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Research Article

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ANALGESIC ACTIVITY OF *FICUS ARNOTTIANA* (MIQ) LEAVES EXTRACT

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ABSTRACT
The methanolic extract of leaves of *Ficus arnottiana* was used to evaluate the analgesic activity. The above activity was evaluated using the eddy's hot plate and heat conduction method and acetic acid induced writing in mice. The dose used for the test of activity (100, 200. 400 mg/kg *i.p*). The extract at all doses tested significantly ($P<0.001$) inhibited acetic acid induced writhing and also significantly ($P<0.05$) prolonged the reaction latency to pain thermally induced in mice by the hot plate. The phytochemical screening revealed the presence of alkaloids, flavonoids, glycosides, saponins and tannins which might be responsible for the observed analgesic and anti-inflammatory activity. This study showed that *Ficus arnottiana* possesses significant anti-inflammatory and analgesic properties in rodents which supported the folkloric claim for the use of the plant as medicine.
KEYWORDS: Analgesic activity, *Ficus arnottiana,* writing test, eddy's hot plate.

INTRODUCTION

Ficus arnottiana commonly known as paras peepal in india. This is an important traditional medicinal plant distributed through out Indian mostly in rocky hills of 1,350 m elevations. It has several vernacular names such as paras papal, beliya neem, kamru. The bark and the leaves of the plants have been used for traditions in the folk and ancient preparations. It has been an oldest and well known plant of medicinal uses in India and have been found in the region of Madhya Pradesh that is Satpura and Vindhyas, in Uttaranchal, Himachal Pradesh and various other parts of Indian sub continents[1-5]. The plant extracts have been evaluated for hyperglycemic, antioxidant and ulcer protective activity[6,7]. The plant has demonstrated positive results in the above screening performed. The present study is to evaluate the anti inflammatory activity and describe the detail pharmacognosy of the plant.

Taxanomy

Domain: Eukaryote
Kingdom: Planate
Subkingdom: Viridaeplantae
Class: Magnoliopsida Dicotyledons
Subclass: Dilleniidae
Superorder: Urticanae
Order: Urticales
Family: Moraceae
Genus: Ficus
Botanical name: *Ficus arnottiana* Miq

Pharmacological Description

Macroscopic

Drug are in cut pieces with or without bark of varying size, 0.5 to 2.0 cm in thickness, external surface brownish in color and slightly rough due to exfoliation of cork, cut surface, yellowish - brown in color. Fracture, fibrous, odour and taste not characteristic[1,5,7].

Microscopic

Transverse section of root shows thick cuticle, single layered epidermis, cells rectangular followed by 3 or 4 layers of cork cells; cork cambium 2 to 4 layered; secondary cortex wide consisting of rectangular to polygonal thin walled pitted cells, some filled with reddish-brown substance, circular to elongated, lignified, elliptical stone cells, a few showing concentric striations present in this region, a few prismatic crystals of calcium oxalate and abundant round to oval starch grains up to about 12µ present in cortical cells, endodermis and pericycle not distinct, secondary phloem shows a wide zone consisting of sieve tubes, companion cells, fibers and ray cells starch grains, latticiferous cells also present in this region, fibers non-lignified, thick walled with narrow lumen; secondary xylem elements thick walled and lignified, vessels and tracheids show bordered pits, medullary rays uni to multiseriate, wide towards peripheral region[1-4].

Traditional uses

The leaves of the plant are used for controlling fertility. Bark of the plant is used as astringent, aphrodisiac, demulcent depurative and emollients. It is also useful in inflammation, diarrhea, and diabetes, burning sensation, leprosy, scabies, wounds and skin diseases, ulcer protective[1,2,8].

PHYTOCHEMICAL PROPERTIES

The phytochemical study of *Ficus arnottiana* leaves was subjected to the preliminary phytochemical screening as per standard procedures[10].

MATERIAL AND METHODS

Plant material and Preparation of Herbal Extract

Ficus arnottiana fresh leaves were collected from the area of mandideep that falls in Raisen district of Madhya Pradesh. Initially these leaves were washed with fresh water to remove adhering dirt and foreign particles and were allowed to dry in shed. The dried leaves were crushed and grinded to get powder and weighed. The weighed powder was then placed with ethanolic solution in a cylinder. 500g of *Ficus arnottiana* powder in 1.0 liter of ethanolic solution were macerated for 7 days. The mensturm was removed and concentrated by vaccum distillation. Again the crude material was allowed to undergo maceration for 4 days followed by 2 days for complete extraction. The mensturm was collected and concentrated by vaccum distillation and then air dried in an evaporating dish till constant weight was obtained. The percent yield of *Ficus arnottiana* leaf extract is 26.52 %.

Test animals

Adult Wister rats, (127-146 g) and Swiss albino mice, (16-30 g) of either sex were used for the experiments. All animals were healthy and obtained from the Animal house facility of Department of Pharmacology, RKDF College of Pharmacy, and Bhopal, INDIA. They were housed in standard polypropylene cages and kept in a well ventilated area. The animals were fed on standard laboratory diet and water *ad libitum*. Food and water were withdrawn during the experimental hours.

Acute toxicity studies

Acute toxicity studies were carried out on Swiss albino mice. Active extract at doses of 100, 300, 500, 1000 and 3000 mg/kg was administered to five groups of mice, each group containing 6 animals. After administration of extracts the animals were observed for the first 3h for any toxic symptoms followed by observation at regular intervals for 24 h up to 7 days. At the end of study the animals were also observed for general organ toxicity, morphological behavior and mortality[11].

Acetic acid-induced writhing test in mice

The method described in the referred research article quoted in the reference, was employed to asses the analgesic activity of the methanol leaf extract of *Ficus arnottiana*[12]. A total of 30 mice were divided into 5 groups of 6 mice each. The mice were treated with normal saline (10 ml/kg), *Ficus arnottiana* extract (100,200 and 400 mg/kg*)* or Diclofenac Sodium (10 mg/kg) intraperitoneally. 30 minutes later, mice in all groups were treated with acetic acid 10 ml/kg of 0.6%v/v intraperitoneally. The number of writhes was counted 5minutes after acetic acid injection for a period of 10 minutes. Percentage inhibition of writhing was calculated using the formula:

% inhibition = Mean No. of writhes (control) –Mean No. of writhes (test)/ Mean No. of writhes (control) X 100.

Hot plate method

The test was carried out using a Eddy's hot plate apparatus, maintained at 50ºC and the method described by reference quoted was employed[13]. Only mice that showed initial nociceptive response within 30 seconds were selected for the experiment. The reaction time of the mice to the thermal stimulus, taken to be the interval between the instant the animal reached the hot plate to the time it licked its paw or jump off the hot plate.

30 mice were selected and divided into 5 groups of 6 mice each. The first group received normal saline 10 ml/kg intraperitoneally. The second, third and fourth groups were given the test extract at 400, 200 and 100 mg/kg via intraperitoneal route respectively. The fifth group received Diclofenac Sodium (10 mg/kg) intraperitoneally.

Thirty minutes after treatment, the reaction time of each mouse was again evaluated as above. The final test mean value (Ta) for each treatment group was calculated which represented the after treatment reaction time and was subsequently used to determine the percentage thermal pain stimulus or protection by applying the formula:

% protection against thermal stimulus = Test mean (Ta) – Control mean (Tb)/ Control means (Tb) X 100.

Statistical analysis

The data was expressed as Mean ± SEM (standard error of mean). Analysis of variance (ANOVA) followed by post hoc and Dunnet-t-test was used to statistically analyze data. P values less than 0.05 ($P<0.05$) were considered as significant.

RESULTS

Phytochemical screening

After phytochemical investigation it was found that extract of the leaves of *Ficus arnottiana* showed the presence of sterols, carbohydrates, phenols, alkaloids and tannins.

Analgesic activity

The extract significantly attenuated the number of acetic acid induced abdominal writhes in mice, dose dependently. The highest percentage inhibition of abdominal constriction (80.5%) was observed at 400 mg/kg ($P<0.001$) and was greater than that of Diclofenac Sodium (45.1%) at 10 mg/kg ($P<0.05$), the standard non-steroidal analgesic and anti-inflammatory drug used. The extract at 100 and 400 mg/kg, significantly protected the mice against thermally induced pain stimulus. The extract (100 and 400 mg/kg), Morphine Sulphate (4 mg/kg), significantly ($p<0.05$) increased pain latency thermally induced by the hot plate. The reaction time at the dose of 300 mg/kg (3.08) was found to be twice that of the control group (1.48).

Table 1: Effect of methanol leaf extract of *Ficus arnottiana* on acetic acid-induced writhing in mice

Treatment mg/kg	Mean number of writing	% inhibition
Normal saline solution	22.17±2.75	
Extract (100)	6.83±1.30*	69.19
Extract (200)	6.67±1.05*	69.91
Extract (400)	4.33±1.43#	80.45
Diclofenac sodium (10)	12.17±2.32	45.11

Each value is Mean ± SEM of 6 mice; * P< 0.05, #P<0.001
One way ANOVA df=4, 25 f=14.421

Table 2: Effect of methanol leaf extract of *Ficus arnottiana* on thermally induced pain in mice

Treatment (mg/kg).	Mean Pain Latency (sec).	% inhibition
Normal saline solution	1.14±0.17	
Extract (100)	2.08±0.12*	40.54
Extract (200)	1.98±0.15*	33.78
Extract (400)	3.08±0.68*	108.11
Morphine Sulphate (4)	8.83±1.88	496.62

Each value is Mean ± SEM of 6 mice; *P< 0.05
One way ANOVA df=4, 25 f=11.398

DISCUSSION

The above conducted study evidenced that ficus arnottiana leaves extract exhibit analgesic properties. The result was demonstrated by using thermal method and acetic acid induced writing test. The acetic acid induced abdominal constriction method is widely used for the evaluation of peripheral antinociceptive activity[14]. It is very sensitive and able to detect anti-nociceptive effects of compounds at dose levels that may appear inactive in other methods like the tail-flick test[15,16]. Local peritoneal receptors are postulated to be partly involved in the abdominal constriction response. The method has been associated with prostanoids in general, e.g. increased levels of PGE2 and PGF2α in peritoneal fluids[17], as well as lipoxygenase products by some researchers[18]. Therefore the results of the acetic acid induced writhing; strongly suggest that the mechanism of action of this extract may be linked partly to lipoxygenases and/or cyclo-oxygenases pathways. The hot plate method is one of the most common tests of nociception that is based on a phasic stimulus of high intensity. Pain induced by thermal stimulus of the hot plate is specific for centrally mediated nociception. The ability of the extract to prolong the reaction latency to pain thermally-induced in mice by the hot plate further suggests central analgesic activity. The extract at the doses tested was shown to possess anti-nociceptive activity evident in all the nociceptive models, signifying it possesses both central and peripherally mediated activities. Flavonoids, saponins and tannins have been shown to exert analgesic effect on acetic acid induced writhing test.

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