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EVALUATION OF FLOWER OF *BARLERIA PRIONITIS* FOR ANTI-INFLAMMATORY AND ANTI NOCICEPTIVE ACTIVITY

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ABSTRACT

Traditionally aerial parts of *Barleria prionitis* Linn. has been used in the inflammation, fever & toothache. The present study was undertaken to evaluate the anti-inflammatory and anti-nociceptive activity of 50% ethanolic extract of the flower of *B. prionitis* (BPF) in experimental animals. The BPF in doses of 50, 100 and 200 mg/kg caused a dose-dependent inhibition of swelling caused by carrageenin equivalent to 17.8–48.6% protection ($P>0.05$–$P<0.001$) and in cotton pellet granuloma, 46.2–36.4% protection ($P<0.01$-$P<0.001$) was observed from inflammation. There was a significant increase in analgesio meter force induced pain in mice equivalent to 26.3–48.23% protection ($P<0.01$-$P<0.001$) & 5.24 - 34.6 % ($P<0.05$–$P<0.001$) protection against Acetic acid induced writhing. Our results shows that flower of *B. prionitis* possess significant anti-inflammatory and anti-nociceptive activity.

KEYWORDS

Barleria prionitis; Anti-inflammatory; Anti-nociceptive; cotton pellet granuloma

INTRODUCTION

Barleria prionitis L. (Family Acanthaceae; commonly known as Vajradanti,), is an annual shrub, 1–3 feet high, found throughout tropical Asia and in South Africa. In indigenous system of medicine in India, the aerial parts (stem, leave & flower) are used in fever, toothache, inflammation & gastrointestinal disorders; bark in whooping cough as an expectorant; the whole plant and especially the roots are used as tonic and diuretic [1,2,3,4]. Leaves, stem and root of *B. prionitis* possess antibacterial and anti-inflammatory activities [5, 6]. Iridoid enriched fraction of aerial parts (leaves and stems) was reported for hepatoprotective activity in various acute and chronic animal models [7].

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An aerial part was reported for barlerinoside, shanzhiside methyl ester, 6-*O*-*trans*-*p*-coumaroyl-8-*O*-acetylshanzhiside methyl ester, barlerin, acetylbarlerin, 7-methoxydiderroside and lupulinoside [8]. Despite the popular use of this specie as a medicinal plant, there are no data about the pharmacological effect of flower of *B. prionitis* on the anti-inflammatory and anti-nociceptive activity. The aim of the present study was to evaluate the potential anti-inflammatory and anti-nociceptive activity of BPF extract on different experimental animal.

MATERIAL AND METHOD

Plant material

The *B. prionitis* flowers were collected from Botanical Garden of National Botanical Research Institute (NBRI), Lucknow, India in month of March & authenticated taxonomicaly by Dr. Sayeeda Khatoon, and the voucher specimens (NAB 180027) were deposited in the departmental herbarium (Pharmacognosy and Ethnopharmacology Division, NBRI, Lucknow) for future reference.

Preparation of extract

The freshly collected *B. prionitis* flowers were washed with distilled water and air-dried under the control conditions and powdered. The powdered plant material was percolated with petroleum ether to remove fatty substances; the marc was further exhaustively extracted with of 50% ethanol for 3 days. The extract was separated by filtration and concentrated on rotavapour (Buchi, USA) and then dried in lyophilizer (Labconco, USA) under reduced pressure and low temperature obtain solid residue (yield 14.10 % w/w).

Preliminary phytochemicals analysis and Finger print profile by HPTLC

The chemical constituents of the *B. prionitis (*BPF) extract were identified by qualitative analysis [9, 10, 11]. This indicates the presence of alkaloids, glycosides and flavonoids.

The high performance thin layer chromatography (HPTLC) studies of BPF, were carried out on precoated silica gel (Merck 60F 254nm) as the stationary phase and ethylacetate: toluene: methanol (9:1:1) as mobile phase. The plate was observed at wavelength 254 nm and was scanned on TLC scanner III using CAT software. The HPTLC profile and fingerprint profile of the extract was illustrated in figure 1.

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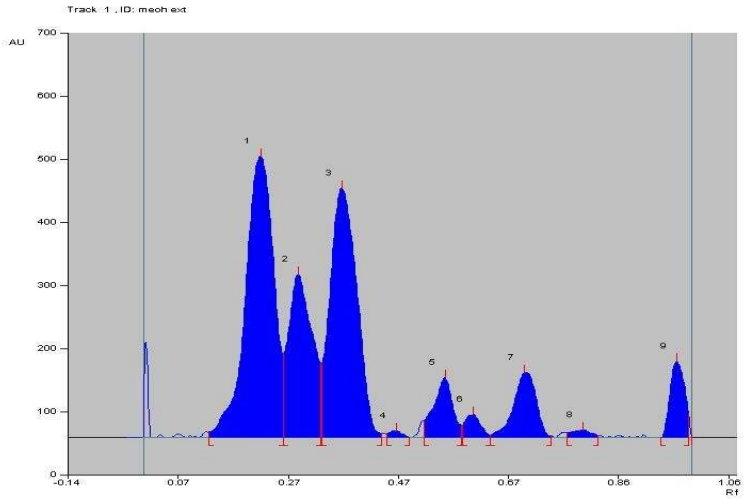

Fig.1. HPTLC fingerprint profile of *B. prionitis* flower extract scan at 254nm.

Test animals

Sprague-Dawley rats (150-175g) and mice (25-30g) of either sex were procured from the animal house of Central Drug Research Institute, Lucknow. They were kept in the departmental animal house at temperature 26 ± 2 °C, relative humidity 44 - 56%, light and dark cycles of 10 and 14 h respectively for one week before and during the experiments. Animals were provided with standard rodent pellet diet (Dayal, India) and the food was withdrawn 18-24 h before the experiment though 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. 222/2000/CPCSEA).

Drug Treatment

The BPF extract (suspended in 1% carboxy methyl cellulose) at the dose levels of 50, 100, 200 mg/Kg body wt., p.o. was administered once daily for three consecutive days. Phenylbutazone (100 mg/Kg; p.o.) was used as standard anti-inflammatory and anti-nociceptive activity. Control group of animals (n=6) received suspension of 1% CMC in distilled water (10 ml/Kg). Experiments were conducted on day 3, 60 min. for anti-inflammatory and 30 min. for anti-nociceptive activity [12].

Evaluation of anti-inflammatory activity

λ Carrageenin-induced paw oedema

Rats were injected with 0.1 ml of 1% λ carrageenin into the sub-planter side of the left hind paw [13]. The paw was marked with ink at the level of lateral malleolus and dipped in perspex cell up to this mark. The paw volume was measured immediately with an Ugo Basile Plethysmometer (No: 61402, 7140 Comerio-varese, Italy) and 3 h after injecting the λ carrageenin suspension. The BPF extract and phenylbutazone was administered

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orally by gavage, 1 h before the λ carrageenin injection. Significant reduction in the paw volume compared to vehicle treated control animals were considered as anti-inflammatory response. Percentage inhibition of oedema was calculated as follows:

$$\% \text{ Inhibition} = (1 - V_T / V_C) \times 100$$

V_T = Paw volume in drug treated rats.

V_C = Paw volume in control group of rats.

Cotton pellet induced granuloma formation

The rats were anesthetized with ether and incision was made on the lumber region [14]. By a blunted forcep subcutaneous tunnel was formed and cotton (100 mg ± 1 mg) was inserted in the groin area. Groups of 6 animals received either test drug (50, 100, 200 mg/Kg body wt., p.o.) or reference drug (100 mg/Kg body wt.) for seven consecutive days from the day of cotton pellet insertion. The animals were sacrificed and the pellets were removed and dried until the weight remained constant on 8th day according to the procedure described and the net dry weight was calculated [15].

Evaluation of anti-nociceptive activity

Analgesio-meter induced pain

The analgesic effect of BPF extract tested in mice of either sex, using an Ugo Basile Analgesy meter (No. 32725, 21025 Comerio-varse, Italy) [16]. This method involves the application of force to the paw of the mice using the analgesy-meter, which exert a force that increase at constant rate. The mice were gently placed between plinth and plunger. The instrument was switched on and a constant motor rate was used to drive the plunger on to the paw of the mice. When the mice struggle, the instrument was switched off and the force at which animal felt pain was read on a scale calibrated in grams x 10 by a pointer. The pre and the post treatment weight causing pain were determined for each mouse. The doses of test drug or reference drug were administered 60 minutes before testing.

Acetic acid induced writhing

Animals received BPF extract (50, 100, 200 mg/Kg) and standard drug orally 30 min before the injection of 0.6% acetic acid (10ml/Kg, i.p) [17]. The number of abdominal contractions (writhing) and stretching with a jerk of the hind limb were counted for 15 minutes after administering acetic acid and % inhibition was calculated.

Gross behavior and acute toxicity studies

Different doses (50-2000 mg/Kg, p.o) of BPF extract were administered to group of 10 mice of each dose, while one group of the same number of mice served as control. The animals were observed continuously for 1 h and then at half-hourly intervals for 4 h, for any gross behavior changes, including general motor activity, writhing, convulsion, response to tail pinching, piloerection, pupilsize fecal output and feeding behavior and further up to 72 h 15 days for any mortality [18].

Statistical analysis

All the data are presented as mean ± SEM and one-way analysis of variance (ANOVA) and Newman-Keuls Multiple Comparison Test were applied for determining the statistical significance between different groups.

RESULTS

The preliminary HPTLC studies revealed that the solvent system ethylacetate: toluene: methanol (9:1:1) was ideal and gave well-resolved sample

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peaks (Fig.1). The spots of the chromatogram were visualized at 254nm with a 400k filter at *Rf* values of 0.23, 0.29, 0.34. These are the major spots which are visualized on TLC plate.

The BPF extract at the dose level of 50, 100 and 200 mg/Kg caused a dose-dependent inhibition of swelling caused by the λ Carrageenin at 3 h equivalent to 17.8–48.6% (P>0.05–P<0.001) protection (Table 1).

Table 1.
Effect of B. prionitis flower extract on λ Carrageenin-induced paw oedema in rats*

Treatment	Dose (mg/Kg)	Paw volume (ml) at 3 h	
		λ Carrageenin	% inhibition
Control	----	1.46 ± 0.21	-----
BPF	50	1.20 ± 0.08*	17.8
BPF	100	0.90 ± 0.10**	38.3
BPF	200	0.75 ± 0.02***	48.6
Phenylbutazone	100	0.62 ± 0.04***	57.5

*Values are mean ± SEM for six rats.
*P> 0.05 compared to control group.
**P< 0.01 compared to control group.
***P< 0.001 compared to control group.

Table 2.
Effect of B. prionitis flower extract on cotton pellet-induced granuloma in rats*

Treatment	Dose (mg/Kg)	Dry weight (mg)
Control	-----	52.5 ± 2.6
BPF	50	46.2 ± 0.80*
BPF	100	39.5 ± 0.70**
BPF	200	36.4 ± 0.43**
Phenylbutazone	100	36.1 ± 0.34**

*Values are mean ± SEM for six rats.
*P< 0.01 compared to control group.
**P< 0.001 compared to control group.

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Table 2. summarises that BPF extract on cotton pellet granuloma in rats. BPF at the dose level of 50, 100 and 200 mg/Kg significantly decreased the granuloma weight from 15.32–36.4% (P<0.01–P<0.001) respectively compared to reference compound phenylbutazone 36.1% (P<0.001), (Table 2).

The BPF extract at the dose level of 50, 100 and 200 mg/Kg caused a significant increase in the analgesio-meter-induced force (P < 0.01 to P < 0.001) and exhibited resistance against pain after 30 min equivalent to 26.3–48.23% protection respectively (Table 3).

Table 3.
Effect of B. prionitis flower extract on force induced pain in mice*

Treatment	Dose	Weight causing pain (g)	
		Before dministration	**After administration**
Control	--	87.1 ± 2.6	86.91± 3.29
BPF	50	86.8 ± 1.7	99.0 ± 4.08*
BPF	100	86.2 ± 1.9	124.5 ± 3.1**
BPF	200	87.4 ± 2.6	147.6 ± 3.1**
Phenylbutazone	100	85.1 ± 3.29	156.6 ± 6.5**

*Values are mean ± SEM for six rats.
*P> 0.05 compared to control group.
**P< 0.001 compared to control group.

The BPF extract at the dose level of 50, 100 and 200 mg/Kg in stretching episodes induced by acetic acid (0.6%) are summarized in Table 4. The BPF showed significant reduction in abdominal cramping and percentage inhibition of abdominal cramping was from 5.24 - 34.6 % (P<0.05–P<0.001) The BPF at the dose of 5 mg/kg was insignificant statistically.

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Table 4.
Effect of B. prionitis flower extract on acetic acid induced pain in mice*

Treatment	Dose (mg/Kg)	No. of writhing	% inhibition
Control	----	24.8 ± 1.4	---
BPF	50	23.5 ± 0.26*	5.24
BPF	100	18.5 ± 0.60**	25.4
BPF	200	17.2 ± 0.66**	30.6
Phenylbutazone	100	16.2 ± 0.37**	34.6

*Values are mean ± SEM for six rats.
*P< 0.05 compared to control group.
**P< 0.001 compared to control group.

The Gross behavior and acute toxicity studies of BPF extract were observed in the surviving mice and rats up to dose of 2000 mg/Kg body weight orally. There were no changes in nature of stool, urine and eye color of all the animals.

DISCUSSION

The present study establishes the anti-inflammatory and anti-nociceptive activity of 50% ethanolic extract of *B. prionitis* flower. The BPF extract showed significant anti-inflammatory activity against λ Carrageenin and Cotton pellet induced granuloma in rats. λ Carrageenin is sulphated polysaccharide obtained from seaweed (Rhodophyceae) is commonly used to induce acute inflammation and is belived to be biphasic. The first phase is due to release of histamine and serotonin. The second phase is caused by the release of bradykinin, protease, prostaglandin and lysosome [19]. It has been reported that the second phase of the edema is sensitive to most clinically effective anti-inflammatory drugs, which has been used frequently to access the anti-edematous effect of natural products [20, 21]. Prostaglandins play a major role in the development of second phase of reaction, which is measured at around 3 h times [22]. The carrageenin induced paw edema model in rats is known to be sensitive to cyclooxygenase (COX) inhibitors and has been used to evaluate the effect of non-steroidal anti-inflammatory agents against which primarily inhibit the enzyme COX involved in prostaglandin synthesis. Based on these report, it can be inferred that the inhibitory effect of BPF extract on carrageenin – induced inflammation in rats could be due to the inhibition of the enzyme COX leading to inhibition of prostaglandin synthesis. But lipo-oxygenase inhibitors also posses significant anti-inflammatory activity against carrageenin induced

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paw edema. In cotton pellet induced granuloma model of sub-acute inflammation, the extract of BPF extract significantly reduced the weight of granulation tissue [23]. This method shown that foreign body granulomas were provoked in rats by subcutaneous implantation of pellets of compressed cotton. This method has been useful for evaluation of steroidal and nonsteroidal anti-inflammatory drugs [24].

The Extract of *B. prionitis* flower showed significant resistance in pain, which is characterized as writhing response. Constriction of abdomen, turning of trunk (twist) and extension of hind legs are taken as reaction to acetic acid induced writhing in mice. Moreover, significant resistance against mechanical pain indicates the potent analgesic activity of BPF. Acetic acid causes an increase in peritoneal fluids of PGE_2 and $PGF_{2\alpha}$ involving in part, peritoneal receptor [25, 26] and is very sensitive method for screening of analgesic compounds [27].

It is also apparent from this study that BPF extract is well tolerated in mice after oral administration and does not cause death or any toxic symptoms. Thus, using the very small doses given orally in the different validated experimental models is encouraging enough to warrant further studies and to explore its possible therapeutic role as an anti-inflammatory and anti-nociceptive activity in modern clinical practice.

CONCLUSION

Our result suggests that the administration of BPF extract showed inhibition of inflammation and pain in the experimental animals. Further studies are in progress to find out exact mechanism of action and responsible active constituents for anti-inflammatory and anti-nociceptive activity.

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