



Research Article

ANTINOCICEPTIVE ACTIVITY OF *ADIANTUM CAPILLUS* IN EXPERIMENTAL ANIMALS

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ABSTRACT

In present study 50% ethanolic extract of whole plant of *Adiantum capillus* (ACE) was evaluated for its analgesic and anti-inflammatory effects in animals at different doses 100, 200 and 300 mg/kg by different method. Eddy's hot plate method, writhing test, formalin test and carrageenan induced revealed that ACE possessed varying degree of analgesic and anti-inflammatory activity significant at 100, 200 and 300 mg/kg and highly significant at 300 mg/kg in comparison to control. In acetic acid induced writhing test, maximum inhibition of writhing was observed at 300 mg/kg where the number of writhes decreased from 40.32 to 18.32 indicating 54.56% inhibition. While formalin induced pain (0.05 ml of 2.5%) was significantly blocked only at higher dose (300 mg/kg) in first phase. ACE significantly blocked pain emanating from inflammation at all the doses in second phase. The reaction time in hot plate was increased significantly and dose dependently whereas pretreatment with diclofenac sodium reduced the analgesic potentials of ACE.

Keywords: *Adiantum capillus*, Analgesic, Anti-inflammatory, Carrageenan.

INTRODUCTION

Inflammation is the state of hyperemia from blood vessels that result of redness, warmth, swelling and discomfort that sign the tissue injury due to biochemical reaction¹. It is occurred by the migration of plasma and white cells comprising monocytes that are locally distinguish into macrophages from blood into wounded tissues. Immune reaction is vital for the body to remove dangerous pathogens and categorized by way of an acute inflammation. In inflammation various pro- and anti-inflammatory agents are produced comprising cytokines, interleukins, reactive oxygen species and chemokine's all of them play serious role in governing the inflammation¹.

Pain encourages the person to avoid both situations deleted to care for a wounded body part until healing and to escape related incidences in future. Most pain subsides quickly as the painful stimulus is removed and the body has repaired, but sometimes may remain regardless of elimination of the stimulus and obvious healing of the body part. Occasionally pain begins in the absence of any detectable stimulus, injury. Pain has been classified in three class's nociceptive, inflammatory and pathological pain caused by damage to the nervous system or by alteration in its function e.g. fibromyalgia, irritable bowel syndrome and tension type headache. The pain pathway usually involves transduction, transmission, modulation and perception. Drugs commonly in use for pain management one classified as opioids analgesics and non-steroidal anti-inflammatory drugs². The medicinal plants have been a major source of a wide variety of biologically active compounds for many centuries have been used extensively in crude form or as pure isolated compounds to treat inflammation³.

Adiantum capillus-veneris also known as Venus hair fern traditionally as well as in English name are avenca and maidenhair^{4,5}. It is commonly grown species from the garden to

the humid coniferous forest. *Adiantum capillus-veneris* is used as traditional medicine for curing various diseases⁶. In Ayurvedic system of medicine for certain health conditions such as cold, tumors of the spleen, liver, skin diseases, bronchitis and inflammation⁷. Capillaries are a main ingredient of *Adiantum capillus* used as a cough syrup and fever as well as used as a stimulant, emollient and purgative also used to improve appetite, digestion, stimulate renal function, regulate menstruation, and facilitate childbirth depurative, emetic, emollient, febrifuge, galactagogue, alopecia and tonic. It is used as a poultice on snake bites and bee sting⁸. In Nepal, a paste made from the fronds is applied to the forehead to relieve headaches and to the chest to relieve chest pains⁹. Externally, it is rubbed to prevent hair loss this hair-stimulant effect has also been observed in the Venezia Giulia region of Italy. It is used as anti-fertility effect, mixed with *Dryopteris normalis* (Polypodiaceae) and drunk for four consecutive mornings⁵.

The extracts of the *Adiantum* leaves have anti-inflammatory, analgesic, anti-microbial activities⁷. The hydro-alcoholic extract of leaves has strong anti-urolithic properties and isolated vascular endothelial growth factor and transforming growth factor beta-1 proteins in normal and streptozotocin-induced diabetic rats, during treatment of formulations of *Aloe Vera*, *Henna*, *Adiantum capillus-veneris*, and *Myrrha* usually claimed^{2,10} have been reported very good antidiabetic as well as low side effect at the different dose of aqueous extracts and methanolic extract, Hair growth-promoting activity was reported by¹¹ on albino mice using a testosterone-induced alopecia model and methanolic extract of whole plant showed the antibacterial activity¹¹. Ethanolic extract have nephroprotective activity against cisplatin induced oxidative stress caused in male wistar rats⁴.

Phytochemical analysis of *Adiantum* leaves revealed the presence of flavonoids, alkaloids, tannins, saponins, glycosides, steroids,

and terpenoids also reported anti-bacterial and anti-fungal activity. *Adiantum* leaves extract contains high level of flavonoids were a good source of antioxidants³ evaluated the antioxidant potential of leaf extract of *Adiantum capillus veneris* Linn as well as isolated thirty seven bioactive compounds which are major compound such as 5- 7A-Isoprenyl-4, 5-dimethyloctahydro-1h-inden-4yl)-3-methyl-2-penta, n-hexadecanoic acid and gamma-sitosterol cis-vaccenic acid, 5-7A-Isopropenyl-4,5-Dimethyl-octahydro-inden-4-yl)-3-methyl-pent-2-EL⁹. Isolated new terpenoids that was 22, 29-epoxy-30-norhopan-13-ol from the *Adiantum* leaves with strong anti-bacterial activity. Seven bioactive compounds, containing 3-coumaroylquinic acid, kaempferol-3-glucosides as major phenolic compounds¹³. Similarly, quercetin, quercetin-3-glucoside and quercetin-3-rutinoside were identified in the leaves and extracts were found helpful against inflammation and hypoglycemia¹⁴.

MATERIAL AND METHOD

Plant collection and identification

Adiantum capillus-veneris (Family: Adiantaceae) (whole plant) were freshly collected from the Shankar nursery, Dubbagga Lucknow, India, in May 2017. The plant part was identified and authenticated taxonomically at National Botanical Research Institute, Lucknow. The voucher specimens (NBRI-SOP-201) for the collected plant part have been deposited in the college departmental herbarium for future reference.

Preparation of extract

Whole plant material was washed with double distilled water to remove dirt and shade dried. The dried materials was powdered and passed through a 10-mesh sieve. The coarsely powdered material was extracted with petroleum ether thrice to remove the fatty material and further marc was extracted thrice with ethanol (50%, v/v). The extracts were filtered pooled and concentrated at reduced temperature (5°C) on a rotary evaporator. Phytochemical screening of 50 % ethanolic extract of *Adiantum capillus* (ACE) was performed for alkaloids, carbohydrates, flavonoids, glycosides, triterpenoids and tannins.

Animals

Swiss albino mice weighing 20 ±5 gm. and Wistar rats weighing 140±20 gm were procured from the animal house of the Indian Toxicological Research Centre, Lucknow. They were kept in departmental animal house in well cross ventilated room at 27±2 and relative humidity 44–56%, light and dark cycles of 10 and 14 h, respectively, for 1 week before and during the experiments. Animals were provided with rodent pellet diet (Amrut, India) and the food was withdrawn 18–24 h before the experiment thought, water was allowed *ad libitum*. All studies were performed in accordance with the guide for the care and use of laboratory animals, as adopted and promulgated by the Institutional Animal Care Committee, CPCSEA, India (Reg. No.54/GO/R Bi/S/99/CPCSEA). All animal experiments were carried out according to NIH and Indian National Science Academy, New Delhi, India guidelines. The study was approved by the Institutional Animal Ethics Committee of the Mahatma Gandhi Institute of Pharmacy, Lucknow.

Antinociceptive activity

Hot Plate

The animals of either sex was weighed and divided into five groups each group six animals. Group first received orally normal

saline solution (10 ml/kg) (i.e. control), group second (pentazocine 30 mg/kg p.o) served as standard and group third, fourth and fifth were treated with 50 % ethanolic extract of *Adiantum capillus* (ACE) at a dose of 100, 200 and 300 mg/kg body weight. The reaction time of animal was noted down on hot plate at 0.5, 1, 2, 3, 4 and 5 hours after the above treatment. The basal reaction was the time taken by observing hind paw licking and jump response in animals while placed on hot plate which was maintained at constant temperature 55°C. A cut off period of 15 second was taken for complete analgesia and to avoid further tissue damage¹⁵.

The percentage inhibition of activity at each interval can be calculated by the formula:

$$\text{Percentage analgesic activity} = (T_a - T_b / T_b) \times 100$$

Where T_b is initial reaction time and T_a is reaction time administration of drugs.

Acetic acid induced writhing reflex

Mice (six per) group were injected intraperitoneally with 0.6% acetic acid at a dose of 10 ml/kg. ACE (100, 200 and 300mg/kg, p.o.), diclofenac sodium (10 mg/kg, i.p), were administered 30 min prior to treatment with acetic acid¹⁶. The writhing was counted for 30min after a latency period of 5min¹⁵. The percentage analgesic activity was calculated as follow:

$$\text{Percentage analgesic activity} = \frac{N - N^1 \times 100}{N}$$

Where N represents the average number of writhing of control group and N^1 the average number of stretching of test group

Formalin Test

The mice were divided into five groups each group containing five mice were administered with orally normal saline solution (10 ml/kg), 50% ethanolic extract of *Adiantum capillus* (ACE) (100, 200 and 300 mg/kg, i.p) and diclofenac sodium (10 mg/kg, s.c). Thirty minutes after this treatment; 50 µL of a freshly prepared 0.6% solution of formalin was injected subcutaneously under the plantar surface of the left hind paw of each mouse. The mice were placed individually in an observation chamber and monitored for one hour. The time (in second) spent in licking and biting of the injected paw was taken as an indicator of pain response. Antinociceptive effect was determined in two phases. The early phase (phase 1) was recorded during the first 5 minutes, while the late phase (phase 2) was recorded during the last 20-30 minutes after formalin injection¹⁶.

Anti-Inflammatory

Carrageenan-induced

The animals of either sex was weighed and divided into five groups of six animals in each. Group first received orally normal saline solution (10 ml/kg) (i.e. control), Group second received diclofenac sodium (10 mg/kg/i.p) as a standard. Group third, fourth and fifth were treated with 50 % ethanolic extract of *Adiantum capillus* (ACE) at a dose of 100, 200 and 300 mg/kg body weight. Half an hour after the oral administration of ACE, 1% carrageenan was injected to the right hind paw of each animal. The volume of paw edema was measured at 0, 1, 2, 3, 4 and 8 hours using Plethysmometer after administration of carrageenan. The left hind paw served as a reference non-inflamed paw for comparison. The average percentage increase in paw volume with time was calculated and compared against the control group¹⁷. Percentage inhibition was calculated using the formula:

$$\% \text{ Inhibition of paw edema} = \frac{V_c - V_t}{V_c} \times 100$$

Where, V_c and V_t represent average paw volume of control and treated animal respectively

Statistical test

Values were represented as mean $\pm$ S.E.M. and data were analyzed by paired-*t*-test using SPSS software for the Windows 10 package.

RESULT

Hot plate method

Hot plate procedure was adopted for the estimation of analgesic activity of the extract of 50 % ethanolic extract of *Adiantum capillus* (ACE) have displayed increase in latency time at 0, 0.5, 1, 2, 3, 4, 5 hour as shown in table 1. Administration of ACE at the dose of dependent increase analgesic activity compared to the standard group *i.e.* 15.5-3.4%, 19.65- 12% and 6.8-6.9% at the three dose (100, 200 and 300) in between 0 hours to 5 hours while standard drug (diclofenac sodium) showed the 7.35-38.9 compared to the control group.

Acetic acid writhing reflex

50 % ethanolic extract of *Adiantum capillus* (ACE) reduced writhing and stretching induced by 0.6 % acetic acid at 10 ml/kg. the significant and dose dependent protective effect were observed as 36.65% ($P < 0.01$), 47.61 ($P < 0.001$), and 54.56% ($P < 0.001$) at dose of 100,200 and 300 mg/kg of ACE respectively compared to control group while standard group *i.e.* 10 mg/kg diclofenac sodium was found to be 59.52% ($P < 0.001$) compared to the control table 2.

Formalin test

In the study formalin induced pain of 50 % ethanolic extract of *Adiantum capillus* (ACE) in mice were dose dependently inhibited the licking response in both the early phase (56.81% ($P < 0.05$), 53.38 ($P < 0.05$) and 63.01% ($P < 0.01$) while in late phase (44.87% ($P < 0.05$), 46.25% ($P < 0.01$) and 51.38 ($P < 0.01$) at dose of 100, 200 and 300 mg/kg respectively compared to control group while standard group *i.e.* 10 mg/kg diclofenac sodium was found to be 61.01% ($P < 0.01$) compared to the control group table 3.

Table 1: Effect of 50% ethanolic extract of *Adiantum capillus* (ACE) on pain induced by hot plate in mice.

Treatment	0 h	0.5 h	1 h	2 h	3 h	4 h	5 h
Control	4.67 $\pm$ 0.26	4.28 $\pm$ 0.12	4.16 $\pm$ 0.19	3.94 $\pm$ 0.26	3.92 $\pm$ 0.22	3.96 $\pm$ 0.21	3.91 $\pm$ 0.23
Standard	4.35 $\pm$ 0.05	4.43 $\pm$ 0.20 *	5.03 $\pm$ 0.11 *	6.76 $\pm$ 0.5 *	7.31 $\pm$ 0.57 *	6.87 $\pm$ 0.48 *	6.39 $\pm$ 0.47 *
ACE 100mg/kg	4.04 $\pm$ 0.20	5.12 $\pm$ 0.43 *	5.49 $\pm$ 0.39 ^b	6.96 $\pm$ 0.45 * * *	5.24 $\pm$ 0.46 *	3.97 $\pm$ 0.63	3.78 $\pm$ 0.33
ACE 200mg/kg	3.78 $\pm$ 0.16	5.19 $\pm$ 0.61 * *	5.70 $\pm$ 0.46 ^c * *	6.39 $\pm$ 0.49 * * *	5.13 $\pm$ 0.23 * *	3.74 $\pm$ 0.54	3.49 $\pm$ 0.63
ACE 300 mg/kg	4.35 $\pm$ 0.05	5.70 $\pm$ 0.46 * * *	6.69 $\pm$ 0.49 * * *	7.24 $\pm$ 0.62 * * *	5.19 $\pm$ 0.37 *	4.67 $\pm$ 0.26	4.20 $\pm$ 0.58

Value expressed as $\pm$ S.E.M, n=6 mice, $P < 0.05$ compare to same group at 0 h., $P < 0.01$ compare to same group at 0 h., $P < 0.001$ compare to same group at 0 h.

Table 2: Effect of the 50% ethanolic extract of *Adiantum capillus* (ACE) on acetic acid induced writhing in mice

Treatment	Dose (mg/ kg)	No. of writhing	% Inhibition
Control	Vehicle	40.32 $\pm$ 3.25	-
Diclofenac sodium	10	16.32 $\pm$ 0.42 ***	59.52
ACE	100	25.54 $\pm$ 1.32 **	36.65
ACE	200	21.12 $\pm$ 1.12 ***	47.61
ACE	300	18.32 $\pm$ 0.75 ***	54.56

Value expressed as $\pm$ S.E.M, n=6 mice, $P < 0.05$ compare to same group at 0 h., $P < 0.01$ compare to same group at 0 h., $P < 0.001$ compare to same group at 0 h.

Table 3: Analgesic effect of 50% ethanolic extract of *Adiantum capillus* (ACE) using the formalin test

Treatment	0-5 min(early phase)	% Inhibition	20-30 min (late phase)	% Inhibition
Control	39		29.5	
Standard	17.85 ***	54.23	11.0	62.7 ***
ACE 100 mg/kg	21.5 ***	44.87	15.5	46.77 *
ACE 200 mg/kg	20.96 **	46.25	13.75	53.38 **
ACE 300 mg/kg	18.96 **	51.38	11.5	61.01 ***

Value expressed as $\pm$ S.E.M, n=6 mice, $P < 0.05$ compare to same group at 0 h., $P < 0.01$ compare to same group at 0 h., $P < 0.001$ compare to same group at 0 h.

Table 4: Effect of 50% ethanolic extract of *Adiantum capillus* (ACE) on carrageenan induced oedema in rat.

Treatment	0 h	0.5 h	1 h	2 h	3 h	5 h
Control	0.62 $\pm$ 0.048	0.75 $\pm$ 0.046	1.41 $\pm$ 0.06	1.79 $\pm$ 0.08	1.67 $\pm$ 0.08	1.59 $\pm$ 0.07
Standard	0.37 $\pm$ 0.037 **	0.36 $\pm$ 0.04	0.57 $\pm$ 0.064 *	0.37 $\pm$ 0.094 **	0.32 $\pm$ 0.07 ***	0.68 $\pm$ 0.04 **
ACE 100 mg/kg	0.45 $\pm$ 0.05	0.46 $\pm$ 0.06	0.68 $\pm$ 0.019 *	0.49 $\pm$ 0.13 **	0.43 $\pm$ 0.06 ***	0.86 $\pm$ 0.04 *
ACE 200 mg/kg	0.42 $\pm$ 0.028 **	0.41 $\pm$ 0.036	0.59 $\pm$ 0.016 *	0.41 $\pm$ 0.035 ***	0.36 $\pm$ 0.03 ***	0.77 $\pm$ 0.02 **
ACE 300 mg/kg	0.41 $\pm$ 0.026 **	0.39 $\pm$ 0.05	0.49 $\pm$ 0.13	0.36 $\pm$ 0.03 ***	0.43 $\pm$ 0.06	0.57 $\pm$ 0.064 *

Value expressed as $\pm$ S.E.M, n=6 mice, $P < 0.05$ compare to same group at 0 h., $P < 0.01$ compare to same group at 0 h., $P < 0.001$ compare to same group at 0 h.

Carrageenan-induced oedema

Carrageenan induced paw edema test in rats, the ethanolic extract of *Adiantum capillus*(ACE) at a doses of 100 mg/kg, 200 mg/kg and 300 mg/kg inhibited the rat paw oedema 74.25% ($P>0.05$) 78.44% ($P>0.001$) and 78.44% ($P>0.001$) at 3 hour when compared to control group. Standard drug diclofenac sodium at 10 mg/kg shows the inhibition of the rat paw oedema 80.8% ($P>0.001$) at 3 hours when compare to control group table 4.

DISCUSSION

Carrageenan induced inflammation is useful orally active anti inflammatory drugs and to determining the value of anti inflammatory agent acting by inhibiting the mediators of acute inflammation¹⁸. The inflammation induced by carrageenan that involves the release of substances in early phase such as histamine, bradykinin and serotonin and prostaglandins in late phase. Thus carrageenan induced oedema is biphasic¹⁹. The first two hours of the oedema is the first phase of carrageenan induced inflammation is occur due to release of serotonin, histamine and cytoplasmic enzymes²⁰ after this is the second phase occur due to the release of prostaglandins, lysosome, bradykinin and proteases and these chemical substances induced the oedema due to increase in vascular permeability and accumulation of fluid²¹. The ability of the 50 % ethanolic extract of *Adiantum capillus* (ACE) almost completely inhibit oedema in the second phase (4th and 5th hour) indicate that it contains bioactive components such as rutin, quercetin, betalains and luteolin present in *Adiantum capillus* which are active against the liberation of prostaglandins and other inflammatory agents usually released in the second phase of carrageenan induced oedema. It could be argued that the suppression of the first phase may be due to inhibition in the release of mediators. Sub plantar administration of carrageenan 1% (0.1 ml/paw) resulted in increased paw (volume in the control animals at all the measured periods 50% ethanolic extract of *Adiantum capillus* ACE) (100 mg/kg, 200 mg/kg and 300 mg/kg) was able to reduce the paw volume more significantly ($p<0.01$) at all the intervals as compared with control. Diclofenac sodium at 10 mg/kg/*i.p* was able to produce more significant reduction of paw volume at all the intervals. Flavonoids and phenolic compounds have been reported to have multiple biological effects such as antioxidant activity and analgesic activity *in vivo*²² anti-inflammatory activity²³ inhibition of platelet aggregation²⁴ inhibition of mast cell histamine release and inhibitory actions on arachidonic acid metabolism as demonstrated by *in vitro* and *in vivo* tests²⁴.

Preliminary qualitative phytochemical screening reveals the presence of flavonoids, glycosides, tannin, protein, sterol, resin and carbohydrates in 50% ethanolic extract of *Adiantum capillus* (ACE) may be responsible for the observed analgesic and anti-inflammatory activity. Tannins, Flavonoids such as rutin, quercetin, were reported to have a role in antinociceptive and or anti-inflammatory activities primarily by targeting prostaglandin²⁵. Formalin induced edema also shows a biphasic response and originate mainly from neurogenic inflammation followed by participation of kinins and leukocytes with their pro-inflammatory factors including prostaglandins. According to²⁸ acute inflammation induced by formalin results from cell damage which provides the production of endogenous mediators likes histamine and bradykinin²⁶. Oedema produced by formalin was significantly ($P < 0.01$) inhibited by 50 % ethanolic extract of *Adiantum capillus* (ACE) (300 mg/kg, *p.o.*). Carrageenan was found to be more potent in inducing oedema than formalin, indicating a more reliable model for inflammation. Thus, it is concluded that 50 % ethanolic extract of *Adiantum capillus* (ACE) possess analgesic and anti-inflammatory properties which

are probably mediated via inhibition of prostaglandin synthesis as well as central inhibitory mechanism and may have a potential benefit for the management of pain and inflammatory disorders²⁷. The 50 % ethanolic extract of *Adiantum capillus* (ACE) at different doses (100 mg/kg, 200 mg/kg and 300 mg/kg) showed significant ($P<0.01$) and dose dependent inhibition of pain responses (36.65%, 47.61 and 54.56% respectively) as compared to control group. The dose dependent inhibition of acetic acid induced writhing by the extracts indicated a peripheral effect. The 50 % ethanolic extract of ACE have shown the central nervous system depressant effect that reduces the number of writhing in acetic acid pain models¹⁷. The hot plate method has been found to suitable for the evaluation of centrally but not of peripherally acting analgesics. The validity of this test has been showing even in the presence of substantial impairment of motor performance. In hot plate test, nociceptive reaction towards thermal stimuli in rat is a well validated model for detection of opiate like analgesic drugs where in pain response is from spinal organ. In our present study the three doses of the 50 % ethanolic extract of *Adiantum capillus* (ACE) had increased the reaction time in dose dependent manner at 300 mg/kg of 50 % ethanolic extract of *Adiantum capillus* (ACE) had exhibited the highest anti-nociceptive effect to the thermal stimulus at 60 min, which is comparable to the effect of standard drug of pentazocine. The present findings of the study indicate that the 50 % of ethanolic extract of *Adiantum capillus* (ACE) may be centrally acting produced significant ($P < 0.001$) analgesic activity at all test doses when compared to that of control²⁸.

CONCLUSION

From the above study it can be concluded that the whole plant extract of *Adiantum capillus* promising anti-inflammatory activity. This effect may be beneficial for the management of pain.

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