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Analgesic and anti-inflammatory properties of the fruits of *Vernonia anthelmintica* (L) Willd.

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ABSTRACT

Objective: To evaluation of analgesic and anti-inflammatory properties of the fruits of *Vernonia anthelmintica* (L) Willd. (*V. anthelmintica*).

Method: Hot plate method in mice, acetic acid induced writhing response in mice, tail immersion test and carrageenan-induced paw edema in rats and cotton pellet induced granuloma in rats method were used for screening analgesic and anti-inflammatory properties of the fruit of *V. anthelmintica* (family: Asteraceae).

Results: The result of the study showed that the ethanolic extract of *V. anthelmintica* (100 and 200 mg/kg body weight, *p.o.*) fruits possessed peripheral and central analgesic activity in animal model. The *V. anthelmintica* fruits extract showed *in vivo* anti-inflammatory activity on acute and chronic anti-inflammatory activity models in rats.

Conclusions: On the basis of result it can be concluded that saponins, steroids, tannins and flavonoids are the major constituents that are present in the fruits of *V. anthelmintica* which may be responsible for its analgesic and anti-inflammatory activity.

1. Introduction

Inflammation or phlogosis is pathological response of living tissue to injuries that leads to the local accumulation of plasmatic fluid and blood cells. Although it is defense mechanism, the complex events and mediators involved in inflammatory reaction can induce, maintain or aggravate many diseases[1]. It is a complex phenomenon, comprising of biochemical as well as immunological factors. Inflammation is recognized by following symptoms, rubor (redness), tumor (swelling), calor (heat), dolor (pain), functio laesa (loss of functions)[2]. In this study an attempt has been made to screen analgesic and anti-inflammatory properties of the fruit of *Vernonia anthelmintica* (L) Willd. (*V. anthelmintica*).

V. anthelmintica is found throughout India up to an

altitude of about 1500 m, and is more common in waste places near habitations. It is an annual herbaceous plant. Stems and leaves are covered with minute hairs. As the scientific name of the plant indicates, it is a valuable medicine as anthelmintic. It is useful in thread worm infections. The seeds are black of a bitter and nausea taste. The seed contains about 20%–30% oil of which more than 70%–74% is epoxy acid or vernolic acid. *V. anthelmintica* fruits are boiled with black pepper and garlic and its decoction is given in diarrhoea. Infusion of the plant is given in stomach worms (round and tape worms). *V. anthelmintica* seeds are used as anthelmintic for the treatment of gastrointestinal trichostrongylids in small ruminants[3], and antifilarial activity of *Centratherum anthelminticum* seed extracts on *Setaria cervi* has been reported by Singhal *et al*[4]. The present study was undertaken to scientifically investigate its therapeutic utility as analgesic and anti-inflammatory activity. The study was thus initiated with the aim of evaluating the effect of ethanolic extract of *V. anthelmintica* seeds and its fractions as analgesic and anti-inflammatory agent in treatment of severe pain management in arthritis, gout and headache.

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2. Materials and methods

2.1. Plant material

The dried fruits of *V. anthelmintica* (L.) Willd. was collected during the month of August from Erode District, Tamilnadu, India. The plant was identified and authenticated by Prof. R. Duraisami, Department of Pharmacognosy, Nandha College of Pharmacy and Research Institute, Erode. The fruits were shade dried, pulverized by a mechanical grinder and stored in a well-closed container for further extraction.

2.2. Preparation of extract

The dried powdered fruits material was extracted with ethanol in a Soxhlet extraction apparatus. The solvent was removed under reduced pressure and semi solid mass was obtained.

2.3. Phytochemical analysis

Ethanolic extract of *V. anthelmintica* (L.) Willd. were subjected to qualitative phytochemical tests for different constituents such as alkaloids, carbohydrates, glycosides, flavonoids, phenolic compounds, tannins, free amino acids, saponins, steroids and triterpenoids.

2.4. Drugs and chemicals

The chemicals used in the present study were carrageenan (S. D. Fine Chemicals Limited, Bombay, India), indomethacin (IPCA, Bombay, India), pentazocine (Neon labs, Bombay, India), naproxen (Ranbaxy, Gurgaon, India).

2.5. Animals

Male Swiss albino mice weighing 20–25 g and male Wister rats weighing 150–200 g were used for this study. The animals were obtained from animal house, IRT Perundurai Medical College, Erode, Tamilnadu, India. On arrival, the animals were placed randomly and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of (24±2) °C and relative humidity of 30%–70%. A 12:12 light: day cycle was followed. All the animals were allowed to free access to water and fed with standard commercial pelleted chaw (M/s. Hindustan Lever Ltd., Mumbai, India). All the experimental procedures and protocols used in this study were reviewed by institutional animal ethics committee (688/2/C–CPCSEA) and were in accordance with the guidelines of the Institutional Animal Ethics Committee.

2.6. Analgesic activity

2.6.1. Hot plate method in mice

The hot plate assay method was employed for the purpose of preferential assessment of possible centrally mediated analgesic effects of ethanolic extract of *V. anthelmintica* (L.) Willd[5]. The central analgesic drug, pentazocine, was used

for positive control group. In this experiment, four groups ($n=6$) of Swiss albino mice (20–25 g) were placed on a hot plate maintained at room temperature for 15 min. Food was withdrawn on the preceding night of the experiment. Group 1–normal control received 0.5% CMC (*p.o.*), and Group 2 received pentazocine (30 mg/kg, *i.p.*), whereas Groups 3 and 4 animals received ethanolic extract *V. anthelmintica* (L.) Willd. (100 and 200 mg/kg, *p.o.* respectively). Each animal was then individually placed gently on Eddy's hot plate at 55 °C. Latency to exhibit nociceptive responses such as licking paws or jumping off the hot plate, were determined 15, 30, 45, 60, and 90 min after administration of the test drug or vehicle.

2.6.2. Acetic acid induced writhing response in mice

This method was used to preferentially evaluate possible peripheral effects of ethanolic extract of *V. anthelmintica* (L.) Willd. as analgesic substance[6]. Groups of four Swiss albino male mice ($n=6$) were fasted overnight prior to the start of the experiment, with free access to water. The peripheral analgesic drug, indomethacin (5 mg/kg), was used as a positive control. Group 1–normal control received 0.5% CMC (*p.o.*), and Group 2 received indomethacin (5 mg/kg, *p.o.*), whereas Groups 3 and 4 animals received ethanolic extract of *V. anthelmintica* (L.) Willd. at doses of 100 and 200 mg/kg (*p.o.*) to mice. Thirty minutes after treatment, the mice were injected intra peritoneally with 0.1 mL of 1% acetic acid solution to induce the characteristic writhing 5 min after acetic acid administration. The mice were then placed in an observation box, and the number of writhing were counted in a 5 min period. The response of the extract and indomethacin treated groups were compared with those of animals in the control group.

2.6.3. Tail immersion test

Tail immersion test was used to assess the analgesic activity of *V. anthelmintica*[7,8]. In this method six rats per group were used. This involved immersing the extreme 3 cm of the rat's tail in a water bath containing water at a temperature of (55.0±0.5) °C. Within a few minutes, the rat reacted by withdrawing the tail. The reaction time was noted on a stop-watch. Each animal served as its own control and two readings were obtained for the control at 0 and 10 min interval. The average of the two values was the initial reaction time. The test groups were given ethanolic extract of *V. anthelmintica* (L.) Willd (100 mg/kg and 200 mg/kg, *p.o.*). Pentazocine (25 mg/kg, *p.o.*) were given for standard group, and 0.5% CMC for control group (10 mL/kg, *p.o.*). The reaction time of the test groups was taken at 0.5, 1.0, 2.0, 3.0 and 4.0 h after a latency period of 30 min following the administration of the tests substances. The cut off time, *i.e.* time of no response was put at 120 seconds. The reaction time was measured and calculated as described above for the hot plate test.

2.7. Anti-inflammatory activity

2.7.1. Carrageenan-induced paw edema in rats

Carrageenan-induced paw edema was used[9]. For

this experiment, the male rats (120–150 g) were divided into four groups ($n=6$). The first group received 0.5% CMC (10 mL/kg *p.o.*), while the second group received indomethacin (8 mg/kg *p.o.*). The third and the fourth groups were treated with the ethanol extract of *V. anthelmintica* (L.) Willd (100 and 200 mg/kg *p.o.*, respectively). Acute inflammation was produced by the sub plantar administration of 0.1 mL of 1% carrageenan (in 1% CMC w/v) in the right hind paw of the rats. The paw thickness was measured at 0 min, 30 min, 60 min, 120 min and 240 min after carrageenan injection by using vernier calipers (Sauravkumar G, Rajesh K, Seenngottuvele S, Chiranjeev G, Hareesh D 2012). The animals were pretreated with the drug an hour before the administration of carrageenan.

2.7.2. Cotton pellet induced granuloma in rats

Cotton pellets, weighing 5 mg each were sterilized. Under ether anesthesia, the pellets were introduced subcutaneously through a skin incision on the back of the animals[9]. Starting from 30 min after the implantation of cotton pellet for all the rats, 0.5% CMC to the normal control group, 25 mg/kg of naproxen to the positive control group and 100, 200 mg/kg of the ethanol extract of *V. anthelmintica* (L.) Willd to the test groups were administered daily for 5 d (*p.o.*). On the fifth day, the animals were sacrificed with chloroform, the granulomas were removed and the weights were determined.

2.8. Statistical analysis

The results and data obtained in this study were evaluated using the One-way analysis of variance (ANOVA) test between two mean groups: control and test groups, followed by dunnet's test. Significant levels were at $P<0.05$ and $P<0.01$ (95% confident limits).

3. Results

Ethanol extract of *V. anthelmintica* (L.) Willd. were subjected to qualitative phytochemical tests for different constituents and the results are shown in Table 1. Results of hot plate method in Swiss albino mice are illustrated in Table 2. Ethanol extract of *V. anthelmintica* (L.) Willd. showed significant analgesic activity at 100 and 200 mg/kg, *p.o.* dose. Analgesic activity was comparable with the standard drug pentazocine. Among the two doses 200 mg/kg showed the maximum analgesic activity at reaction time 90 min which was slightly lower than the standard drug pentazocine. In this analgesic testing model, pentazocine significantly prolonged the reaction time of animals with relatively extended duration of stimulation, confirming centrally mediated activity. Thermic painful stimuli is known to be selective to centrally active drugs. In the present study, all the extracts showed significant ($P<0.05$ and $P<0.01$) analgesic activity but among the two doses, 200 mg/kg showed highest analgesic activity at reaction time 90 min.

Table 1

Phytoconstituent present in different extracts of *V. anthelmintica*.

| Phytochemicals | Tests | Presence or absence |
|----------------|-----------------------|---------------------|
| Alkaloids | Dragendroff's test | + |
| | Mayer's test | – |
| | Carbohydrate | |
| | Molisch's test | + |
| | Fehling's test | + |
| Proteins | Biuret test | – |
| | Millon's test | – |
| Saponins | Foam test | + |
| Glycosides | Keller–killiani test | + |
| Flavonoids | Lead acetate test | + |
| | Sodium hydroxide test | + |
| Tannins | Ferric chloride test | + |
| | Lead acetate test | + |

–: Absence; +: Presence.

Table 2

Analgesic effect of ethanolic extract of *V. anthelmintica* (100 and 200 mg/kg), and pentazocine (30 mg/kg) on heat stimulating response in the hot plate test in Swiss albino male mice.

| Treatment group | Dose (mg/kg) | Reaction time (min) | | | | |
|-----------------|--------------|---------------------|-----------|-----------|-----------|------------|
| | | 15 | 30 | 45 | 60 | 90 |
| Control | 10 | 4.0±0.3 | 4.0±0.4 | 4.1±0.2 | 3.9±0.2 | 4.2±0.2 |
| Pentazocine | 30 | 5.6±0.5** | 6.5±0.3** | 7.3±0.7** | 8.6±0.7** | 10.6±0.2** |
| VA–I | 100 | 3.4±0.4 | 3.8±0.4 | 4.1±0.2 | 4.6±0.8* | 5.3±0.4* |
| VA–II | 200 | 5.6±0.4* | 6.0±0.3** | 6.7±0.2** | 7.6±0.3** | 9.1±1.7** |

The data represent the mean±SEM ($n=6$). *: $P<0.05$, **: $P<0.01$, compared to corresponding control. Control: 0.5% CMC (*p.o.*); Standard drug: Pentazocine (*i.p.*); VA: Ethanolic extract of *V. anthelmintica* Willd (*p.o.*).

The extracts (100 and 200 mg/kg, *p.o.*) significantly reduced pain induced by acetic acid and tail immersion writhing responses (Tables 3 and 4). The numbers of writhing episode in treated mice decreased significantly ($P<0.05$ and $P<0.01$) and were comparable to indomethacin. Among the two doses 200 mg/kg showed slightly lower analgesic activity than standard drug indomethacin. It was observed that the onset of writhing was delayed and duration of writhing was shortened. There was a significant reduction of painful sensation due to tail immersion in warm water, after a latency period of 0.5 h following oral administration of the plant extract at the dose of 200 mg/kg. The maximum inhibitory effect of *V. anthelmintica* (L.) Willd. was significant ($P<0.05$ and $P<0.01$) at 1 h post dose in 200 mg/kg. The maximum antinociceptive properties of the plant extract were not as effective as that of pentazocine.

The anti-inflammatory activity of *V. anthelmintica* against acute paw oedema (induced by carrageenin) are shown in Table 5 and the results were comparable to that of indomethacin, a prototype of nonsteroidal anti-inflammatory agent. The ethanol extract showed maximum inhibition of 37.58% at the dose of 200 mg/kg body weight after 30 min of drug treatment in carrageenin induced paw edema (Table 5) where as the standard drug produced 49.35% of inhibition. The effect of the extract on granuloma pouch in rats is shown in Table 6. The ethanol extract of *V. anthelmintica* significantly inhibited granuloma formation in rats ($P<0.01$) in a dose dependant

manner. The ethanol extract produced the maximum inhibition of 68.3% at the dose of 200 mg/kg body weight when compared with that of the control group.

Table 3

Analgesic effect of ethanolic extract of *V. anthelmintica* Willd. (100 and 200 mg/kg) and indomethacin (5 mg/kg) on acetic acid induced writhing test in Swiss albino male mice.

| Treatment group | Dose (mg/kg) | Total number of writhes | Inhibition (%) |
|-----------------|--------------|-------------------------|----------------|
| Control | 10 | 68.50±0.50 | – |
| Indomethacin | 5 | 16.00±0.50** | 76.65 |
| VA–I | 100 | 36.66±0.50* | 46.48 |
| VA–II | 200 | 25.33±0.70** | 63.03 |

The data represent the mean±SEM ($n=6$). *: $P<0.05$, **: $P<0.01$, compared to corresponding control. Control: 0.5% CMC ($p.o.$); Standard drug: Indomethacin ($p.o.$); VA: Ethanolic extract of *V. anthelmintica* Willd ($p.o.$).

Table 4

Analgesic effect of ethanolic extract of *V. anthelmintica* (L.) Willd. (100 and 200 mg/kg), and pentazocine (25 mg/kg) on tail immersion method in rats.

| Treatment group | Dose (mg/kg) | 0 h | 0.5 h | 1 h | 2 h | 3 h | 4 h |
|-----------------|--------------|-----------|-------------|-------------|-------------|-------------|-------------|
| Control | 10 | 2.20±0.23 | 2.50±0.23 | 3.00±0.21 | 2.30±0.17 | 2.00±0.18 | 2.40±0.18 |
| Pentazocine | 25 | 3.00±0.24 | 3.61±0.36** | 4.90±0.31** | 4.11±0.14** | 3.67±0.19** | 3.42±0.46** |
| VA–I | 100 | 2.33±0.23 | 2.70±0.38 | 3.16±0.21 | 2.70±0.12 | 2.22±0.20* | 1.93±0.16* |
| VA–II | 200 | 2.66±0.22 | 3.20±0.41* | 4.46±0.19* | 3.85±0.12* | 3.47±0.22* | 2.84±0.12* |

The data represent the mean±SEM ($n=6$). *: $P<0.05$, **: $P<0.01$, compared to corresponding control. Control: 0.5% CMC ($p.o.$); Standard drug: Pentazocine ($i.p.$); VA: Ethanolic extract of *V. anthelmintica* Willd ($p.o.$).

Table 5

Anti inflammatory activity of ethanolic extract of *V. anthelmintica* (L.) Willd (100 and 200 g/kg) and indomethacin (8 mg/kg) on carrageenan induced paw edema method in Wister rats.

| Treatment Group | Dose (mg/kg) | Paw thickness (mm) | | | | | Inhibition (%) |
|-----------------|--------------|--------------------|-------------|-------------|-------------|-------------|----------------|
| | | 0 min | 30 min | 60 min | 120 min | 240min | |
| Control | 10 mL/kg | 4.83±0.06 | 5.89±0.03 | 6.85±0.02 | 7.78±0.05 | 8.63±0.01 | – |
| Indomethacin | 8 | 4.27±0.06** | 3.51±0.01** | 3.27±0.05** | 3.15±0.04** | 3.01±0.02** | 49.35 |
| VA–I | 100 | 4.48±0.12 | 4.59±0.09** | 4.51±0.04** | 4.37±0.01** | 4.21±0.18** | 34.78 |
| VA–II | 200 | 4.62±0.03 | 4.41±0.04** | 4.29±0.06** | 3.96±0.10** | 3.93±0.10** | 37.58 |

The data represent the mean±SEM ($n=6$). *: $P<0.05$, **: $P<0.01$, compared to corresponding control. Control: 0.5% CMC ($p.o.$); Standard drug: Indomethacin ($p.o.$); VA: Ethanolic extract of *V. anthelmintica* Willd ($p.o.$).

frequently associated with pain and involves several events, such as the increase of muscular permeability, increase of granulocytes and mono nuclear cells migration, as well as the granulomatus tissue proliferation[10]. Pain is subjective experience, which is difficult to define exactly eventhough we all experience it. Pain is distinguished as two types, peripheral or neurogenic pain may involve the following pathological states: peripheral nociceptive afferent neurons which are activated by noxious stimuli and central mechanism which is activated by afferent inputs pain sensation[6]. The hot plate test was selected to investigate central antinociceptive activity because it had several advantages particularly the sensitivity to strong antinociceptive and limited tissue damage. Prostaglandins and bradykinins were suggested to play an important role in pain. Phenolic compounds are reported to inhibit prostaglandin synthesis[11]. A number of flavonoids and tannins have been reported to produce analgesic activity[12]. Other studies have demonstrated that various flavonoids such as rutin, quercetin, luteolin, biflavonoids and triterpenoids produced significant antinociceptive[13], as

Table 6

Anti inflammatory effect of *V. anthelmintica* (L.) Willd ($p.o.$) ethanol extract (100 and 200 mg/kg), and naproxen (25 mg/kg) on cotton pellet–induced granuloma in rats.

| Treatment group | Dose (mg/kg) | Weight of cotton pellet (mg) | Inhibition (%) |
|-----------------|--------------|------------------------------|----------------|
| Control | 10 mL/kg | 130.7±2.6 | – |
| Naproxen | 25 | 31.8±0.2** | 6.0 |
| VA–I | 100 | 55.2±0.5** | 57.4 |
| VA–II | 200 | 41.4±0.5** | 68.3 |

The data represent the mean±SEM ($n=6$). *: $P<0.05$, **: $P<0.01$, compared to corresponding control. Control: 0.5% CMC ($p.o.$); Standard drug: Naproxen; VA: Ethanolic extract of *V. anthelmintica* Willd ($p.o.$).

4. Discussion

The inflammation is a complex process, which is

phytochemical tests showed presence of flavonoids and/or tannins in ethanolic extract of *V. anthelmintica* (L.) Willd, they might suppress the formation of prostaglandin and bradykinins[12].

Acetic acid is known to trigger the production of noxious substances within the peritoneum, which induces the writhing response[14]. The effect of the extracts against the noxious stimulus may be an indication that it depressed the production of irritants and thereby reduction in number of writhes in the animals. The writhing induced by chemical substances is due to sensitization of nociceptors by prostaglandins[15]. The abdominal constriction response induced by acetic acid is a sensitive procedure to establish peripherally acting anti nociceptives. This response is thought to involve local peritoneal receptors[16]. This result indicates that the analgesic effect of ethanolic extract of *V. anthelmintica* (L.) Willd might be mediated by inhibiting the synthesis or action of prostaglandins.

The centrally acting analgesic activity of the extract was also corroborated in our study by the tail immersion test results. The fact that in thermal stimuli (hot plate and tail

immersion tests); the anti nociceptive effect should be shown by acting centrally on opioid receptors. Since the drug had shown the analgesic activity in tail immersion test, it seems that the ethanolic extract can act centrally. Taking this in to consideration the ethanolic extract of *V. anthelmintica* (L.) Willd possesses peripheral and central analgesic properties. Obtained results showed that ethanolic extract of *V. anthelmintica* (L.) Willd has anti-inflammatory activity on an acute inflammatory process like in carrageenan induced paw edema in rat paws. It is well known that leukocytes migration to the injured tissues is an important aspect of the inflammatory process. Histamine and serotonin are responsible for the immediate inflammation response, whereas Kinins and prostaglandins mediate prolonged response. Anti-inflammatory activity of many plants has been attributed to their high sterol/triterpene or flavonoids contents^[13]. The anti-inflammatory effect of ethanolic extract of *V. anthelmintica* (L.) Willd in rats with carrageenan-induced paw was significant^[9]. It is known that the inflammatory granuloma is a typical response of a chronic inflammatory process and it has been established that the dry weight of the pellets is well correlated with the granulomatous tissue. The chronic inflammation occurs by means of the development of proliferative cells. These cells can be either spread or in granuloma form. The *V. anthelmintica* (L.) Willd. extract showed significant anti-inflammatory activity in cotton pellet induced granuloma and thus found to be effective in chronic inflammatory conditions. It reflected its efficacy in inhibiting the increase in the number of fibroblasts and synthesis of collagen and mucopolysaccharide during granuloma tissue formation^[17].

It is concluded that the ethanolic extracts of aerial parts of *V. anthelmintica* (L.) Willd. have central and peripheral antinociceptive effects. Opioid receptors and the inhibition of cyclo-oxygenase enzyme may mediate these activities. The extracts also have activity against acute and especially chronic inflammation. The antinociceptive and anti-inflammatory effects of the extracts may be due to their content of flavonoids, tannins and sterol/triterpene. However, the chemical constituents responsible for the pharmacological activities remain to be investigated.

Conflict of interest statement

We declare that we have no conflict of interest.

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