

ANTINOCICEPTIVE AND ANTI-INFLAMMATORY ACTIVITY OF ALKALOID FRACTION OF *TRICHOPUS ZEYLANICUS* GAERTN.

SAMBATH KUMAR R^{1*}, PERUMAL. P², BACHHAV R.SHANKAR.³

J. K. K. Nataraja College of pharmacy, Komarapalayam, 638183, Namakkal, Tamilnadu, India. ^{1,2} Department of Pharmaceutical Chemistry,³Department of Pharmacology. Email: sambathkumar121@rediffmail.com

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ABSTRACT

The whole plant material of *Trichopus zeylanicus* is defatted and successively extracted with methanol. The alkaloid fraction of *Trichopus zeylanicus* (AFTZ) was obtained from methanolic extract. Up to the dose of 2000 mg/kg b. w. per orally AFTZ did not show any mortality or toxicity. Diclofenac sodium (20 mg/kg p.o.) and Pentazocine (10mg/kg i. p.) was used as reference standard for antinociceptive and anti-inflammatory activity. The AFTZ at a dose of 75,150 and 300 mg/kg p.o. in acetic acid induced writhing and in hot plate analgesic method showed significant (***) dose dependent inhibition of writhing and elevated mean basal reaction time in hot plate method respectively. In carrageenan induced rat paw edema and cotton pellet induced granuloma method the AFTZ at a dose of 75,150 and 300 mg/kg p.o. showed significant (***) decrease in paw edema volume and weight of granulomatous tissue respectively. AFTZ showed antinociception in acetic acid induced writhing method may be by inhibiting peripheral pain receptor present on cell lining of peritoneal cavity. In hot plate method, the activity of AFTZ may be by involvement of opioid receptor. The carrageenan induced inflammation, AFTZ possibly act by inhibiting release and/or action of histamine, serotonin, kinin and prostaglandin like substances. The decrease in weight of granuloma of tissue may be due to both, the ability of AFTZ reducing the number of fibroblast and synthesis of collagen and mucopolysaccharide.

Keywords: *Trichopus zeylanicus*, Anti-inflammatory, Antinociceptive, pentazocine

INTRODUCTION

Trichopus zeylanicus Gaertn is a perennial herb, belongs to the family Trichopodaceae popularly known as "Arogyapacha" or "Arokyapachilai" in Malayalam literally meaning "the green that gives strength". The plant is found in the Agasthyar hilly forest of Kerala. This plant is used as health tonic. The Kani tribes are using this plant for increasing the stamina (Pushpangadan et al., 1988). *Trichopus zeylanicus* have shown various pharmacological activities. Moreover, this plant increases the resistance of rodent against a variety of stress and also shows antiulcer activity. ¹ The methanol extract of *T. zeylanicus* showed Hepatoprotective activity² and stimulates the male sexual behaviour in mice.³ The plant also possesses immunomodulatory activity⁴ This plant also possessed antifatigue, antioxidant and adaptogenic properties.^{5,6} The leaves of *Trichopus zeylanicus* is used by Kannikars for scabies and ring worm infection.⁷ On the basis of traditional use and literatures, the aim of the present study was to evaluate the antinociceptive and anti-inflammatory activity of Alkaloidal fractions of *Trichopus zeylanicus*.

MATERIALS AND METHODS

Plant Material

The plant *Trichopus zeylanicus* Gaertn, (Family: Trichopodaceae) subspecies travancoricus was collected from Agasthyar hills of Kerala in the month of September 2008, and authenticated by taxonomist of NIPER nursery, Mohali, Chandigarh, India. A voucher specimen No.NIP-159 has been deposited at that institute.

Extraction and Phytochemical screening

The whole plant material of *Trichopus zeylanicus* (3.0 Kg) was dried. It was ground to coarse powder and defatted with pet ether then it successively extracted with 90% methanol at room temperature for three times. The methanol extract was concentrated by using rotary evaporator. The 350 gm of methanol extract was further analyzed by various phytochemical tests for carbohydrates, proteins, saponin, glycosides, alkaloids, flavonoids, steroids and triterpenoids.⁸ The methanol extract shows positive test for saponins, alkaloids, flavonoids, steroids, triterpenoids, carbohydrates and proteins. The (110 gm) methanolic extract was then moistened with 25% ammonia solution and allow to stand overnight then the extract was concentrated on rotary evaporator.

Method for Isolation of Alkaloid fraction

The brownish solid mass (100 gm) of crude methanol extract was dissolved in 10% acetic acid (500ml). After shaking and filtering, the acidic solution was washed with chloroform (500ml), its pH adjusted to 9 by addition of 25% ammonia, and rewashed with chloroform (1.2 l). The latter extract was dried over anhydrous disodium sulphate (Na₂SO₄) and after removal of the solvent crude alkaloid fraction (10gm, 1% w/w).⁹

Drugs

Diclofenac Sodium (Research Lab, Mumbai), pentazocine (Fortwin, Ranbaxy), carrageenan (Sigma, Mumbai), were used in the study. Alkaloidal fraction of *Trichopus zeylanicus* (AFTZ) (75,150 and 300, mg/kg), carrageenan and diclofenac sodium (20 mg/kg, p.o.) were prepared in 1% carboxy methyl cellulose suspension before oral administration (p. o), pentazocine (10 mg/kg) was mixed in saline before intraperitoneal administration (i. p)

Animals

Male Swiss albino mice weighing 25-75 gm were selected for the study of anxiolytic and antidepressant activity. Adult Albino rats (Wistar strain) of either sex weighing between 120-200 gm were used for study. The animals were obtained from the Department of Pharmacology, J.K.K Nataraja College of pharmacy, Komarapalayam. The animals were housed in well ventilated colony cages in the departmental animal house at (25±2° C, 12:12 hr L: D (Light and Dark) cycle). The animals were fed with standard rodent pellet diet and water ad libitum. All the experimental procedure and protocols used in the study were approved by IAEC of J.K.K Nataraja College of Pharmacy, Komarapalayam, and as per CPCSEA guidelines.

Acute toxicity

Acute toxicity assay was performed as per OECD guidelines 423. Six female wistar albino rats (three animals in each step) were randomly selected. The animals were kept fasting for overnight providing only water. The test drug was administered orally at one dose level of 2000 mg/kg b. w. after that rats were observed continuously for the first 4 hours and then periodically upto 24 hr for toxic symptoms and mortality.¹⁰

Analgesic Activity

Acetic Acid Induced Writhing Method.

In this method, mice were divided in five groups six each. The animals were pretreated with drugs 45 min before induction of writhings. The animals received standard drug diclofenac sodium (20mg/ kg, p.o.) which serve as reference standard. Analgesic activity of reference standard and AFTZ of (75,150,300mg/ kg, p.o.) was assessed by counting the number of writhes induced by 0.6% acetic acid (10 ml /kg i. p.).¹¹ The number of writhes per animal was counted for next 20 min.^{12, 13} Percentage protection against abdominal constriction was taken as index of analgesia.

It is calculated as:

$$\frac{\text{No of writhing in control group} - \text{No of writhing in treated group}}{\text{No of writhing in control group}} \times 100$$

Hot Plate Method

In this method, mice were divided in five groups six each. The animals were pretreated with drugs 60 min before experimentation. The animals received standard drug Pentazocine (10 mg/ kg, i. p.) which serve as reference standard. Analgesic activity of reference standard and AFTZ (75,150,300 mg/kg, p.o.) were assessed by placing the animal on a hot plate maintained at a temperature of 55 ± 0.5 °C. The latency to flick the paw or lick or jump from the hot plate was the reaction time.^{14,15} The reaction time was noted at 0, 60, 120, and 240 min. The cut off time was considered as 15 sec.

Anti-inflammatory Activity

Carrageenan-induced paw edema

In this method, rats were divided in five groups of six each. The animals were pretreated with drugs 60min before carrageenan (0.1ml of 1%) injection. Carrageenan was injected into the sub plantar tissue of left hind paw of each rat.¹⁶ Swellings of carrageenan-injected foot were measured at 1, 2, 3, and 4 hours using Plethysmometer (UGO Basile 7140, Italy). The right hind paw was injected with 0.1 ml of vehicle. The animals were received the standard drug diclofenac sodium (20 mg/ kg, p.o.) served as a reference standard. Percentage decrease in paw edema is calculated as:

$$\frac{\text{Increase in paw edema of control group} - \text{Increase in paw edema of treated group}}{\text{Increase in paw edema of control group}} \times 100$$

Cotton pellet-induced granuloma

The effect of AFTZ on the chronic or proliferative phases of inflammation was assessed in the cotton pellet induced granuloma model. The rats were divided in to five groups of six each. The rats were anaesthetized with ether, and then incision was made under lumbar region. By a blunted forceps, sterilized cotton pellet (100 ± 1 mg) was implanted on each side of the abdomen. They were administered the vehicle, AFTZ and diclofenac sodium once daily for seven consecutive day from the day of cotton pellet insertion. On the 8th day, the animals were euthanized with ether. Each implanted cotton pellet was removed with surrounding fibrovascular tissues and dried at 60 °C for 24 hours.¹⁷ Afterwards the dried weight was measured and the result was expressed as the difference between initial implanted cotton pellet weight and final dry mass of cotton pellet and fibrovascular tissue.

$$\frac{\text{Increase in granuloma wt. of control group} - \text{Increase in granuloma wt. of treated group}}{\text{Increase in granuloma wt. of control group}} \times 100$$

Statistical Analysis

Results were expressed as mean \pm SEM. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Dunnett's test.*P<0.05 was considered statistically significant.

RESULTS

Acute toxicity

The AFTZ did not show any mortality and toxicity up to dose of 2000 mg/kg b. w., p. o. in female rat of any group of animal.

Acetic Acid Induced Writhing Method

The AFTZ (75, 150 and 300, mg/ kg, p.o.) significantly (P<0.001***) reduced the number of writhing induced by acetic acid, which is compared to the control group. Maximum percentage of inhibition of writhing response shown by AF of *T. zeylanicus* (300 mg/ kg) was 43.42%, which was comparable to Diclofenac sodium (20 mg /kg).

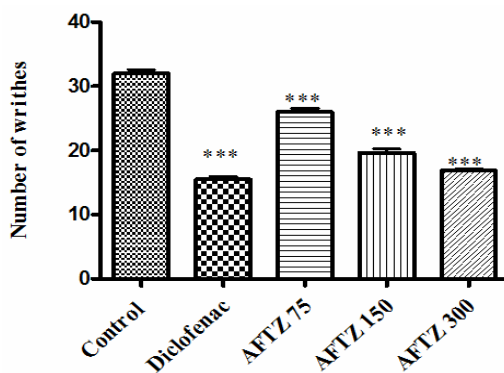


Fig. 1: Effect of AFTZ (75, 150, 300, mg /kg) on Acetic acid induced writhing method in mice.

Values are mean \pm SEM (n=6). *p< 0.05, **p<0.01, ***p<0.001 as compared to control (ANOVA followed by Dunnett's test). AFTZ = Alkaloidal Fraction *Trichopus zeylanicus*

Hot Plate Method

The AFTZ (75, 150, and 300 mg /kg, p.o.) showed significantly (P<0.001***) elevated the mean basal reaction time in hot plate method as compared to control.

The highest nociception inhibition of stimulus exhibited by AFTZ (300 mg /kg) was observed at 120 min.

Carrageenan Induced Rat Paw Edema

The AFTZ 75, 150, and 300 mg/ kg, p.o.) significantly (P<0.001***) inhibited carrageenan induced rat paw edema. Maximum inhibition of paw edema was observed in AFTZ (300 mg/ kg) and percentage inhibition 67.39% at 4 h when compared to the control group. Diclofenac sodium as a reference standard showed percentage inhibition of paw edema 79.34% at 4hr.

Cotton pellet induced granuloma

In the method of chronic inflammation using cotton pellet induced granuloma. AFTZ showed (75,150 and 300 mg /kg) significantly (P<0.001***) inhibited the formation of granulomatous tissue in a

dose dependent manner. In this model AFTZ possessed inhibition rate 56.81% at the dose of 300 mg /kg and diclofenac sodium inhibition rate 63.63% at the dose of 20 mg /kg as compared to control group.

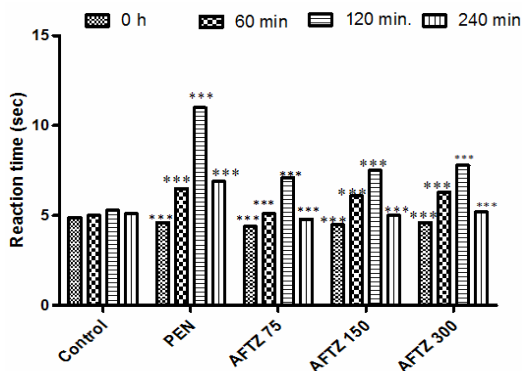


Fig. 2: Effect of AFTZ (75, 150, 300, mg /kg) on Hot plate method in mice.

Values are mean ± SEM (n=6). *p< 0.05, **p<0.01, ***p<0.001 as compared to control (ANOVA followed by Dunnett’s test). AFTZ = Alkaloidal Fraction *Trichopus zeylanicus*.

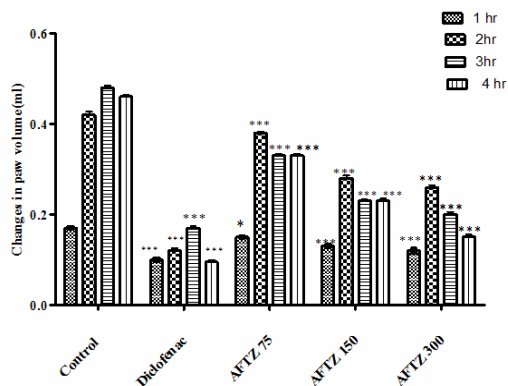


Fig. 3: Effect of AFTZ (75, 150, 300, mg/kg) on Carrageenan Induced Rat Paw Edema .

Values are mean ± SEM (n=6). *p< 0.05, **p<0.01, ***p<0.001 as compared to control (ANOVA followed by Dunnett’s test). AFTZ = Alkaloidal Fraction *Trichopus zeylanicus*

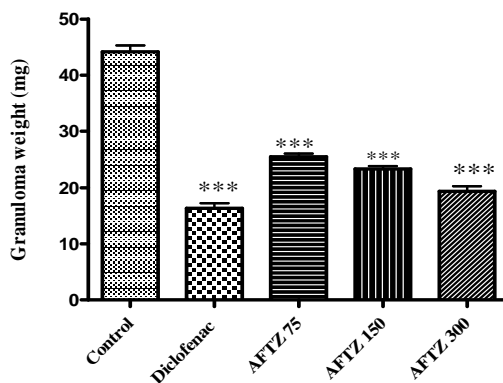


Fig. 4: Effect of AFTZ (75, 150, 300, mg/ kg) on Cotton pellet-induced granuloma.

Values are mean ± SEM (n=6). *p< 0.05, **p<0.01, ***p<0.001 as compared to control (ANOVA followed by Dunnett’s test). AFTZ = Alkaloidal Fraction *Trichopus zeylanicus*.

DISCUSSION

The AFTZ did not show any toxicity and behavioral changes in rats up to 2000 mg /kg hence dose of AF of *T.zeylanicus* of (75, 150 and 300 mg/ kg; p. o.) were selected for the present study.

Antinociceptive activity of AFTZ was investigated using acetic acid induced writhing and hot plate method. The acetic acid induced writhing is a simple and reliable method for evaluating analgesic drug.¹⁸ On intraperitoneal administration of acetic acid produced abdominal constriction followed by extension of hind limb. It is due to sensitization of pain receptor by increasing the concentration of prostaglandins (PGE₂ and PGF_{2α}) in peritoneal fluid.^{19,20} AFTZ showed dose dependent antinociceptive activity in acetic acid induced writhing method may be by inhibiting peripheral nociceptors present on cell lining of peritoneal cavity. To investigate the role of centrally mechanism in inhibition of pain threshold in mice by using hot plate method. Centrally acting drug exerts their antinociceptive effect via supraspinal and spinal receptors. At constant temperature two types of behavioral response integrated through supraspinally such as paw licking and jumping response.^{21,22} In present study AFTZ showed significant antinociceptive activity compared with control group and reference standard pentazocine. The results suggest that AFTZ may show their antinociceptive activity partly may be by involvement of opioid receptor. The anti-inflammatory activity of AFTZ was evaluated using acute inflammatory model i.e. carrageenan induced paw edema and chronic inflammatory model i.e. cotton pellet induced granuloma. The carrageenan induced edema is sensitive models for non steroidal anti-inflammatory drugs and has long been accepted as a useful pharmacologic tool for investigating new anti-inflammatory drug.²³ AFTZ produced significant inhibition of carrageenan induced paw edema comparable with diclofenac sodium as standard. Carrageenan induced inflammation consist of three phases including an initial release of histamine and serotonin, a second phase is mediated by kinin and a third phase involving prostaglandin.^{24, 25} The study showed that AFTZ possibly act by inhibiting release and /or action of histamine, serotonin, kinin and prostaglandin like substance.^{20, 26} The AFTZ decreased the weight of cotton pellet compared with control group. The decrease in weight of granuloma of tissue may be due to both the ability of AFTZ reduces the number of fibroblast and synthesis of collagen and mucopolysaccharide, which are natural proliferative agent involve in formation of granuloma tissue.^{27,28}

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