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Reversal of Morphine Tolerance and Dependence by *Passiflora incarnata* – A Traditional Medicine to Combat Morphine Addiction

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Abstract

This paper describes the use of *Passiflora incarnata* Linneaus in reversing the development of dependence and tolerance of morphine in mice. A fraction (BZF) derived from the methanol extract of *P. incarnata* that had exhibited good anxiolytic activity, delayed the development of tolerance to the analgesic effect of morphine when administered at 10, 50 and 100 mg/kg doses along with 10 mg/kg dose of morphine for 9 days. A single dose of *P. incarnata* bioactive fraction (BZF) also decreased the naloxone-precipitated withdrawal jumps in mice that had already been rendered tolerant due to chronic treatment with 10 mg/kg of morphine.

Keywords: Addiction, analgesia, dependence, morphine, *Passiflora incarnata* Linneaus, withdrawal effects.

Introduction

Abuse of morphine and its di-acetyl derivative heroin is the most alarming socio-economic problem being faced by health management agencies all over the world. In general, pharmacological or psychosocial approaches have been used, either separately or together, in the treatment of morphine-dependent persons (Katzung, 1992; UNDCP, 2001). Chronic users require pharmacological methods; those with a short history of morphine abuse are more amenable to psychosocial interventions. The former approach comprises the substitution of morphine with a longer-acting, orally active, pharmacologically equivalent drug with no abuse-potential, stabilizes the patient on that drug, and then gradually withdraws the substituted drug. Methadone is the most prevalent drug of choice for this method of de-toxification. Most recently, the morphine antagonist (viz., naltrexone, for its morphine-blocking potential) has been used for morphine de-addiction therapy. The psycho-social treatments include psychotherapy, didactic approaches, alternative life-styles through work or communal living, and so forth, but the chances of the person resorting to morphine again are always present.

In the traditional system of medicine in India, *Passiflora incarnata* Linn. (Passifloraceae; synonyms: passionflower, maracuja, maypops, *Krishan-Kamala*) attracted our attention due to various reports regarding the use of the plant in breaking down the morphine habit in addicts (Felter & Lloyd, 1983; Lad, 2000; Vasudev, 1955). *P. incarnata* is otherwise used (Bergner, 1995) in all parts of the world as a plant-derived anxiolytic, sedative, CNS-depressant and forms an active constituent of as many as 130 herbal, homoeopathic and allopathic medicinal preparations used in the treatment of various cardiovascular, respiratory and nervous system disorders (Reynolds, 1996). In recent studies by the authors, the methanol extract of aerial parts of *P. incarnata* has been reported to exhibit significant anxiolytic properties at 125 mg/kg, p.o. dose, in mice using the elevated plus-maze model of anxiety (Dhawan et al., 2001a). The leaves exhibited the maximum anxiolytic properties when the segregated plant parts were evaluated for their activity in mice (Dhawan et al., 2001b). The methanol extract of leaves of *P. incarnata* also exhibited good anti-tussive properties against SO_2-induced cough in mice (Dhawan & Sharma, 2002) and also had aphrodisiac properties at 100 mg/kg, p.o. dose, in male mice (Dhawan et al., 2001c). The same dose prevented acetylcholine-induced bronchospasm in guinea pigs, thus,

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inferred about anti-asthmatic and spasmolytic properties in the methanol extract of leaves of *P. incarnata* (Dhawan et al., 2001d). Resorting to bioactivity-directed fractionation and chromatographic procedures, the authors were able to separate and identify a new phyto-moiety from the methanol extract of aerial parts of *P. incarnata* that comprised a benzoflavone nucleus (BZF) and that was responsible for the anxiolytic and other biological effects of *P. incarnata* (Dhawan et al., 2001e; Dhawan et al., 2001f; Dhawan et al., 2001g). The BZF moiety exhibited significant anxiolytic effects at a dose of 10 mg/kg, p.o. in mice, using the elevated plus-maze model of anxiety (Dhawan et al., 2001e). The anxiolytic effects exhibited by the BZF moiety were better than the anxiolytic dose of (2 mg/kg, p.o.) of a standard anxiolytic diazepam. The BZF moiety was found to be potentially useful in the prevention of cannabinoid (Δ^9-tetrahydrocannabinol) dependence in mice at 10 and 20 mg/kg, p.o. doses (Dhawan et al., 2002).

Prompted from various literature reports pertaining to morphine-breaking properties of *P. incarnata*, and also the fact that morphine-addiction has become a global menace that has to be dealt with by the medical professionals, the present studies were designed to evaluate the effect of the bioactive benzoflavone moiety of *P. incarnata* on (1) development of tolerance to the anti-nociceptive effect of morphine, and (2) development of dependence to morphine assessed by the naloxone-precipitated withdrawal jumps in mice. In the present study, three different doses (10, 50 and 100 mg/kg) of the bioactive BZF fraction of *P. incarnata* were evaluated against morphine-induced tolerance and withdrawal effects in mice.

Materials and methods

Plant material

Aerial parts of *P. incarnata* were obtained in January 1999 from a cultivation source Rati Ram Nursery at the village Khurammpur via Kalsia in district Saharanpur (UP, India). The identity of the procured plant material was confirmed from the Department of Systematic Botany, Forest Research Institute, Dehradun (UP). A voucher specimen (code no. 1325/2000) was deposited in the Herbarium-cum-Museum of the Forest Research Institute, Dehradun.

Extraction, fractionation and isolation of the bioactive BZF

The aerial parts were dried in shade and powdered (# 60) and 100 g of the dried powder was Soxhlet extracted successively with petroleum ether (60–80 °C), chloroform (Ranbaxy Laboratory Chemicals), methanol (sd Fine-Chem Limited) and distilled water. All these extracts were dried using a Buchi 461 Rotary Vacuum Evaporator and were stored in a vacuum desiccator containing anhydrous silica blue. The weight of various extracts after drying was calculated as: petroleum ether extract (6.8875 g), chloroform extract (8.2314 g), methanol extract (11.8787 g) and water extract (4.8876 g). The four different extracts of *P. incarnata* were suspended in a vehicle comprising simple syrup I.P. and 1% w/w carboxymethylcellulose (CMC) as suspending agent. Five sets of doses (viz., 300, 200, 125, 100 and 75 mg/kg of each extract of *P. incarnata*) were prepared by suspending the dried extracts in the vehicle under vigorous stirring to form a uniform suspension. The weight of the dried extracts was so adjusted as to administer 0.25 ml of the suspension of the extracts. Simple syrup containing CMC (0.25 ml) was used as control. Amongst the four extracts, only the methanol extract of *P. incarnata* showed a significant anxiolytic activity at a dose of 125 mg/kg whereas the remaining three extracts did not exhibit anxiolytic activity statistically comparable to that of the standard anxiolytic (diazepam, 2 mg/kg in vehicle, p.o.). The bioactive methanol extract was processed and purified further by resorting to bioactivity directed fractionation using column chromatographic procedures until a fraction which exhibited significant anxiolytic activity at a dose of 10 mg/kg in mice was obtained. This fraction (yield = 332 mg, 0.33%) gave positive tests for the presence of flavones. The UV, LC-MS, GC-MS, IR, ^{1}H-NMR, ^{13}C-NMR characterization studies have confirmed the presence of a benzoflavone moiety (BZF), never reported from *P. incarnata* earlier, that has been accounted for the CNS properties of *P. incarnata* (Dhawan et al., 2001f). The exact structure and chemical identity of BZF is not being presented here due to patent considerations.

In the present study, the morphine reversal effects of *P. incarnata* were examined at three different doses of BZF (viz., 10 and 50 and 100 mg/kg).

Animals

Swiss albino mice (either sex) procured from the Disease Free Small Animals House, College of Veterinary Sciences, Haryana Agriculture University, Hisar, India, were bred at the Central Animal House of the Panjab University, Chandigarh. The mice were allowed standard laboratory feed and water *ad libitum*. Groups of five mice (20–24 g) were used in all sets of experiments. Tolerance and dependence to morphine was induced in mice by a repeated administration of morphine sulfate (10 mg/kg, p.o.) twice a day at 09:00 and 17:00 h for 9 days.

Drugs

Morphine sulfate (Pharma-Chemico Laboratories, Solan, Himachal Pradesh), naloxone (Sigma, USA) and *P. incarnata* bioactive fractions (BZF) were suspended in the vehicle comprising simple syrup IP with the aid of 1% carboxymethylcellulose (CMC). All the test drugs were administered orally 1 h before testing tail-flick latency or naloxone (2 mg/kg, p.o.) challenge.

Treatment schedule

For assessing of the effects of BZF on the induction of morphine tolerance and dependence, the different doses of BZF were co-administered with 10 mg/kg of morphine (M) throughout the induction (day 1–9) period and on day 10, only morphine (2.5 mg) (M-2.5 mg) was administered. To the other group of mice that had been rendered tolerant to morphine (day 1–9), different doses of BZF were administered 10 min prior to morphine on day 10 (Vogel & Vogel, 1997).

Assessment of morphine tolerance and dependence

Development of tolerance to the antinociceptive effect of morphine was assessed on days 1 and 10 by the tail-flick test (Vogel & Vogel, 1997). The reaction time (tail-flick) to radiant heat (analgesiometer, Inco, Ambala, India) was recorded as nociceptive response. Each mouse was tested twice before drug administration on the first day and the reaction times were averaged to obtain a baseline reaction time. Only mice showing a baseline reaction time of 2–3 s were used. A cut-off time of 10 s was imposed to prevent any tissue damage. A minimum of three trials was recorded for each mouse 1 h after morphine injection. Development of dependence to morphine was assessed by the naloxone-precipitated withdrawal jumps. Immediately after the tail-flick test on day 10, naloxone (2 mg/kg, p.o.) was administered and the animals were individually placed in a Plexiglass box (45 × 30 × 30 cm) and observed for withdrawal jumps for a period of 20 min.

Effect of chronic treatment with BZF on morphine-induced anti-nociception

Chronic administration (twice a day × 9 days) of BZF (10, 50 and 100 mg/kg) had no effect on an acutely administered sub-maximal dose of morphine (2.5 mg)-induced (M-2.5 mg) anti-nociception. None of the animals treated with BZF alone showed an analgesic effect, nor did naloxone (2 mg/kg, p.o.) elicit any jumps in these animals.

Effect of oral administration of BZF on induction of morphine tolerance and dependence

Table 1 shows the effect of the three doses of BZF on the induction of tolerance to morphine. When co-administered with morphine (10 mg/kg) (M) during the induction phase, BZF (10–100 mg/kg, p.o.) reversed the development of tolerance to morphine. Concurrent administration of BZF (10–100 mg/kg, p.o.) with morphine during the induction phase attenuated naloxone (2 mg/kg) precipitated withdrawal jumps.

Effect of acute administration of BZF (three dose levels) on the expression of morphine tolerance and dependence

Table 2 shows the effects of the three doses of bioactive phyto-moiety of *P. incarnata* (i.e., BZF) on the expression of morphine tolerance in mice. The expression of morphine tolerance was not affected by acute treatment (single treat-

Table 1. Effect of oral administration of BZF (i.e., the bioactive fraction of *P. incarnata*) on the induction of morphine tolerance and dependence.

Induction regimen (1–9 days)	Tail flick latency	No. of jumps after naloxone administration
Control (vehicle 0.25 ml)	9.4 ± 0.51*	–
M	3.4 ± 0.41	23.8 ± 3.83
BZF (10) + M	6.9 ± 0.51*	3.2 ± 0.84**
BZF (50) + M	7.1 ± 0.50*	2.4 ± 0.55**
BZF (100) + M	6.9 ± 0.73*	2.8 ± 0.45**

The data are expressed as mean ± S.D. ($n = 5$); ANOVA ($P < 0.05$) followed by Fischer's-LSD test; * = significantly more than the corresponding value for group M; ** = significantly less than the corresponding value for group M; vehicle (simple syrup IP containing 1% w/w of carboxymethylcellulose).

Table 2. Effect of acute administration of three doses of BZF on the expression of morphine tolerance.

Induction regimen (1–9 days)	Acute treatment (10th day)	Tail flick latency	No. of jumps after naloxone administration
M	Vehicle + M-2.5 mg	3.4 ± 0.26	24.2 ± 3.27
M	BZF (10) + M-2.5 mg	3.5 ± 0.47*	18.4 ± 3.65
M	BZF (50) + M-2.5 mg	3.8 ± 0.34*	4.6 ± 0.89**
M	BZF (100) + M-2.5 mg	3.6 ± 0.37*	2.4 ± 0.55**

The data are expressed as mean ± S.D. ($n = 5$); ANOVA ($P < 0.05$) followed by Fischer's LSD test. * = significantly equivalent to control (vehicle + M-2.5 mg) group; ** = significantly less than corresponding value for control group. vehicle (simple syrup IP containing 1% w/w of carboxymethylcellulose).

ment, on 10th day) with BZF (10–100 mg/kg). However, physical dependence (withdrawal jumps) was attenuated by BZF in a dose-dependent manner.

Results and discussion

It becomes clear from the above studies that co-administration of bioactive phyto-moiety (BZF) of *P. incarnata* during the induction phase (day 1 to 9) delayed the development of tolerance to the analgesic effect of morphine and it also reversed naloxone-induced withdrawal jumps. Secondly, administration of BZF phyto-moiety of *P. incarnata* during the expression phase (day 10) of morphine tolerance reversed only naloxone-induced withdrawal jumps in a dose-dependent manner without affecting tolerance to its analgesic effect (Dhawan et al., 2001h).

These observations, though, confirm the possible use of *P. incarnata* in breaking down the morphine habit, yet the available reports on *P. incarnata* are too meager to make us opine about the possible mode of action of *P. incarnata*. The fraction that has been used in this study comprises a benzoflavone phyto-moiety and, to date, benzoflavone compounds have been used as aromatase inhibitors (Aldrich Catalog, 1996). Since aromatase inhibitors are potential antioxidants and alter the steroid hormone metabolism (Kao et al., 1998), the mode of morphine-reversal action of the benzoflavone moiety of *P. incarnata* could be speculated to be a neuro-steroidal phenomenon. Still, at this stage, except for mere speculations, nothing can be concluded about the potential usefulness of *P. incarnata* in morphine de-addiction. The authors invite potential organizations/researchers who could help in developing this viable project, so that this traditional time-tested remedy could be completely explored, and to make this research replicable in human beings for a noble cause. There are approximately 187 million opiate addicts (NIMH, 2001) who are in direct need of such cheap, safe and effective medicines to give up their addiction habits.

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