever, other aspects also warrant concern, such as the patient's comfort and physiologic responses. Drugs commonly used in modern medicine for suppression of pain and fever such as non-steroidal anti-inflammatory drugs and corticosteroids provide only symptomatic relief, and long-term use of the drugs is associated with serious adverse effects. Hence, the search for a new, safe analgesic and anti-inflammatory drug is always going on ability to inhibit the activity of the cyclooxygenase COX) enzyme, which in turn results in a diminished synthesis of pro inflammatory prostaglandins (Vane 1971). This action is considered not the sole but a major factor of the mode of action of NSAIDs. The pathway leading to the generation of prostaglandins has been elucidated in detail. Within this process, the COX enzyme. Traditional medicine and folk medicine have offered us with significant drugs in the therapy of various diseases and are more and more subjected to scientific research. One such herb extensively used in Unani, Ayurvedic, and Siddha systems for centuries for various indications including pain, inflammation, and fever.

Vitex Castofolia is an small tree growing from 2 to 8 m (6.6 to 26.2 ft) in height. The bark is blockish brown leaves are violet colour with five lanceolate leaflets, sometimes three. Each leaflet is around 4 to 10 cm (1.6 to 3.9 in) in length, with the central leaflet being the largest and possessing a stalk. The leaf edges are toothed or serrated, and the bottom surface is covered in hair. It is black or purple when ripe. The active constituents of the leaf juice are casticin, isoorientin, chrysophenol D, luteolin,para hydroxybenzoic acid and D-fructose. The main constituents of the oil are sabinene, linalol, terpinen-4-ol, beta-caryophyllene, α-guaiene constituting 61.8% of the oil. is an important medicinal plant and is used for treatment of a wide spectrum of health disorders in traditional and folk medicine; some of which have been experimentally validated. It is widely planted as a hedge plant along the roads. Traditionally it is reported to have multifarious activities such as antioxidant, insecticidal, antimicrobial, anticancer, tonic, febrifuge, expectorant and diuretic .3 The current study was designed to provide scientific evidence to the ethnobotanical uses of the plant in the treatment of pyrexia, analgesia and inflammation in various animal models.

METHODS

Chemicals

Paracetamol, Diclofenac sodium, Acetic acid, Brewer's yeast, Carrageenan (Sigma Lambda, USA), Histamine (Alfa Aesar - A Johnson Matthey Company), Naloxone (Acent Scientific Company), Tramadol[R] . Sterile normal saline was used in all experiments as control while butanol fraction was prepared in normal saline.

Animals

Rats either sex were used in all experiments the animals were maintained in standard laboratory conditions (25°C and light/dark cycles i.e., 12/12h) and were fed with standard food and water *ad libitum*. The experimental protocols were approved by the ethical committee of the Pharmacy Department.

Plant material

The fresh leaves of *Vitex Castofolia* were collected in November 2019, from forest area of adilabad, telanagan, India. The plant was identified authenticated by the Dr. K.Raju , professor, Department of Botany; Kakatiya university warangal collection number1005 . For further confirmation, the microscopic characters of this plant were studied and compared with available literature as mentioned above. The collected whole plant (12kg) was air dried and powder. The powder was extracted by maceration with methanol at room temperature for 14days with occasional shaking. The methanolic extract was filtered and concentrated under vacuum using rotary evaporator at low temperature (45°C). The methanolic extract was dissolved in distilled water and further fractionated with *n*-butanol.

Acute toxicity

The acute toxicity test was carried out for v.castofolia leaf butanol fraction to evaluate any possible toxicity. rats (n = 6) of either sex were treated with different doses (500, 1000 and 2000mg/kg, p.o.), while the control group received saline (10ml/kg). All the groups were observed for any gross effect for first 4h and then mortality was observed after 24h.

Antipyretic test

The antipyretic activity was evaluated for fraction of *Vitex Castofolia* using (150-250g) of either sex. The selected animals were healthy and were acclimatized to laboratory conditions before the start of experiment. The animals were divided into five groups each of six mice. The normal body temperature of each mouse was recorded using digital thermometer and then pyrexia was induced in all mice by injecting 20% aqueous suspension of Brewer's yeast (10ml/kgs.c.). All groups were fasted overnight but allowed free accesses to drinking water and after 24h rectal temperature of each mouse 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 experiment5. Group I received saline (10ml/kg) as a negative control,

Group II received paracetamol (150mg/kg) as a standard drug while the remaining groups III, IV and V received 50, 100 and 200mg/kg i.p. *Vitex Castofolia* leaf fractions, respectively. After drugs administration, rectal temperature was again recorded periodically at 1, 2, 3, 4 and 5h of drugs administration. The percent reduction in pyrexia was calculated by the following formula.

Percent reduction = B – C_n/B – A × 100

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

Analgesic activity (4)

Acetic acid induced writhing test.

Rats of either sex (n = 6) weighing 18–22g were used. All animals were withdrawn from food 2h before the start of experiment and were divided in five groups. Group I was injected with normal saline (10ml/kg) as control, Group II received standard drug diclofenac sodium (10mg/kg) while the remaining groups III, IV and V were injected with 50, 100 and 200mg/kg i.p. of *Vitex Castofolia* butanol fraction, respectively. After 30min of saline, diclofenac sodium and plant fraction injection, the animals were treated i.p. with 1% acetic acid. The number of abdominal constrictions (writhes) were counted after 5min of acetic acid injection for the period of 10 min.

Hot plat test

Rats of either sex (n = 6) weighing 18–22g were acclimatized to laboratory conditions one hour before the start of experiment with food and water available *ad libitum*. Animals were then subjected to pre-testing on hot plate (Havard apparatus) maintained at 55 ± 0.1°C. Animals having latency time greater than 15 s on hot plate during pre-testing were rejected (latency time) . All the animals were divided in eight groups each of six mice. Group I was treated with saline (10ml/kg), group II was treated with Tramadol^R (30mg/kg i.p). Group III, IV and V were treated with 50, 100 and 200mg/kg *Vitex Castofolia* leaf butanol fraction i.p. respectively. After 30min of treatment the animals were placed on hot plate and the latency time (time for which mouse remains on the hot plate (55 ± 0.1°C) without licking or flicking of hind limb or jumping) was measured in seconds. To prevent the tissue, damage a cut-off time of 30 s were imposed for all animals. To find out the opiod analgic mechanism in the analgesic activity of *Vitex Castofolia* leaf butanol fraction, Groups VI and VII were treated with naloxone (0.5mg/kg s.c.) and after 10min these groups were treated with *Vitex Castofolia* leaf butanol fraction (100 and 200mg/kg, i.p), while group VIII was treated with Tramadol^R (30mg/kg i.p.) after 10min of naloxone injection. The latency time for all groups was recorded at 0, 30, 60, 90 and 120min. Percent analgesia was calculated using the following formula: % Analgesia = (Test latency – control latency)/(Cut – off time – control latency) × 100

Tail immersion test

Animals of either sex were divided into five groups each of six animals (18–22g). Saline (10ml/kg), *Vitex Castofolia* leaf butanol fraction at the dose of 50, 100 and 200mg/kg, and Tramadol^R (30mg/kg) were administered intraperitoneally. The animal was kept in vertical position to hang the tail, which was up to 5cm into a pot of hot water maintained at 55 ± 0.5°C. The time in seconds to withdraw the tail out of water was taken as the reaction time (Ta). The reading was taken after 0, 30, 60, 90 and 120min of administration of the test drugs. The cut-off time, i. e. time of no response was put at 30s, while Tb was considering the reaction time for control group.

Percentage analgesic activity = Ta – Tb/Tb × 100

Anti-inflammatory activity4

Carrageen induced paw edema

The anti-inflammatory activity was performed on mice of either sex (25–30g). The animals were randomly divided in five groups each of six animals. Group I was treated with normal saline (10ml/kg), group II with diclofenac sodium (10mg/kg), rest of the groups were treated with VC leaf butanol fraction (50, 100, and 200mg/kg, i.p). After thirty minutes of the above intraperitoneal administration, carrageenan (1%, 0.05ml) was injected subcutaneously in the sub plantar tissue of the right hind paw of each mouse. The inflammation was measured using plethysmometer , immediately after injection of carrageenan and then 1,2,3,4 and 5h. The average foot swelling in drug treated animal as well as standard was compared with that of control and the percent inhibition (anti-inflammatory activity) of edema was determined using the formula.

Percent inhibition = A-B/A × 100, where A represent edema volume of control and B as paw edema of tested group.

Histamine induced paw edema

Animals were divided as in the previous experiment and inflammation was induced by subcutaneous injection of 0.1ml of freshly prepared solutions of histamine (1mg/ml) into the hind paws of the mice 6 . The percent inhibition of paw edema induced by each test sample was calculated as described in case the carrageenan induced paw test.

Phytochemical status

Preliminary phytochemical tests were performed for VC leaf butanol fraction . The presence of alkaloid content was determined by performing Mayer's test; white precipitate (ppt) indicated the presence of alkaloids7. Flavonoids were determined when

addition of few drops of sodium hydroxide solution, formed intense yellow coloration that became colorless after addition of dilute acetic acid. Saponins were identified by formation of froth upon simple shaking (frothing test). Tannins and phenols were identified on addition of ferric chloride to the extract solution; the appearance of blue or green ppt indicated the presence of tannins. Sterols and triterpenoids were identified on addition of few drops of acetic anhydride to the extract solution, boiled, cooled and then add concentrated sulphuric acid, producing brown ring at the junction of two layers, the turning of upper layer to green indicated sterols and deep red color indicated triterpenoids8.

Statistical analysis

The results obtained were expressed as mean ± SEM (Standard error of mean) of six animals. For statistical analysis, ANOVA was followed by post hoc Dunnett's test for multiple comparisons. Effects were significant at the $P < 0.05$ level.

RESULTS

Acute toxicity

Vitex Castofolia leaf butanol fraction was found safe at all test doses (500, 1000 and 2000mg/kgi.p.). During 24h assessment time, test animals were found normal.

Antipyretic test

The *Vitex Castofolia* leaf butanol fraction markedly ($P < 0.01$) attenuated hyperthermia induced by yeast. The inhibition was dose dependent and remained significant up to 3h of administration as shown in Table 1. The maximum antipyretic effect was observed at 200mg/kg i.e. 78.23% while, the antipyretic effect of paracetamol was 90%.

Table 1: Effect of *Vitex Castofolia* leaf butanol fraction at 50, 100 and 200mg/kg i.p. in yeast induced pyrexia

Treatment	Dose mg/k	Rectal temperature (°C)						
		After administration of drug						
		Normal (A)	after 24h (B	1h (C1)	2h (C2)	3h (C3)	4h (C4)	5h (C5)
Saline	10mL	36.66 ± 0.11	38.92 ± 0.34	38.82 ± 0.21	38.78 ± 0.11	38.68 ± 0.20	38. 68 ± 0.20	38.72 ± 0.15
Paracetamol	150mg	37.08 ± 0.08	39.44 ± 0.04	39.22** ± 0.1	37.10** ± 0.3	37.08** ± 0.2	37.05** ± 0.2	37.03** ± 0.4
VC leaf	100mg	37.80 ± 0.02	38.70 ± 0.4	38.4± 0.2	37.8* ± 0.2	37.4** ± 0.1	37.1** ± 0.1	37.2** ± 0.1
butanol fraction	200	37.00± 0.1	38.4 ± 0.2	38.30 ± 0.3	37.7* ± 0.1	37.4** ± 0.4	37.1** ± 0.1	37.0 0± 0.1

Data are reported as mean ± S.E.M. for group of six animals Antipyretic effect of *Vitex Castofolia* leaf butanol fraction presents the percent inhibition of pyrexia after 1,2,3,4 and 5h of the treatment with paracetamol (150mg/kg) and butanol fraction (100–200mg/kg). The data was analyzed by ANOVA followed by Dunnett's test. Asterisks indicated statistically significant values from control. *$P < 0.05$, **$P < 0.01$.

Analgesic activity

Acetic acid induced test

The results showed that the pain relief was achieved in a dose dependent manner, at all test doses (50, 100 and 200mg/kg i.p.) as shown in Table.2. Maximum inhibition (78.90%) was observed at 200mg/kg dose of VC leaf butanol fraction. The percent inhibition of writhing is effect of paracetamol (96.22%) was greater than that of the highest dose of *Vitex Castofolia* leaf butanol fraction.

Table.2: Effect of *Vitex Castofolia* leaf butanol fraction 50, 100 and 200mg/kg in acetic acid induced test

Treatment	Dose (mg/kg i.p.)	No. of writhing (10min)
Saline	10ml/kg	71.10 ± 0.01
	50	40.02 ± 0.5**
VC leaf butanol fraction	100	26.00 ± 0.1**
	200	20.00 ± 0.1**
Diclofenac	10	12.1 ± 0.3**

Values are reported as mean ± S.E.M. for group of six animals. The data was analyzed by ANOVA followed by Dunnett's test. Asterisks indicated statistically significant values from control. *$P < 0.05$, **$P < 0.01$.

Hot plat test

The results of the hot plat test revealed that the latency time was significantly ($P < 0.05$) increased from 17.22% to 68.58% at the dose of 50 to 200mg/kg. The effect was dose dependent, and the maximum effect was observed after 60min as shown in Table . The most significant ($P < 0.01$) increase in latency time noticed against 200mg/kg of *Vitex Castofolia* leaf butanol fraction was 68.58% whereas, the percent inhibition of the standard opioid analgesic (Tramadol^{R}) was 76.73% as shown in In the presence of naloxone, the analgesic effect of Tramadol^{R} (30mg/kg) and VC leaf butanol fraction (100 and 200mg/kg) was reversed profoundly.

Table.3: Effect of VC leaf butanol fraction 50, 100 and 200mg/kg in hot plat test

Group	Treatment /kg	0min	30min	60min	90min	120min
Saline	10ml	10.62 ± 0.2	10.22 ± 0.8	10.16 ± 0.9	10.20 ± 0.3	10.12 ± 0.01
Tramadol^{R}	30mg	9.10 ±0.5	24.30** ± 0.4	25.01*** ± 0.6	25.80*** ± 0.7	25.77*** ± 0.3
VC leaf	50mg	9.81 ±0.2	14.42 ± 0.4	15.20* ± 0.8	15.78* ± 0.4	15.5* ± 0.3
butanol	100mg	9.93 ±0.6	15.8 ± 0.7	15.51** ± 0.4	16.3** ± 0.8	16.09** ± 0.9
fraction	200mg	10.94 ±0.7	24.76* ± 0.2	25.04** ± 0.1	25.10** ± 0.1	25.98** ± 0.6
Analgesic effect of Tramadol^{R} and VC leaf butanol fraction antagonized by Naloxone						
VC leaf butanol fraction	100mg	10.55 ±0.05	11.78** ± 0.5	12.80** ± 0.3	13.91** ± 0.8	14.8** ± 0.7
	200mg	12.82 ±0.7	14.81** ± 0.8	15.7** ± 0.9	15.82** ± 0.7	15.86** ± 0.9
Tramadol^{R}	30mg	12.23 ±0.02	12.22** ± 0.05	15.02*** ± 0.9	15.24*** ± 0.03	15.05*** ± 0.9

Values are reported as mean ± S.E.M. for group of six animals. The data was analyzed by ANOVA followed by Dunnett's test. Asterisks indicated statistically significant values from control. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Tail immersion test

The analgesic effect of the VC leaf butanol fraction was also significant ($P < 0.05$) in tail immersion test and was dose dependent like hot plat test. The reaction time of all doses and Tramadol^{R} is given in Table4. The maximum analgesic effect was noticed at 60min after the dose administration. The percent inhibition of pain was 17.22, 22.29 and 68.58 at 50, 100 and 200mg/kg of VC leaf butanol fraction, respectively. Tramadol^{R} which is a centrally acting opioid analgesic showed marked activity (76.73%) as shown.

Table.4: Effect of *Vitex Castofolia* leaf butanol fraction 100, 200 and 300mg/kg in Tail immersion test

Group	Per Kg	0min	30min	60min	90min	120min
Saline	10ml	5.02 ± 0.2	5.23 ± 0.4	5.31 ± 0.4	5.48 ± 0.10	5.75 ± 0.2
VC leaf butanol fraction	50	6.01 ± 0.7	6.50 ± 0.2	5.99* ± 0.2	6.79 * ± 0.6	6.71* ± 0.2
	100	7.22 ± 0.2	7.58* ± 0.1	7.95** ± 0.2	7.91** ± 0.4	7.85 ** ± 0.9
	200	7.25 ± 0.6	8.02* ± 0.2	8.28** ± 0.7	8.49 ** ± 0.6	8.91** ± 0.7
Tramadol^{R}	30mg	3820 ± 0.1	8.60*** ± 0.3	8.85*** ± 0.3	8.79*** ± 0.8	8.71*** ± 0.3

Values are reported as mean ± S.E.M. for group of six animals. The data was analyzed by ANOVA followed by Dunnett's test. Asterisks indicated statistically significant values from control. *$P < 0.05$, **$P < 0.01$, ***P < 0.001.

Anti-inflammatory activity

Edema induced by Carrageenan.

The anti-inflammatory activity at test doses (50, 100 and 200mg/kg i.p.) of VC leaf butanol fraction is presented in Table.5 with the average volume of the paw edema. The percent protection of inflammation is presented. The injection of the carrageenan in paw created an inflammatory edema which increased gradually. The VC leaf butanol fraction at the dose of 200mg/kg exhibited an anti-inflammatory activity that became significant ($P < 0.01$) 2 h after the injection of carrageenan and was maintained all along the experiment with a maximum effect of 60.880%. The VC leaf butanol fraction (100 and 200mg/kg) induced significant ($P < 0.01$) anti-inflammatory effect and the anti-inflammatory effect of diclofenac sodium (10mg/kg) was greater than that of the extract as presented.

Table.5: Effect of intraperitoneal administration of VC leaf butanol fraction at 50, 100 and 200mg/kg in carrageenan and histamine induced paw edema test.

Treatment	Dose mg/kg	NPS	0h	1h	2h	3h	4h	5h
Saline	10ml	0.0940 ± 0.1	0.2861 ± 0.4	0.2080 ± 0.1	0.2090 ± 0.5	0.2140 ± 0.1	0.2040 ± 0.3	0.2000 ± 0.17
Diclofenac	10mg	0.0910 ± 0.2	0.2190 ± 0.3	0.1681* ± 0.2	0.980** ± 0.8	0.1099** ± 0.3	0.1620** ± 0.6	0.0801** ± 0.6
Anti-inflammatory effect against carrageenan induced paw edema								
VC leaf butanol fraction	50	0.0900 ± 0.2	0.2040 ± 0.2	0.2080 ± 0.9	0.1902 ± 0.16	0.1409* ± 0.3	0.1180 ± 0.1	0.1480 ± 0.2
	100	0.0910 ± 0.1	0.2090 ± 0.1	0.1023* ± 0.2	0.1300* ± 0.2	0.0918** ± 0.31	0.1105* ± 0.4	0.1210* ± 0.2
	200	0.099 ± 0.2	0.2110 ± 0.2	0.1001* ± 0.2	0.1227* ± 0.3	0.0299** ± 0.1	0.0910** ± 0.3	0.1001** ± 0.1
Anti-inflammatory effect against histamine induced paw edema								
	100	0.0980 ± 0.1	0.2090 ± 0.4	0.1688* ± 0.4	0.1420* ± 0.2	0.0999** ± 0.2	0.1001* ± 0.3	0.1209* ± 0.1

VC leaf butanol fraction	200	0.0947 ± 0.3	0.2192 ± 0.5	0.1085* ± 0.8	0.1055* ± 0.2	0.0710** ± 0.71	0.0805** ± 0.3	0.1013** ± 0.2

NPS (Normal Paw size). Values are reported as mean ± S.E.M. for group of six animals. The data was analyzed by ANOVA followed by Dunnett's test. Asterisks indicated statistically significant values from control. *$P < 0.05$, **$P < 0.01$.

Phytochemical test

The VC leaf butanol fraction was found to contain alkaloids, saponins, flavonoids, tannins, proteins, and phenolic compounds.

DISCUSSION

Results of the present study showed that the VC leaf butanol fraction has marked antipyretic, analgesic and anti-inflammatory effects with a reasonable safety profile. Subcutaneous injection of Brewer's yeast induces pyrexia by increasing the synthesis of prostaglandin. It is considered as a useful test for the screening of plants materials as well as synthetic drugs for their antipyretic effect 9. Yeast-induced pyrexia is called pathogenic fever and its etiology could be the production of prostaglandins10. The inhibition of prostaglandin synthesis could be the possible mechanism of antipyretic action as that of paracetamol and the inhibition of prostaglandin can be achieved by blocking the cyclo-oxygenase enzyme activity. There are several mediators for pyrexia and the inhibition of these mediators are responsible for the antipyretic effect11. The intraperitoneal administration of VC leaf butanol fraction significantly attenuated rectal temperature of yeast induced febrile mice. Thus, it can be postulated that VC leaf butanol fraction contained pharmacologically active principle(s) that interfere with the release of prostaglandins. Furthermore, the presence of salicylic acids in the other species of the genus *Vitex12* and the antipyretic action of the *n-butanol* fraction of VC leaf butanol fraction supplement the antipyretic activity of our tested fraction. Acetic acid-induced writhing is a well recommended protocol in evaluating medicinal agents for their analgesic property. The pain induction caused by liberating endogenous substances as well as some other pain mediators such as arachidonic acid via cyclooxygenase, and prostaglandin biosynthesis 13. This pain paradigm is widely used for the assessment of peripheral analgesic activity due to its sensitivity and response to the compounds at a dose which is not effective in other methods. The local peritoneal receptor could be the cause of abdominal writhings14. Pain sensation in acetic acid induced writhing paradigm is elicited by producing localized inflammatory response due to release of free arachidonic acid from tissue phospholipids via cyclo-oxygenase (COX), and producing prostaglandin specifically PGE2 and PGF2α, the level of lipoxygenase products may also increase in peritoneal fluids. These prostaglandin and lipoxygenase products cause inflammation and pain by increasing capillary permeability. The substance inhibiting the writings will have analgesic effect preferably by inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition. Regarding the results of our extract in acetic acid-induced abdominal constriction assay, a prominent inhibition of writhing reflux was observed. These findings strongly recommend that VBME has peripheral analgesic activity, and their mechanisms of action may be mediated through inhibition of local peritoneal receptors which may be the involvement of cyclooxygenase inhibition potential. The profound analgesic activity of VC leaf butanol fraction may be due to the interference of their active principle(s) with the release of pain mediators. Thermal nociception models such as hot plat and the tail immersion tests were used to evaluate central analgesic activity. VC leaf butanol fraction showed significant ($P < 0.01$) analgesic effect in both the hot plate and tail immersion tests, implicating both spinal and supraspinal analgesic pathways. In these pain paradigms Tramadol^{R}, which is similar to the action of opioid agonists (e.g. morphine), raised the pain threshold level within 30min of administration. In contrast, VC leaf butanol fraction showed maximum analgesic effect after 60min of administration. This difference in the maximum analgesic point could be explained by difference in the metabolic rate of each drug or may be the potency of each drug as the analgesic potential of Tramadol^{R} is higher than VC leaf butanol fraction (200mg/kg). Moreover, VC leaf butanol fraction showed a maximum effect after 60min and remain up to 120min in both thermal tests. When the nonselective opioid receptor antagonist naloxone was applied, the analgesic effect of VC leaf butanol fraction was also antagonized by naloxone after 30min of administration; it means that the analgesic effect of this extract is due to activation of the opioid receptor stimulation. Carrageenan-induced paw edema is a well-established animal model to assess the anti-inflammatory effect of natural products as well as synthetic chemical compounds. Edema formation due to carrageenan in paw is a biphasic event, during 1–5h; the initial phase (1h or 1.5h) is predominately a non-phagocytic edema followed by a second phase (2–5) h with increased edema

formation that remained up to 5h 15. The initial phase has been induced due to the action of mediators such as histamine, serotonin and bradykinin on vascular permeability 16. The late phase or second phase edema has been shown to be the result of overproduction of prostaglandins 17. The result of pre-treatment of VC leaf butanol fraction demonstrated that the extract (100 and 200mg/kg i.p.) is effective in the early phase of inflammation which is due to release of histamine and serotonin primarily. The anti-inflammatory effect of the extract remains significant up to 5th h of the experiment. VC leaf butanol fraction showed significant activity against histamine induce edema in both phases. During preliminary phytochemical screening of the crude extract, important therapeutic principles like alkaloids, saponins, flavonoids, tannins etc. were detected. Therefore, the current findings can be attributed to these groups of chemical compounds. Further study is need on VC leaf butanol fraction to find the exact mechanism of action for its antipyretic, analgesic and anti-inflammatory effects.

CONCLUSION

In conclusion, our current findings demonstrated scientific rationale for the folk use of the plant as antipyretic, analgesic and anti-inflammatory. Interestingly the VC leaf butanol fraction exhibited both peripheral as well as central analgesic effect which might have been attributed to the presence of such active principles, due to which it has proven folk use in various nervous disorders. Nevertheless, the isolation of pure secondary metabolites from the plant will help us further in understanding the mechanism of these activities and identification of lead compounds of clinical utility.

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