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Anti-inflammatory and anti-nociceptive activities of ethanolic extract and its various fractions from *Adiantum capillus veneris* Linn.

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ABSTRACT

Aim of the study: To investigate the anti-inflammatory and anti-nociceptive activities of the crude ethanolic extract of *Adiantum capillus veneris* Linn. (Adiantaceae) and its various fractions.
Materials and methods: The ethanolic extract and its fractions were given at a dose of 200 mg/kg po and 300 mg/kg po for testing their anti-inflammatory activity by carrageenan induced hind paw edema. The analgesic activity of the ethanolic extract and its fractions has been carried out by tail-flick method and writhing test at a dosage of 300 mg/kg po. Gastric ulceration studies have been further carried out to study the antiulcer effect of the ethanolic extract and its various fractions at dose of 900 mg/kg body weight.
Results: Amongst the tested fractions, the ethyl acetate fraction exhibited better inhibition (67.27%) at 300 mg/kg po dosage when compared to the standard drug Indomethacin (63.63%) after 3 h in the carrageenan induced hind paw edema. The anti-inflammatory activity of the ethanolic extract and its various fractions appear to be related to the inhibition of NO release, and the decreasing TNF-α level. The ethanolic extract and all its fractions especially the ethyl acetate ($p < 0.01$) showed significant analgesic activity with insignificant ulceration as compared to the standard drug, i.e. ibuprofen. The histopathological study of ethanolic extract and its fractions reveals that none of them cause ulcer.
Conclusion: The present study indicates that *Adiantum capillus veneris* Linn. has significant anti-inflammatory and analgesic effect.

1. Introduction

Adiantum is a large genus of ferns which are widely distributed throughout the world. Ethno medicinally, the genus is important and popularly known as "Hansraj" in the Ayurvedic System of Medicine. About nine species of *Adiantum* are found in India (Mohini et al., 1990). *A. capillus veneris* Linn. has been shown to exhibit antimicrobial activity against different bacterial and fungal strains (Singh et al., 2007). This genus has been used as tonic and diuretic; in treatment of cold, fever, cough and bronchial disorders, as stimulant, emollient, purgative, demulcent, general tonic and hair tonic, in addition to skin diseases, tumors of spleen, liver and other viscera (Singh et al., 2008) and in treatment of jaundice and hepatitis (Abbasi et al., 2009). *A. capillus veneris* Linn. is traditionally used in the Unani system of medicine for the treatment of inflammatory diseases (Kabeeruddin, 1937; Farah et al., 2005). Chemical analysis of *A. capillus veneris* Linn. reveals an array of compounds including triterpenes, flavonoids, phenylpropanoids and carotenoids (Berti et al., 1969; Takahisa et al., 1999). Khare (1996) and Vasudeva (1999) have described the use of *Adiantum* species for the treatment of various diseases. On the basis of the common uses of this plant in traditional folk medicine, the present investigation was carried out to evaluate the anti-inflammatory and antinociceptive potential of *Adiantum capillus veneris* Linn. in experimental animal models.

2. Materials and methods

2.1. Plant preparation and ethanolic extraction

The fronds of *Adiantum capillus veneris* Linn. were collected from a nursery in Ashok Vihar area of New Delhi in the month of April.

Abbreviations: NO, nitric oxide; LPS, lipopolysaccharide; NED, N (1-naphthyl ethylene diamine dihydrochloride); PBS, phosphate buffer saline; TMB, tri-methyl benzidine; TNF-α, tumor necrosis factor-α; NSAIDS, non-steroidal anti-inflammatory drugs; NOS, NO synthase.

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The plant was authenticated by Dr. H.B. Singh, taxonomist, NISCAIR, CSIR, New Delhi (Voucher Number 2009-10/1199/03). The plant was shade dried and powdered. It was then extracted with ethanol (95% v/v) in a Soxhlet apparatus and dried under vacuum at a temperature of 45 °C in a rotary evaporator. The yield of the ethanolic extract was (11.43% w/w). The extract was then fractionated by different solvents of increasing polarity using petroleum ether, ethyl acetate and methanol. The yields of petroleum ether, ethyl acetate and methanolic fractions were 35% (w/w), 31.25% (w/w) and 33.75% (w/w) respectively.

2.2. Animals

Albino Wistar rats of either sex (150–200 g) were obtained from Central Animal House, Hamdard University, New Delhi. The animals were kept in cages at the room temperature and fed with food and water ad libitum. Fourteen hours before the start of the experiment the animals were sent to lab and fed only with water ad libitum. The experiments were performed in accordance with the rules of Institutional Animals Ethics Committee (registration number 173-CPCSEA).

2.3. Drugs

Indomethacin, Ibuprofen, Carrageenan, Carboxymethylcellulose, N (1-naphthyl ethylene diamine dihydrochloride), glutamine, penicillin, streptomycin were purchased from Sigma–Aldrich Chemicals Pvt. Limited, Bangalore, India. ELISA kits for the determination of TNF-α were provided by e-Bioscience (San Diego, CA).

2.4. Toxicity study

The selected albino rats were used to determine the dose. The animals were fasted overnight before the start of the experiment. The animals were divided in to six groups of six in each. The karbers method (Kale and Kale, 1994) was used to determine the dose. Carboxymethylcellulose (1% w/v) was used as vehicle to suspend the extracts and administered orally. The other group received the extract in one of the following doses – 50, 100, 200, 300, 500 and 1000 mg/kg. Immediately after dosing, the animals were observed continuously for first four hours for behavioral changes and for mortality at the end of 24 h, 48 h and 72 h respectively. The toxicity study showed that the ethanolic extract at a minimum dose of 300 mg/kg onwards shows the reaction in experimental animals. However, no mortality was reported even after 72 h. This indicates that the ethanolic extract is safe up to a single dose of 3 g/kg body weight.

2.5. Anti-inflammatory activity

The rat paw edema was induced by subcutaneous injection of 0.1 mL of 1% freshly prepared saline solution of carrageenan into the right hind paw of rats (Winter et al., 1962). The standard drug Indomethacin (20 mg/kg po) was given orally as a positive control. The control group was administered orally with 0.9% of 0.1 mL of saline solution only. The test groups were administered orally with the ethanolic extract and its various fractions at the dosages of 200 and 300 mg/kg body weight, one hour before the administration of carrageenan. The paw volumes were measured using plethysmometer (Ferreira, 1979) at interval of 3 h and 5 h.

2.6. Isolation of peritoneal macrophages

Balb/C mice were injected i.p. with 10 mL (ice cold) of incomplete medium RPMI containing antibiotic and antimycotic mixture as well as heparin (5 U/mL) or ice cold PBS. The abdomen was gently massaged and peritoneal cells were lavaged out in tubes. The peritoneal cells were washed thrice with incomplete RPMI by centrifugation at 1000 rpm, 10 min in cold and finally suspended at 2×10^6 cells/mL in complete RPMI containing 10% FCS (heat inactivated). 100 μL cell suspension/well were dispensed in triplicates using a 96 well flat-bottomed culture plate. Macrophages were allowed to adhere to the bottom of the wells at 37 °C for 24 h in CO_2 (5%) incubator. Non-adherent cells were removed and macrophages were stimulated by LPS (10 μg/mL) in 100 (l volume for 24 h incubation at 37 °C in CO_2 incubator.

2.6.1. The estimation of release of nitric oxide (NO) ex vivo

Nitrite estimation was done by Griess reagent for assaying the macrophage function in the supernatant of unstimulated and stimulated macrophages (Anamika et al., 2007). Griess reagent was prepared by mixing N (1-naphthyl ethylene diamine dihydrochloride) (NED) (0.1%) in distilled water and sulfanilamide (1%) in 5% phosphoric acid in equal volume (prepared fresh). 50 μL of test supernatant from macrophages was added to 96 well microtitre plate in triplicate with complete medium as blank. 50 μL of sulfanilamide solution was added and incubated for 5 min and then 50 μL of NED was added to reaction mixture in each well. Plate was kept for 5 min at room temperature for colour development and stabilization. Absorbance at 550 nm was measured using an ELISA reader. A standard curve was generated using sodium nitrite to quantitate the test results. Results are expressed in (M.

2.7. Analysis of cytokines in spleen cell culture supernatant (TNF-α) ex vivo

Spleens from Balb/C mice were removed aseptically and single splenocyte suspension in RPMI 1640 containing 10% FBS, 1 M HEPES, 2 mM glutamine, 100 U/mL penicillin and 100 mg/mL streptomycin was obtained by passage through a stainless mesh. Red blood cells were lysed with lysis buffer for 5 min. After washing with PBS, cells were cultured in 96-well plates at 2×10^6 cells/mL and cytokines were then measured from the supernatants by ELISA. The assay was performed according to the manufacturer's instruction with multipoint analysis (Malik et al., 2007).

Briefly, 100 μL of diluted capture antibody was added to each well in a 96 well plate and was allowed to adhere overnight for 4 °C. Plates were washed and then blocked with 1× PBS supplemented with 10% FBS for 1 h at room temperature. After washing, serial dilutions of the standard and samples were prepared in the plates and were then incubated for 2 h at room temperature. Then, plates were washed and working detector solution (including detector antibody and avidin–horse radish peroxidase reagent) was added into each well. Plates were then sealed and incubated for 1 h at room temperature. After washing, 100 μL of tri-methyl benzidine (TMB) substrate was added into each well. Stop solution (2 N H_2SO_4) was finally added after incubation in the dark for 30 min at room temperature. The absorbance was read at 450 nm. The result was analyzed using softmax program and values determined against the standard provided by the manufacturer.

2.8. Antinociceptive activity

2.8.1. Tail immersion method

In the present study analgesia was assessed by employing tail immersion method (Distasi et al., 1988). Prior to the experiment the animals were screened for the sensitivity test by immersing the tail of the rats gently in hot water maintained at 55 °C (Agrahari et al., 2010). The animals flicking their tail from hot water in 5 s were selected for the study in order to avoid any thermal injury to the tail. The selected rats were then divided into six groups of six rats each. The control group was administered orally with 0.9% of 0.1 mL