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Research Article

Evaluation of the anti-nociceptive potential of ethanolic extract of leaves of *Bryophyllum pinnatum* in experimental animals

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ABSTRACT

Background: The plant *Bryophyllum pinnatum* is traditionally used for the treatment of pain and inflammation. The present study was carried out to evaluate the antinociceptive effect of the ethanolic extract of the leaves of *B. pinnatum* (EEBP) using a hot plate method and acetic acid induced writhing test in mice.
Methods: In the hot plate analgesiometer method, the time between the placement on the hot plate and the occurrence of licking of the paws, shaking or jumping off from the plate was recorded as response latency. Total numbers of stretching episodes for 30 mins immediately after acetic acid injection in all the groups were recorded in acetic acid induced writhing method. Pentazocine (10 mg/kg intraperitoneal) and aspirin (500 mg/kg) were used as the standard drugs in the hot plate and acetic acid induced writhing method, respectively. Extract was used in 200, 300 and 400 mg/kg doses.
Results: At all the three doses the EEBP showed significant ($p<0.01$) anti-nociceptive activity in experimental models of Eddy's hot plate analgesiometer and acetic acid induced writhing method in mice.
Conclusion: The observed pharmacological activities provide the scientific basis to support traditional claims, as well as exploring some new and promising leads in the management of pain.

Keywords: Anti-nociceptive, *Bryophyllum pinnatum*, Hot plate test, Writhing test

INTRODUCTION

The plant-based traditional medicine system continues to play an essential role in health care with about 80% of the world's inhabitants relying mainly on traditional medicines for their primary healthcare.[1] The International Association for the Study of Pain's widely used definition states: "pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage." Pain is a common situation and is one of the most frequently observed symptoms of different pathology and the management of pain is challenging.[2] It is a major symptom in many medical conditions and can significantly interfere with a person's quality of life and general functioning.[3] Pain is an unpleasant sensation no doubt, but pain is mainly a protective mechanism for the body. It occurs whenever any tissues damage or nerve damage or dysfunction in the peripheral or central nervous system, and it causes the individual to react to remove the pain stimulus. Analgesics are the drug that selectively relieves pain by acting in the central nervous system or on peripheral pain mechanisms, without significantly altering consciousness.[4] Analgesic drugs act in various ways on the peripheral and central nervous systems. Nociceptive pain is caused by stimulation of peripheral nerve fibers that respond only to stimuli approaching or exceeding harmful intensity (nociceptors), and may be classified according to the mode of noxious stimulation. Neuropathic pain is caused by damage or disease affecting any part of the nervous system involved in bodily feelings (the somatosensory system).[5] Peripheral neuropathic pain is often described as "burning," "tingling," "electrical," "stabbing," or "pins" and needles."[6]

Bryophyllum pinnatum (Crassulaceae) is a widely used medicinal plant in the traditional system with a wide range of biological activities.[7] It is a crassulescent herb of about 1 m in height, with opposite, glabrous leaves (with 3-5 deeply crenulated, fleshy leaflets),[8] distributed worldwide, but growing primarily in the rain forest.[9,10] It grows widely and is used as folk medicine in tropical Africa, India, China,

Australia, America, Madagascar, Asia, and Hawaii.[11,12] Flavonoids, polyphenols, and triterpenoids have been identified from the leaves of *B. pinnatum*. Quecetin-3-α-L-rhu-β-D-xyl; a flavonoid; bryophyllin B, a novel potent cytotoxic bufadienolide, and malic acid were isolated from the leaves of *B. pinnatum*.[13] The leaves and bark are bitter tonic, astringent to bowels, analgesic, carminative, and are useful in diarrhea and vomiting. Antimicrobial, antifungal, antiulcer, anti-inflammatory, and analgesic activities of leaf extract were reported. Juice of the fresh leaves is used very effectively for the treatment of jaundice in folk medicine of Bundelkhand region of India.[14] A water extract of Bryophyllum leaves administered topically and internally has been shown to prevent and treat leishmaniasis.[15] The plant is used traditionally for the treatment of earache, in ophthalmic preparations, sprains, and in dysmenorrhea.[16] The juice from fresh leaves is used to treat smallpox, otitis, cough, asthma, palpitations, headache, convulsion, and general debility.[17] It is well known for its hemostatic and wound healing properties.[18] Methanol extract of the leaves have histamine receptor (H1) antagonism in the ileum, peripheral vasculature, and bronchial muscle.[19]

Currently available analgesic drugs such as opiates and non-steroidal anti-inflammatory drugs (NSAIDs) though useful, have their own side effect profile. Opiate analgesic such as morphine has strong addictive potential and other side effects while NSAIDs are well known for their ability to produce gastrointestinal bleeding, ulceration, etc., therefore, search for new analgesic drugs with promising pharmacological actions has become an urgent need.

METHODS

Experimental animals

Swiss albino mice of either sex, weighing between 20 and 30 g, were procured from animal house of Department of Pharmacology, Gauhati Medical College. The animals were housed at 25±2°C with 12 hrs light and dark cycle and allowed to standard diet and water *ad libitum*. They were acclimatized to laboratory condition for 1-week before the study. The study was approved by the Institutional Animal Ethics Committee of Gauhati Medical College and Hospital. CPCSEA guidelines were adhered during the experiment.

Plant materials

The leaves of *B. pinnatum* were collected from in and around Guwahati. Authentication of the plant was done in the Department of Botany, Gauhati University and a voucher specimen was preserved for further reference. The whole plant was thoroughly washed, shade dried, and then chopped to a coarse powder using a mixer grinder. Powder (200 g) was tightly packed in Soxhlet apparatus and extracted employing ethanol as solvent for 5 days at a temperature of 40-60°C using a heating mantle. The extract was filtered using Whatman filter paper no.1 and the filtrate was evaporated on a water bath until it gets concentrated. The jelly-like extract of the leaves was collected in a Petri dish. A final yield of 40.5 g was obtained. The percentage yield of *B. pinnatum* was 20.25% (w/w) with respect to the original dried powder. The extract was stored in a refrigerator at 4°C in labeled air-tight containers for further use.

Drugs and chemicals

Pentazocine, aspirin, acetic acid, gum acacia, ethanolic extract of *B. pinnatum* (EEBP)

Acute toxicity study

Acute toxicity study was done according to OECD 425 guidelines. The animals were found to be alive at 2000 mg/kg per oral feeding of the EEBP.

Methodology

Test for anti-nociceptive activity

Hot plate method

The central analgesic activity of the test drug is studied against thermal stimuli using this method. A total of 30 animals were divided into five groups containing six in each. They were fasted for 24 hrs before the test with free access to water. Animals were placed in Eddy's hot plate analgesiometer (Labotech) maintained at 55±0.5°C. The time between the placement on the hot plate and the occurrence of licking of the paws, shaking or jumping off from the plate was recorded as response latency. The reaction time was measured before administration and at 30 and 60 mins after administration of the extract. Cut off time was 20 sec.

Group 1: Control, received normal saline at a dose of 10 ml/kg per orally.

Group 2: Standard, received pentazocine 10 mg/kg intraperitoneal (i.p).

Group 3: EEBP at a dose of 200 mg/kg per orally.

Group 4: EEBP at a dose of 300 mg/kg per orally.

Group 5: EEBP at a dose of 400 mg/kg per orally.

Acetic acid induced writhing test

The painful stimulus is induced by i.p injection of an irritant substance, acetic acid, and peripheral analgesic activity is evaluated. The i.p injection of acetic acid results in constriction of abdominal muscle together with stretching of hind limbs known as writhing syndrome. 1-hr prior to i.p injection of acetic acid (10 ml/kg of 0.7% v/v solution) standard drug, aspirin, and extracts at all the three different doses were administered. Total numbers of stretching episodes for 30 mins immediately after acetic acid injection in all the groups were recorded.

Group 1: Control, received normal saline at a dose of 10 ml/kg per orally.

Group 2: Standard, received aspirin dissolved in 5% gum acacia in a dose of 500 mg/kg per orally.
Group 3: EEBP at a dose of 200 mg/kg per orally.
Group 4: EEBP at a dose of 300 mg/kg per orally.
Group 5: EEBP at a dose of 400 mg/kg per orally.

Statistical analysis

Mean±standard error of mean (SEM) values were calculated for each group. Significant differences between the groups were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test and results were found to be significant ($p<0.05$). All analysis were done using GraphPad prism software version 5.01.

RESULTS

Results are presented as mean±SEM of six animals in each group in the following Tables 1 and 2 & Figures 1 and 2.

Table 1 and Figure 1 show the effect of EEBP in a hot plate model of analgesia. The paws of rats are sensitive to heat and they respond by jumping, withdrawal of paws, and licking of paws. The baseline recordings were first taken in all the groups. After feeding the extract at all the three doses and injecting the standard drug, pentazocine i.p, the recordings were taken.

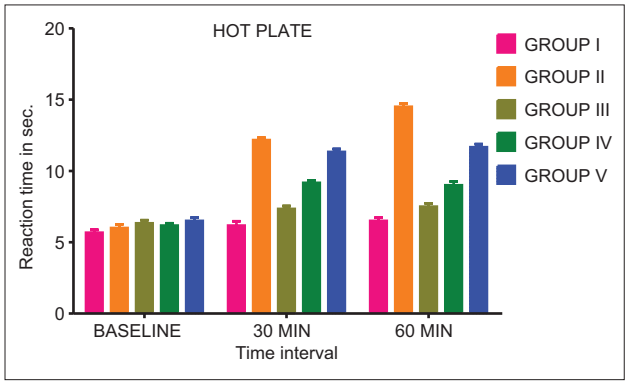

Figure 1: Hot plate test using Eddy's hot plate analgesiometer.

At the initiation when baseline recordings were taken in the hot plate, the mean reaction time of normal control group was 5.66±0.210 sec, in standard group it was 6.00±0.258 sec, in the Group 3 (EEBP 200 mg/kg) it was 6.33±0.210 sec, in Group 4 (EEBP 300 mg/kg) it was 6.16±0.166 sec, and in Group 5 (EEBP 400 mg/kg) the mean reaction time was 6.16±0.166. There was no significant difference in reaction time in between the different groups.

At 30 mins, the mean reaction time of normal control group was 6.16±0.307 sec, reaction time in the standard group was 12.16±0.166 sec, in the Group 3 (EEBP 200 mg/kg) it was7.33±0.210 sec, in the Group 4 (EEBP 300 mg/kg) it was 9.16±0.166, and in the Group 5 (EEOC 400 mg/kg) it was 11.33±0.210 sec.

At 60 mins, the mean reaction time of normal control group was 6.50±0.223 sec, mean reaction time of standard group was 14.5±0.223 sec, in the Group 3 (EEBP 200 mg/kg) it was 7.5±0.223 sec, in the Group 4 (EEBP 300 mg/kg) it was 9.0±0.258 sec, and in the Group 5 (EEBP 400 mg/kg) it was 11.66±0.210 sec.

It can be inferred that at 30 and 60 mins the reaction time in seconds i.e. withdrawal latency was maximum with the nociceptive standard, followed by Group 5 (EEBP 400 mg/kg), and then Group 4 (EEBP 300 mg/kg), and Group 3 (EEBP 200 mg/kg). An increase in the time interval was indicative of analgesia. One-way ANOVA of the data, when done the p value was found to be significant. It was followed by Dunnett's test. There was a significant difference in the groups when normal control was taken as the control. The analgesic effect of the extract increased in a dose-dependent manner.

The analgesic activity was also determined by observing the number of writhing movements in all the groups. The mean number of writhing movements with the normal control group was found to be 67.16±0.600, in the standard group it was found to be 30.33±0.421, with the Group 3 (EEBP 200 mg/kg) it was 61.16±0.307, with Group 4 (EEBP 300 mg/kg) it was found to be 50.66±0.333, and it was 40.66±0.210 in Group 5 (EEBP 400 mg/kg). One-way

Table 1: Analgesic activity of EEBP in mice using hot plate method.

Groups	Reaction time in seconds (mean±SEM)		
	Baseline	30 mins	60 mins
Group 1 (normal control)	5.66±0.210	6.16±0.307	6.50±0.223
Group 2 (pentazocine 10 mg/kg)	6.00±0.258	12.16±0.166$^{\#}$	14.5±0.223$^{\#}$
Group 3 (EEBP 200 mg/kg)	6.33±0.210	7.33±0.210$^{\#}$	7.5±0.223$^{\#}$
Group 4 (EEBP 300 mg/kg)	6.16±0.166	9.16±0.166$^{\#}$	9.0±0.258$^{\#}$
Group 5 (EEBP 400 mg/kg)	6.50±0.223	11.33±0.210$^{\#}$	11.66±0.210$^{\#}$
ANOVA			
F	2.202	136.2	202.9
df	4,25	4,25	4,25

$^{\#}p<0.05$ when compared with the control group (Group 1). EEBP: Ethanolic extract of *Bryophyllum pinnatum*, SEM: Standard error mean

ANOVA of the data when done, the p value was found to be significant. It was followed by Dunnett's multiple comparison tests. There was a significant difference in the groups when normal control was taken as the control. The number of writhes was significantly reduced in the test groups and the standard group. It was minimum with the standard drug, followed by 400 mg/kg dose of the extract, 300 mg/kg, and then 200 mg/kg dose of EEBP. Thus, the anti-nociceptive effect of the extract increased in a dose-dependent manner in acetic acid induced writhing method as well.

Table 2 and Figure 2 show the effect of EEBP in acetic acid induced writhing method of analgesia.

DISCUSSION

In the study to look for the anti-nociceptive effect, both the central and peripheral models of analgesia were used. The hot plate method was used to look for the central mechanism of analgesia, whereas to look for the peripheral mechanism acetic acid-induced writhing response was used. It was seen that at 30 and 60 mins, the reaction time in seconds., i.e. withdrawal latency was maximum with the nociceptive standard pentazocine, followed by EEBP 400 mg/kg, EEBP 300 mg/kg and then EEBP 200 mg/kg. An increase in the time interval was indicative of analgesia. With the increase in dose of the extract, it was seen that the reaction time was increased. In the peripheral model, acetic acid was injected i.p., which resulted in writhing response in mice due to the irritant nature of acetic acid. The number of writhing was significantly reduced in both the test groups and the standard group. It was minimum with the standard drug, aspirin followed by the highest dose of the extract 400 mg/kg. The analgesic effect of the extract increased in a dose-dependent manner. The anti-nociceptive activity demonstrated by the extract might be due to the presence of flavonoids and tannins in the extract. This was supported by other workers who found that flavonoids and tannins were found to have anti-nociceptive and/or anti-inflammatory activities.[20] Abdominal constriction responses were found to partly involve local peritoneal receptors.[21] The method has been associated with prostanoids in general, e.g. increased levels of prostaglandins E2 (PGE2) and PGF2α in peritoneal fluids,[22,23] as well as lipoxygenase products.[24,25] The abdominal contraction is related to the sensitization of nociceptive receptors to PG. It is therefore possible that *B. pinnatum* exerts its analgesic effect probably by inhibiting the action of PG. Previous phytochemical analysis on various plants used as an analgesic have revealed that one of the largest groups of chemicals present are the alkaloids and their amazing effect on humans has led to the development of powerful painkiller medications. The presence of alkaloids in *B. pinnatum* might be responsible for its anti-nociceptive property.

Table 2: Analgesic activity of EEBP on acetic acid-induced writhing response in mice.

Groups	Number of writhing movements (mean±SEM)
Group 1 (normal control)	67.16±0.600
Group 2 (aspirin 500 mg/kg p.o)	30.33±0.421#
Group 3 (EEBP 200 mg/kg p.o.)	61.16±0.307#
Group 4 (EEBP 300 mg/kg p.o)	50.66±0.333#
Group 5 (EEBP 400 mg/kg p.o)	40.66±0.210#
ANOVA	
F	1416
df	4,25

#p<0.05 when compared with the control group (Group 1). EEBP: Ethanolic extract of *Bryophyllum pinnatum*, SEM: Standard error mean

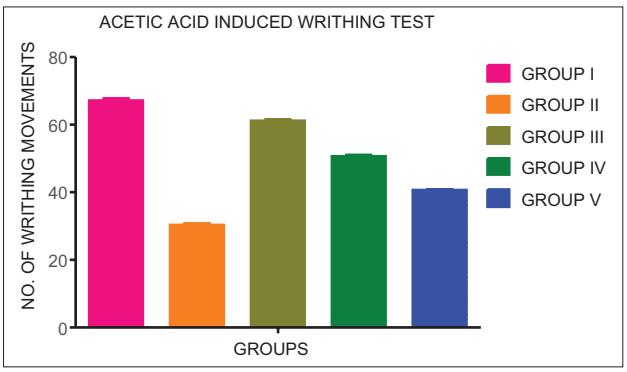

Figure 2: Acetic acid-induced writhing in mice.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Animal Ethics Committee

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