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# Evaluation of antiinflammatory and analgesic activities of ethanolic extract of roots *Adhatoda vasica* Linn

*[1]Wahid A Mulla, [1]Suyog D More, [2]Suraj B Jamge, [1]Ajinkya M Pawar, [3]Mukhtar S Kazi, [1]Madhukar R Varde

[1]Government College of Pharmacy, Vidyanagar, Karad 415 124, Maharashtra, India.
[2]MGM College of Agricultural Biotechnology, Aurangabad - 431 003,India.
[3]Santkrupa College of Pharmacy, Ghogaon, 415111, Maharashtra, India.

*Corres. author: wahidmulla@gmail.com
Tel: +919970171304

**Abstract:** The study was designed to evaluate the anti-inflammatory and analgesic activities of ethanolic extract of roots of *Adhatoda vasica* Linn. (Acanthaceae). The anti-inflammatory potential of ethanolic extract has been determined by using carrageenan-induced paw edema assay, formalin-induced paw edema assay in albino rats. The analgesic activity was tested by using acetic acid-induced writhing response, hot plate method and tail flick method in albino rats. The administration of extract at doses of 200 and 400 mg/kg, by oral administration, significantly ($P < 0.05–0.01$) inhibited both carrageenan- and formalin-induced inflammation. Also, the acute treatment of extract produced a significant ($P < 0.05–0.01$) antinociceptive effect in the acetic acid-induced writhing, formalin-induced pain licking and hot-plate-induced pain. The experimental data demonstrated that ethanolic extract of roots of *Adhatoda vasica* Linn. possess remarkable anti-inflammatory and analgesic activities.
**Keywords:** Acanthaceae, *Adhatoda vasica* Linn., Analgesic, Anti-inflammatory.

## INTRODUCTION

Many Indian ethno botanic traditions propose a rich repertory of medicinal plants used by the population for the treatment, management and/or control of different types of pain.[1] However, there were not enough scientific investigations on the anti-inflammatory and analgesic activities conferred to these plants. One of such plant from Indian flora is *Adhatoda vasica* Linn. is an indigenous herb belonging to family Acanthaceae. The plant has been used in the indigenous system of medicine in worldwide as herbal remedy for treating cold, cough, whooping cough, chronic bronchitis, asthma, as sedative expectorant, antispasmodic, anthelmintic, rheumatism and rheumatic painful inflammatory swellings. The drug is employed in different forms such as fresh juice, decoction, infusion and powder; also given as alcoholic extract and liquid extract or syrup. The leaf juice is stated to cure diarrhoea, dysentery and glandular tumor and the plant is an emmenagogue. The powder is reported to be used as poultice on rheumatic joints as counter-irritant on inflammatory swelling, on fresh wounds, urticaria and in neuralgia.[2] Roots of *Adhatoda vasica* Linn. are used in formulation of Maharasnadhi Quathar, a polyherbal preparation recommended by Ayurvedic medical practitioners for treatment of arthritic conditions.[3] *Adhatoda vasica* Linn. has antioxidant, hepatoprotective, sedative expectorant, antispasmodic and anthelmintic properties.[4] It is an official drug and is mentioned in the Pharmacopoeia of India (1966).[5] This plant contains alkaloids, tannins, flavonoids, terpenes, sugars, and glucosides.[6] Since no scientific data are available to justify the traditional anti-inflammatory and analgesic potentials of the plant, the present study was planned to validate the therapeutic use of this plant in treatment of inflammatory conditions.

## MATERIAL AND METHODS

**Chemicals.** All reagents and chemicals of analytical grade and were purchased from Loba Chemical Ltd, Mumbai unless otherwise designated.

**Experimental animals.** Wistar albino rats weighing 175-225 g of either sex were obtained from Krishna Institute of Medical Sciences, Karad, Dist- Satara (Maharashtra), India and were acclimatized for 10 days under standard housing conditions (24° ±1°C; 45-55% RH with 12:12 h light/dark cycle). The animals had free access to rat food (Hindustan Lever Ltd., Mumbai, India) and water. The animals were habituated to laboratory conditions for 48 h prior to experimental protocol to minimize any nonspecific stress. The experimental protocol was approved by the Institutional Animal Ethics Committee of Government College of Pharmacy, Karad, (MS), India and animals were maintained under standard conditions in the animal house approved by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA).

**Plant material.** Roots of *Adhatoda vasica* Linn. collected from different places at Karad were authenticated at Botany Dept., Yashwantrao Chavan College of Science, Karad, (MS), India. A voucher specimen was deposited at the Institute's Herbarium.

**Preparation of ethanolic extract.** The ethanolic extract of leaves of *A. vasica* Linn. was prepared by soxhletion. The powdered plant material (250 g) was repeatedly extracted in a 1000 ml round bottomed flask with 500 ml ethanol (95 %). The reflux time for each solvent was 40 cycles for complete extraction. The extracts were cooled at room temperature, filtered and evaporated to dryness under reduced pressure in a rotary evaporator and kept under refrigeration at – 4°C till further use. The percentage yield was found to be 4.75 with respect to the initial dried plant material. The ethanolic extract of *A. vasica* Linn. was referred as EEAV.

### Anti-inflammatory activity

***Carrageenan-induced paw edema model***

Carrageenan-induced hind paw edema model was used for determination of anti-inflammatory activity. 120 min after the oral administration of a EEAV (200 and 400 mg/kg) or dosing vehicle, each rat was injected with freshly prepared suspension of carrageenan (0.5 mg/25 µL) in physiological saline (154 nM NaCl) into subplantar tissue of the right hind paw.[7] The control group received 25 µL saline solutions. Indomethacin (10 mg/kg) was used as the reference drug. The paw volume was measured plethysmometrically (Ugo Basile, Italy) at 0 and 3 h after the carrageenan injection. The difference between the two readings was taken as the volume of edema, and the percentage anti-inflammatory activity was calculated using following equation:

Percentage anti-inflammatory activity = $(V - Vi/Vi) \times 100$, where $V$ is the paw volume 3 h after the carrageenan injection and $Vi$ is the initial paw volume.

*Formalin-induced paw edema assay:* The same procedure as mentioned above in carrageenan-induced paw edema assay was followed except that acute inflammation was produced by administration of 20 µl formalin in physiological saline (154 nM NaCl) into the subplanter area of right hind paw of rat.[8]

### Analgesic activity

***Writhing test:*** Writhing was induced in rat (n = 6) by intraperitoneal injection (10 ml/kg) of 0.6% acetic acid. The number of writhings was counted over a 20 min period as previously reported.[9] Animals were treated through oral route 30 min before injection of acetic acid with EEAV (200 and 400 mg/kg) or acetylsalicilic acid (200 mg/kg). The control group received only vehicle (3 ml/kg) of 1% suspension of Tween-80.

***Tail immersion test****:* Six rats were administered orally with vehicle (3 ml/kg), pentazocine (30 mg/kg), and EEAV (200 and 400 mg/kg). The distal part of the tails of the animals was immersed in hot water maintained at 55.0 ± 1.0 °C. The time taken to withdraw the tail was noted as reaction time.[10] A cut-off time of 10 s was maintained at 55 °C to prevent tissue damage. The reaction time was measured at 0, 15, 30, 45, and 60 min after treatment, respectively.

***Hot plate test:*** Rats were placed on an aluminum hot plate kept at 55 ± 0.5 °C for a maximum time of 30 sec.[11] Reaction time was recorded when the animals licked their fore- and hind paws and jumped; at before (0) and 15, 30, 45, and 60 min after intraperitoneal administration of EEAV (200 and 400 mg/kg) to different groups of six animals each. Morphine 10 mg/kg was used as the reference drugs.

### Statistical analysis

The statistical significance was assessed using one way of variance (ANOVA) followed by Bonferrini's multiple comparison tests. The values are expressed as mean ± SE and $P < 0.05$ was considered significant.

## RESULTS

### Anti-inflammatory activity

The ethanolic extract of *A. vasica* (200 and 400 mg/kg) and Indomethacin (10 mg/kg) produced dose-dependent inhibition of carrageenan and formalin-induced paw edema as compared to the control ($P < 0.05$) [Table 1].

**Analgesic activity**

Oral administration of the ethanolic extract of *A. vasica* (200 and 400 mg/kg) significantly ($P < 0.05$) reduced the number of writhings induced by acetic acid in rat [Table 2]. The activity was comparable to that of acetylsalicilic acid (200 mg/kg, p.o.) used as a reference drug. Moreover, the extract induced protection in rat in tail immersion test [Table 3] that is comparable with the standard drug pentazocine (30 mg/kg, p.o.). The results of hot plate test presented in Table 4 showed that the i.p. administration of ethanolic extract of *A. vasica* at the doses of 200–400 mg/kg and Morphine (10 mg/kg) a reference drug significantly raised the pain threshold at observation time of 45 min in comparison with control ($P < 0.001$).

**Table 1: Effects of ethanolic extract of roots of *A. vasica* Linn. (200 mg/kg, 400 mg/kg) and Indomethacin (10 mg/kg) on carrageenan-induced rat paw edema and formalin-induced paw edema.**

| Group | Carrageenan-induced rat paw edema | | Formalin-induced rat paw edema | |
|---|---|---|---|---|
| | Increase in paw volume (mean ± SEM) in ml | % inhibition of paw edema | Increase in paw volume (mean ± SEM) in ml | % inhibition of paw edema |
| Control | 0.56 ± 0.11 | - | 0.52 ± 0.11 | - |
| EEAV (200 mg/kg ) | 0.30 ± 0.05* | 46.42 | 0.26 ± 0.05* | 50.1 |
| EEAV (400 mg/kg ) | 0.22 ± 0.04* | 60.71 | 0.17 ± 0.03* | 67.3 |
| Indomethacin (10 mg/kg) | 0.15 ± 0.03* | 73.2 | 0.12 ± 0.01** | 76.92 |

*$P < 0.05$, **$P < 0.01$ vs. control.
[Values are mean ± SE from 6 animals in each group.]

**Table 2: Effect of the ethanolic extract of roots of *A. vasica* Linn. (200 and 400 mg/kg) on acetic acid induced writhing in rat.**

| Group | Dose (mg/kg) | No. of writhings | Inhibition (%) |
|---|---|---|---|
| Control | -- | 46.2 ± 1.2 | |
| Acetylsalicilic acid | 200 | 10.8 ± 2.1* | 76.62 |
| EEAV | 200 | 20.5 ± 1.2** | 55.62 |
| | 400 | 12.5 ± 1.7* | 73.59 |

*$P < 0.05$, **$P < 0.01$ vs. control.
[Values are mean ± SE from 6 animals in each group.]

**Table 3: Effect of ethanolic extract of roots of *A. vasica* Linn. (200 and 400 mg/kg) on tail immersion method in rat.**

| Treatment | Dose (mg/kg) | Average tail withdrawing time (s) | | | | |
|---|---|---|---|---|---|---|
| | | 0 min | 15 min | 30 min | 45 min | 60 min |
| Control | -- | 3.93 ± 0.11 | 3.95 ± 0.18 | 3.97 ± 0.17 | 3.97 ± 0.15 | 3.98 ± 0.18 |
| Pentazocine | 30 | 3.92 ± 0.30* | 4.09 ± 0.21* | 5.31 ± 0.41* | 6.54 ± 0.22* | 7.10 ± 0.17* |
| EEAV | 200 | 3.94 ± 0.21* | 4.13 ± 0.15** | 5.17 ± 0.14* | 5.15 ± 0.15** | 6.27 ± 0.12** |
| | 400 | 3.91 ± 0.16* | 4.14 ± 0.21* | 5.82 ± 0.28** | 6.25 ± 0.22* | 7.18 ± 0.17** |

*$P < 0.05$, **$P < 0.01$ vs. control.
[Values are mean ± SE from 6 animals in each group.]

**Table 4: Effect of ethanolic extract of roots of *A. vasica* Linn. (200 and 400 mg/kg) on rat submitted to the hot plate test.**

| Treatment | Dose (mg/kg) | Time 0 (min) | Time 15 (min) | Time 30 (min) | Time 45 (min) | Time 60 (min) |
|---|---|---|---|---|---|---|
| Control | – | 6.52 ± 0.25 | 6.22 ± 0.21 | 6.38 ± 0.31 | 7.25 ± 0.38 | 6.16 ± 0.54 |
| Morphine | 10 | 6.56± 0.23* | 11.45 ± 0.38* | 14.65 ± 0.36** | 14.79 ± 0.81** | 15.45 ± 0.56** |
| EEAV | 200 | 6.45 ± 0.27** | 9.16 ± 0.50* | 10.25 ± 0.41* | 11.17 ± 0.35** | 12.16 ± 0.50* |
| | 400 | 6.23 ± 0.48** | 10.10 ± 0.31** | 12.56 ± 0.23* | 12.86 ± 0.72** | 13.67 ± 0.18** |

$^{*}P < 0.05$, $^{**}P < 0.01$ vs. control.
[Values are mean ± SE from 6 animals in each group.]

## DISCUSSION

Carrageenan-induced edema is a biphasic response in which the involvement of the cyclo-oxygenase products of arachidonic acid metabolism and the production of reactive oxygen species are well established.[12] The first phase is mediated through the release of histamine, serotonin, and kinins, whereas the second phase is related to the release of prostaglandin oxygen-derived free radicals and production of inducible cyclo-oxygenase which peak at 3 h.[31] The EEAV extract produced dose-dependent and significant inhibition of carrageenan-induced paw edema comparable in magnitude with the inhibitory action of diclofenac. The formalin-induced paw edema assay defines distinctive biphasic nociceptive response termed neurogenic and inflammatory phases.[13]The ability of EEAV to have effect on both phases shows that it contains active anti-inflammatory principle acting both centrally and peripherally.

The ethanolic extract of *A. vasica* Linn. at the doses of 200–400 mg/kg protected rat against both chemical- and thermal-induced noxious stimuli, which were evidenced from the acetic acid-induced writhing, tail immersion, and hot plate tests. Acetic acid induces writhing syndromes and causes analgesia by releasing of endogenous substances, which then excite the pain nerve endings; the abdominal constriction is related to the sensitization of nociceptive receptors to prostaglandins.[14] Hot plate test was also assayed to characterize the analgesic activity of extract. It is possible that ethanol extract of *A. vasica* Linn. exerts an analgesic effect probably by inhibiting the synthesis of prostaglandins. Variation in order of activity for ethanol extract of AI fractions in acetic acid-induced writhing, tail immersion, and hot plate tests indicated that the different constituents present in different fractions may be responsible for central and peripheral analgesia.

Recent studies suggest that the inflammatory tissue damages are due to the liberation of reactive oxygen species from phagocytes invading the inflammation sites.[15-17] In addition to this, nitric oxide is also implicated in inflammation, cancer, and other pathological conditions.[18] Interactions between superoxide and nitric oxide regulate the vascular tone or inflammation.[19]

*A.vasica* Linn. contains alkaloids, tannins, flavonoids, terpenes, sugars, and glucosides. Flavonoids have been shown to possess various biological properties related to antioxidant, antinociceptive, and anti-inflammatory mechanisms by targeting reactive oxygen species and prostaglandins which are involved in the late phase of acute inflammation and pain perception.[20-22] It can be concluded that ethanolic extract of *A.vasica* possess anti-inflammatory and antinociceptive activities may be due to the presence of flavonoids and other polyphenolic moieties present in it, which seems to support the use of this plant in traditional medicine.

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