ORIGINAL ARTICLE

Evaluation of analgesic activity of the leaves of *Passiflora incarnata* Linn

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Passiflora incarnata also known as 'Passion flower' is used as an anxiolytic and sedative throughout the world from ancient time. The plant is used as an analgesic, antispasmodic, sedative- hypnotic and narcotic. It is also used in neuralgia, epilepsy, insomnia, ulcers, haemorrhoids and neurosis in many parts of the world. There was no report on analgesic activity of *P. incarnata*. Hence, the present study is designed to assess analgesic activity of leaves of *P. incarnata* using sodium chloride-induced eye wiping test and formalin test. In formalin test, n-butanol extract of leaves of *P. incarnata* (BEPI) in the doses of 150 and 300 mg/kg as well as BEPI-F1 showed significant reduction in duration of paw licking in neurogenic and inflammatory phase($P<0.001$). Pretreatment with naloxone reversed the analgesia induced by BEPI, while atropine did not reverse the analgesia induced by BEPI significantly ($P\leq0.001$). In eye wiping test, BEPI in the doses of 150 and 300 mg/kg, i.p. exerted significant reduction ($P\leq0.001$) in number of eye wipes compared to control group. Thus, the result concludes that BEPI and the fraction separated, BEPI–F1 has significant analgesic activity, which may be mediated through central mechanism by modulation of opioid receptors and nicotinic receptors.

Key words: Analgesic activity, eye wiping test, *Passiflora incarnata*, trigeminal neuralgia

INTRODUCTION

Passiflora incarnata Linn (Passifloraceae), also known as Maypop or Passion flower, is a plant used as an anxiolytic and sedative since a long time throughout the world.[1] *P. incarnata* is a popular traditional European remedy as well as a homoeopathic medicine for insomnia and anxiety. It is used as a sedative tea in North America and as an analgesic, antispasmodic, antiasthmatic, wormicidal, and sedative in Brazil; as a sedative and narcotic in Iraq; for conditions such as dysmenorrhea, neuralgia, epilepsy, insomnia, and neurosis in Turkey; to treat hysteria and neurasthenia in Poland; and for diarrhoea, neuralgia, burns, haemorrhoids, and insomnia in the United States.

The main constituents of *P. incarnata* leaves are flavonoid (0.25%) such as vitexin, isovitexin, orientin, isoorientin, apigenin and kampferol. Besides flavonoid, various indole alkaloids based on ß-carboline ring system are present, which include harman, harmine, harmalol and harmaline. Various other phytoconstituents present in *P. incarnata* include carbohydrates, essential oil, amino acids and a cyanogenic glycoside gyanocardin.[2]

Access this article online	
Quick Response Code:	Website: www.greenpharmacy.info
	DOI: 10.4103/0973-8258.97121

P. incarnata Linn (Passifloraceae) has been known to possess anxiolytic, sedative-hypnotic, anticonvulsant, anti-inflammatory, antitussive, antiasthmatic and aphrodisiac activity.[3-8] The traditional claims about *P. incarnata* suggest its potential as analgesic and an important candidate for neuralgia. However, there were no scientific reports presented on the analgesic activity of *P. incarnata*. Hence, the present study is designed to validate the uses of *P. incarnata* in trigeminal neuralgia and as an analgesic.

MATERIALS AND METHODS

Plant Materials

The fresh leaves of *P. incarnata* were collected in the month of June, July and August from local nursery in Pune Maharashtra, India. The plant was identified and authenticated (Voucher Specimen No: PASSIN 3) by Botanical Survey of India, Pune, India. Shade dried leaves (1000 g), powdered and macerated with ethanol for 48 hrs. The extract was evaporated to dryness. The leaf extract was suspended in water and extracted successively with hexane, chloroform, ethyl acetate and n-butanol. The n-butanol (BEPI) extract was evaporated to dryness (22.5% yields). The n-butanol (BEPI) extract

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Received: 21-01-2012; **Accepted:** 21-02-2012

(1 g) was subjected to column chromatography on silica gel G (mesh size 60-120) column using chloroform:methanol (1:1) solvent system for separation of phenolic compounds and 1 liter fraction was collected and concentrated to dryness under reduced pressure (BEPI-F1-50% yield).

Drugs and Chemicals

Ethanol (Baker, Germany), naloxone (Sigma, St. Louis, USA), Formalin, atropine, mecamylamine and sodium chloride (S.D. Fine Chem. Limited, Mumbai, Maharashtra, India) of highest quality were used.

Animals

All the experimental protocols were approved by the Institutional Animal Ethical Committee (Protocol No: ICP/IAEC/10-11/P-15 and P-16) and carried out as per CPCSEA guidelines. Swiss albino mice weighing between 25-30 g of either sex were used. Animals were housed under standard conditions of temperature (24±2°C) and relative humidity (30-70%) with a 12:12 light: Dark cycle. The animals were fed with standard pellet diet and water *ad libitum*.

METHODS

Formalin Test

Experimental group of mice (n=6) were injected 20 µl (0.02 ml) of 1% formalin (in 0.9% saline) into sub-plantar space of the hind paw. The duration of paw licking was determined between 0-5 min (first phase) and 15-30 min (second phase) after formalin injection. Animals were treated intraperitoneally with vehicle (normal saline), pentazocine (10 mg/kg), BEPI (150 and 300 mg/kg) and BEPI-F1 (300 mg/kg) 30 minutes before formalin injection. Naloxone (1.5 mg/kg, s.c.) was given 30 minutes before BEPI and atropine (5 mg/kg, i.p.) and mecamylamine (1 mg/kg, i.p.) was given 15 minutes before BEPI. The paw licking time of treated animals was compared to control group and represented as percent inhibition.[9,10]

$$\% \text{ inhibition} = \frac{N - N^t}{N} \times 100$$

Where, N=Average duration of licking of control group
N^t = Average duration of licking of test group

Eye Wiping Test

Experimental group of mice (n=6) were placed on a 50×50 cm Table for 10 min habituation period. One drop (40 µl) of 5M NaCl was put into the right or left eye of animal and the number of eye wipes was counted during 30 seconds. Animals were treated intraperitoneally either with vehicle, carbamazepine (15 mg/kg), BEPI (150 and 300 mg/kg) and BEPI-F1 (300 mg/kg) 30 minutes before putting 5M NaCl into the eye. Naloxone (1.5 mg/kg, s.c.) was given 30 minutes before BEPI and atropine (5 mg/kg, i.p.) and mecamylamine (1 mg/kg. i.p.) was given 15 minutes before intraperitoneal injection of BEPI.[11]

Statistical Analysis

All observations are expressed as mean±SEM. Statistical analysis of data received was performed using the software Primer of Biostatistics (Primer of Biostatistics, Version 4, Stanton A. Glantz). Calculation of the statistical significance (P value) was done by using ANOVA followed by Dunnett's test. A value of P<0.001 indicated a significant difference compared with the control.

RESULTS AND DISCUSSION

Pain is a subjective experience, which is difficult to define exactly even though we all experience it. Pain is distinguished as two types-peripheral or neurogenic.[12] The formalin test distinguishes two (early and late) phases of pain, which can reveal mechanisms of pain and analgesia. While centrally acting drugs such as narcotics could inhibit both phases equally, the peripherally acting drugs like aspirin inhibit only the late phase indicating a possible development of an inflammatory response and the release of algesic mediators.[13]

The formalin test is an important animal model in the study of acute long-lasting inflammatory pain. In this model, subcutaneous injection of diluted formalin into a hind paw elicits a biphasic pattern of pain-related behaviors, an early short-lasting neurogenic phase followed by a second and more sustained inflammatory phase. The formalin model is very useful for elucidating mechanism of pain and analgesia.[14,15]

In formalin test, BEPI in the doses of 150 and 300 mg/kg, i.p. exerted significant inhibitory effect on both phases of the formalin test. The maximum effect occurred at dose of 300 mg/kg, in which the duration of paw licking reduced from 182.75±19.7 to 71.5±11.05 (60% inhibition) in first phase and from 353±53.29 to 212.2±20.38 (39% inhibition) in second phase. Whereas, pentazocine (10 mg/kg, i.p.), exhibited about 63% and 37% inhibition in the first and second phase. A fraction separated from BEPI (BEPI-F1) produced 60% inhibition in first as well as second phase of formalin test, respectively [Figure 1]. Thus, BEPI in the doses of 150 and 300 mg/kg as well as BEPI-F1 showed significant reduction in duration of paw licking in neurogenic and inflammatory phase. This indicates that BEPI and BEPI-F1 has significant central analgesic activity.

Study of trigeminal pain and analgesic effects on trigeminal acute pains such as headache, muscle spasms, dental problems or post-surgery pain seems to be more problematic. The cornea is used for nociception studies in trigeminal system, since corneal nociceptive receptors have a large representation in the trigeminal ganglion through the

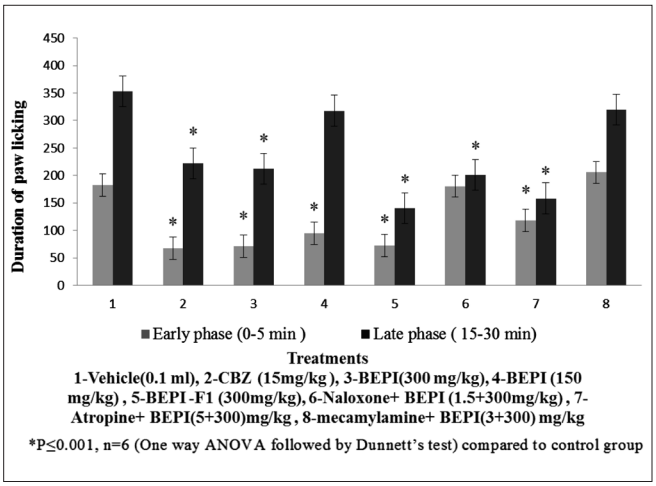

Figure 1: Effect of n-butanol extract of leaves of *Passiflora incarnata* (BEPI) on duration of paw licking in formalin test

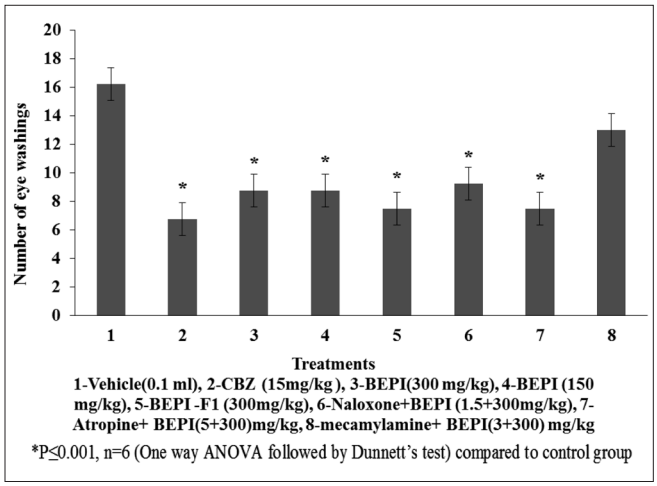

Figure 2: Effect of n-butanol extract of leaves of *Passiflora incarnata* (BEPI) on eye wiping test

ophthalmic branch of trigeminal nerve. Thin myelinated fibres as well as unmyelinated fibres in cornea respond to chemical, mechanical and thermal noxious stimuli. In rat, wiping the eye with forelimb is an obvious withdrawal response to corneal chemical stimuli. Some researchers have used eye wiping test for investigating the chemical pungency or the presence of C-fibre activity. Eye wiping test is a phasic analgesic test and is sensitive to centrally acting analgesics.[11]

In the eye wiping test, n-butanol extract of *P. incarnata* leaves in the doses of 150 and 300 mg/kg, i.p. exerted significant reduction in number of sodium chloride induced eye wipes compared to vehicle-treated group. BEPI significantly reduced number of wipes in mice from 16.25±2.65 to 8.75±1.43 in a dose of 300 mg/kg and to 8.75±0.25 in a dose of 150 mg/kg. Carbamazepine (15 mg/kg, i.p) also reduced the number of eye wiping significantly. A fraction separated from n-butanol extract of *P. incarnata* (BEPI-F1) exhibited maximum effect on eye wiping [Figure 2].

There are evidences[16] which suggest that cholinesterase inhibitors such as neostigmine and physostigmine, and atropine, a muscarinic receptor antagonist are involved in modulation of pain. Hence, to study exact mechanism involved in analgesic action of BEPI, naloxone (narcotic antagonist), atropine (muscarinic antagonist) and mecamylamine (nicotinic antagonist) were used. In formalin test, pretreatment with naloxone (1.5 mg/kg, s.c.) and mecamylamine (1 mg/kg, i.p.) reversed the analgesia induced by BEPI (300 mg/kg) significantly, while atropine (5 mg/kg) did not reverse the analgesia induced by BEPI (300 mg/kg). Therefore, the n-butanol extract of leaves of *P. incarnata* (BEPI) and BEPI-F1 was found to have central analgesic activity, which may be mediated through modulation of opioid receptors and nicotinic cholinergic receptors.

In eye wipe test, pretreatment with naloxone (1.5 mg/kg, s.c.) and atropine (5 mg/kg) do not reverse the effect of BEPI but pretreatment with mecamylamine (1 mg/kg, i.p.) decreased the antinociceptive effect of BEPI (P<0.001). Thus, both opioidergic and nicotinic cholinergic systems seem to be involved in central analgesic activity of BEPI. In our phytochemical study, we have shown that n-butanol extract of *P. incarnata* leaves contain tannins, glycosides, alkaloids, and flavonoid. Total flavonoid content was estimated as 45.33 mg chrysin equivalent/100 g of dry weight determined by aluminum chloride colorimetric assay (unpublished data). Several flavonoid isolated from medicinal plants are known to possess significant antinociceptive activity. It is, therefore, possible that analgesic activity of BEPI may be attributable to its flavonoid content.

CONCLUSION

In conclusion, this study has shown that n-butanol extract of *P. incarnata* leaves possess significant analgesic effect in laboratory animals at the doses investigated. The n-butanol extract of *P. incarnata* is effective and potent in the same way as that of reference analgesic drug. The analgesic potential is partly through an opioid-mediated mechanism and nicotinic cholinergic mechanism. Furthermore, the results of eye wipe test support the traditional use of this plant in relieving painful conditions like neuralgia. The analgesic activity may be attributed to flavonoid content of the plant. Further studies are in progress to isolate and characterize the active principles of the extracts.

ACKNOWLEDGMENTS

Authors are grateful to University of Pune for financial support and the Management of Indira College of Pharmacy, Pune- 411 033, Maharashtra, India for providing laboratory facilities.

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How to cite this article: Ingale S, Kasture S. Evaluation of analgesic activity of the leaves of *Passiflora incarnata* Linn. Int J Green Pharm 2012;6:36-9.

Source of Support: Authors are grateful to University of Pune for financial support., **Conflict of Interest:** None declared.