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PSYCHOPHARMACOLOGICAL PROFILE OF *PASSIFLORA INCARNATA* LINN IN MICE

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ABSTRACT

Passiflora incarnata L. commonly known as passion flower belongs to Passifloraceae family and is one of the most popular and widely used medicinal plants in traditional medicine. The plant has a long history of application in herbal medicine as an anxiolytic and sedative-hypnotic. Extracts prepared from *P. incarnata* have been reported for their diverse ranges of pharmacological actions including antitussive, analgesic, anticonvulsant, anti-inflammatory, antiasthmatic and aphrodisiac activity. Documents related to antipsychotic actions were not found in a comprehensive literature survey. Therefore, we aimed to investigate the effect of n-butanol extract of *P. incarnata* leaves (BEPI) and flowers (BEPIF) on experimentally induced psychosis using some psychopharmacological tests. Psychopharmacological profile of BEPI and BEPIF were studied using amphetamine induced stereotyped behavior, swimming induced grooming behavior and lithium induced head twitches. Significant differences between groups were determined by analysis of variance test followed by Dunnett's test. When BEPI and BEPIF was administered i.p. at 150, 300 and 600 mg/kg inhibited amphetamine induced stereotyped behavior, reduced swim induced grooming behavior and decreased number of head twitches induced by lithium sulphate dose dependently. The results of present investigation revealed antipsychotic/ neuroleptic potential of BEPI and BEPIF. The observed antipsychotic effect of BEPI and BEPIF in present study has been attributed to flavonoids. Furthermore, it may act synergistically or other minor constituents may also contribute to the observed activity.

Keywords: *Passiflora incarnata*, Antipsychotic activity, Swim induced grooming.

INTRODUCTION

Passiflora incarnata L. commonly known as passion flower belongs to Passifloraceae family and is one of the most popular and widely used medicinal plants in traditional medicine in West India, Mexico, Netherland and South America. *P. incarnata* is a popular traditional European remedy as well as a homoeopathic medicine for insomnia and anxiety and is taken as a sedative tea in North America. The plant has a long history of application in herbal medicine as an anxiolytic and sedative- hypnotic which dates back to ancient time (Dhawan *et al*., 2004). Aerial parts of *P. incarnata* have been used as sedative, anxiolytic, antispasmodic, analgesic, anticonvulsant, and wormicidal and also in whooping cough, bronchitis, asthma, and other tough coughs. Extracts prepared from *P. incarnata* have been reported for their diverse range of pharmacological actions including antitussive (Dhawan *et al*., 2002a), analgesic, anticonvulsant, anti-inflammatory (Dhawan *et al*., 2003a), antiasthmatic (Dhawan *et al*., 2003b) and aphrodisiac activity (Dhawan *et al*., 2002b). *P. incarnata* has also been reported to possess antihypertensive effects (Patel *et al*., 2009).

The newly reported benzoflavone (BZF) compound from the methanol extract of aerial parts of *P. incarnata* has also been known to possess significant anxiolytic properties (Dhawan *et al*., 2001), and to attenuate and suppress the dependence/addiction caused

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by morphine and cannabinoids in the recent pharmacologic studies performed on mice (Dhawan *et al*., 2002c; Dhawan *et al*., 2002d).

The main chemical constituents of the Passion flower are the flavonoids (0.25%) such as vitexin, isovitexin, orientin, isoorientin, apigenin, kaempferol and quercetin and indole alkaloids (0.1%) based on the beta-carboline ring system such as harman, harmin, harmalin, harmol and harmalol. Some other isolated plant constituents have been identified such as glycosides, carbohydrates, amino acids, benzopyrones, cyanogenic glycosides such as gyanocardin, pyrone derivatives such as maltol and ethyl maltol. Two important constituents like chrysin and tri-substituted benzoflavone moiety (BZF) also have been isolated (Patel *et al*., 2009).

Psychoses (schizophrenic, schizoaffective, and affective illnesses) are common with a lifetime prevalence of 2–3% and account for a high percentage of the serious morbidity. The magnitude of their social impact stems from their onset in early adult life coupled with a strong tendency to recur, or sometimes, to persist and even progress (Crow and Harrington, 1994). A report suggests that phytotherapies which potentially have significant use in psychiatry, and urgently require more research is *Rhodiola rosea* (roseroot), *Crocus sativus* (saffron), *Passiflora incarnata* (passionflower), *Scutellaria lateriflora* (scullcap), *Zizyphus jujuba* (sour date) and *Piper methysticum* (kava) (Sarris, 2007) .On the other hand, documents related to antipsychotic actions were not found in a comprehensive literature survey. Therefore, we aimed to investigate the effect of *P. incarnata* on experimentally induced psychosis performing some psychopharmacological tests. To the best of our knowledge, this is the first experimental study related to the antipsychotic potential of *P. incarnata.*

MATERIALS AND METHODS

Plant materials

The fresh leaves and flowers of *P. incarnata* were collected in the month of June, July and August from local nursery in Pune region India. The plant was identified and authenticated by Dr. Dinesh Shirodkar, Botanical Survey of India, Pune, where voucher specimens were deposited (Voucher Specimen No: PASSIN 3).

Extraction and isolation

Shade dried leaves of *Passiflora incarnata* (1000 gm) were 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. n-butanol (BEPI) extract was evaporated to dryness (22.5% yields). Shade dried flowers of *P. incarnata* (1000gm) also subjected to maceration with ethanol followed by successive extraction with hexane, chloroform, ethyl acetate and n- butanol. n-butanol (BEPIF) extract was evaporated to dryness (25.5% yields).

Pharmacological studies

Animals

Swiss albino mice weighing between 25-30 g of either sex obtained from Serum Institute of India, Pune, India were used for this study. 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*. Experimental protocols were approved by Institutional Animal Ethical Committee (IAEC) of Indira College of Pharmacy, Pune-411033.

Drugs and chemicals

Ethanol (Baker, Germany), hexane, chloroform, ethyl acetate, n-butanol, amphetamine, haloperidol, lithium sulfate and risperidone used in this study were procured from S.D. Fine Chem. Limited, Mumbai, and Maharashtra, India.

Methods

Swim induced grooming in mice

The animals were divided into eight groups and were treated i.p. with vehicle (normal saline), haloperidol (1 mg/kg), BEPI (150, 300 and 600 mg/kg) and BEPIF (150, 300 and 600 mg/kg). Sixty minutes after treatments, mice were placed individually in swimming cylinders (8x8x18cm high) filled with water (32°C) for three min. They were then removed and dried with towel for 30 seconds and placed immediately into single perspex boxes. The number and the total duration of grooming episodes in seconds were recorded for 15 min. (Kedves *et al*., 2008).

Amphetamine-induced stereotyped behavior in mice

The animals were divided into eight groups, each containing six animals. They were treated with vehicle (normal saline), chlorpromazine (2 mg/kg), BEPI (150, 300 and 600 mg/kg) and BEPIF (150, 300 and 600 mg/kg) and placed individually in the cage. Thirty minutes after treatments, Amphetamine (1 mg/kg, i.p.) was administered. The mice were allowed a maximum of 30 min to get acclimatized to the observation cage, prior to the experiment. Amphetamine-induced stereotypy was scored for 30 minute using following scoring system.

Stereotypy scoring –: 0-absence of stereotyped behavior; 1-intermittent sniffing; 2-constant sniffing; 3-constant sniffing with intermittent licking or false biting; 4-constant licking or false licking; 5-constant licking; 6-constant biting and moving around; 7- constant biting and restricted to a small area in the cage; 8- rearing - was used (Yadav and Nade, 2008).

Lithium induced head twitches

The animals were divided into eight groups, each containing six animals. They were treated i.p. with vehicle (normal saline), risperidone (2 mg/kg), BEPI (150, 300 and 600 mg/kg) and BEPIF (150, 300 and 600 mg/kg). Thirty minute after treatments, lithium sulfate (200 mg/kg i.p.) was administered. The number of head twitches was observed for 60 min after the administration of lithium sulfate (Wielosz and Kleinrok, 1979).

Statistical analysis

Statistical analysis of data received was performed using the software Primer of Biostatistics (Primer of Biostatistics, Version 4, Stanton A. Glantz). Results were expressed as mean ± SEM. Significant differences between groups were determined by analysis of variance followed by Dunnett's test. Differences between data sets were considered as significant when *p* value was less than 0.05.

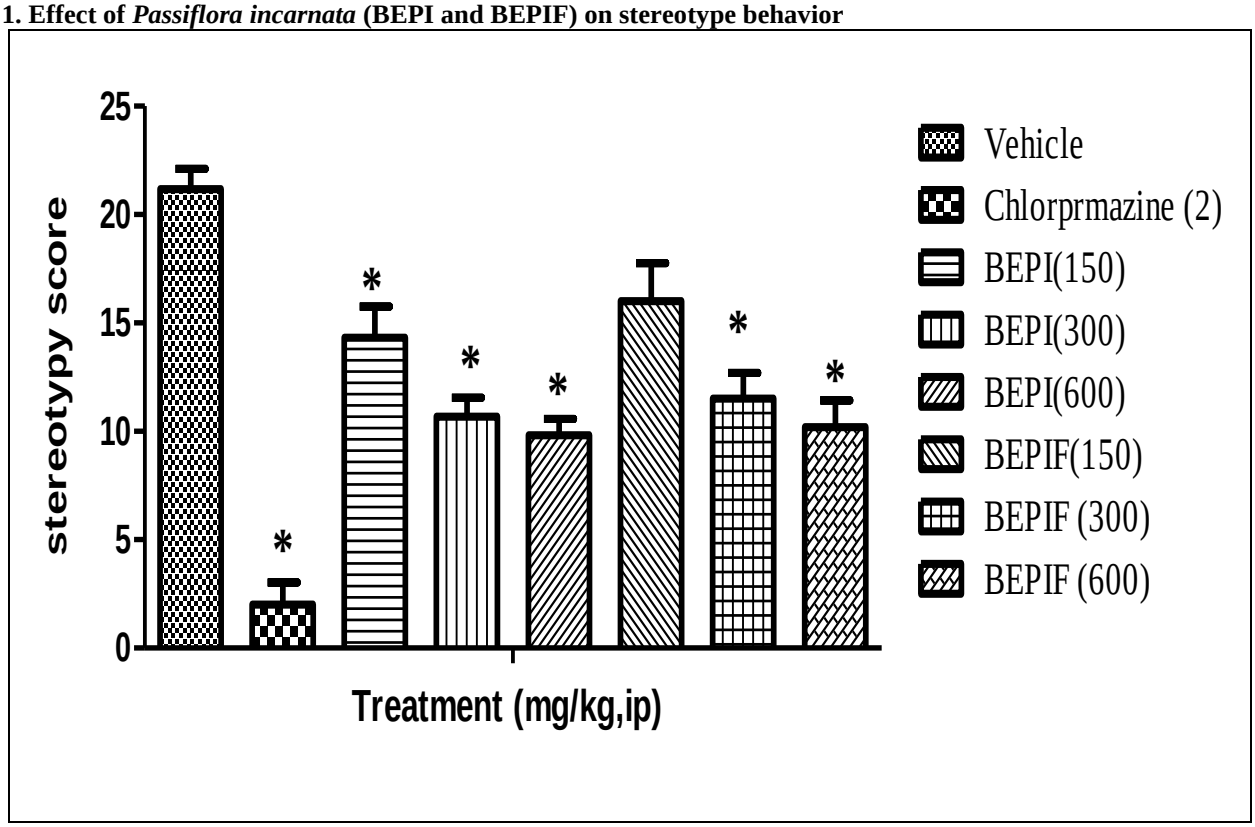
RESULTS

Figure 1 depicts the results of amphetamine induced stereotyped behavior. Amphetamine (1 mg/kg, i.p.) induced stereotyped behavior in mice characterized by sniffing, licking, biting, moving around, restricted to a small area in the cage and rearing. Pretreatment with BEPI (150, 300 and 600 mg/kg, i.p.) decreased the stereotypic scores significantly compared to vehicle treated group. Pretreatment with BEPIF in a dose of 300 and 600 mg/kg, also reduced the stereotyped behavior to a great extent .Whereas, BEPIF in a dose of 150 mg/kg was found to be ineffective.

Figure 2 depicts effect of BEPI and BEPIF on duration of grooming episodes and total number of grooming. In swim induced grooming model, swimming in water induced characteristic grooming behavior in animals. Pretreatment with BEPI (150, 300 and 600 mg/kg, i.p) and BEPIF (150, 300 and 600 mg/kg, i.p) significantly decreased the number of grooming and total duration of grooming episodes compared to vehicle treated group.

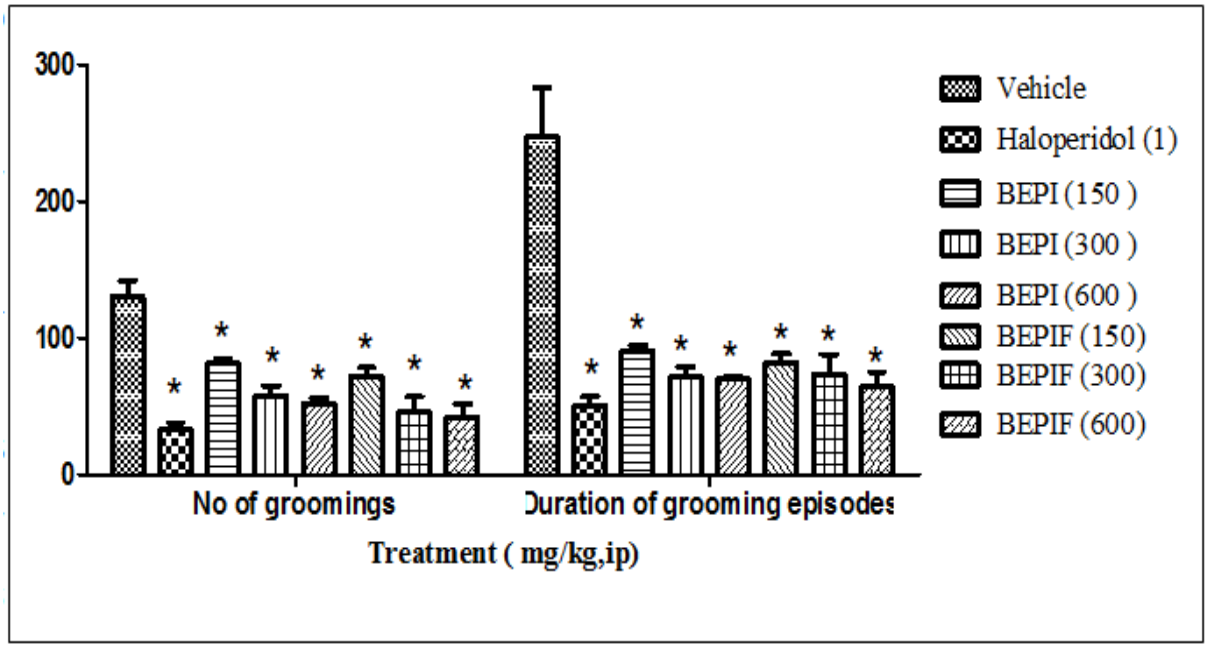
To identify the possible mechanism of antipsychotic action, lithum induced head twitches model was explored. In this model, BEPI (150, 300 and 600 mg/kg, i.p.) significantly reduced the lithium induced head twitches. BEPIF (150, 300 and 600 mg/kg, i.p.) also decreased the lithium induced head twitches significantly. Comparative results of lithium induced head twitches are given in figure 3.

Fig 1. Effect of *Passiflora incarnata* (BEPI and BEPIF) on stereotype behavior



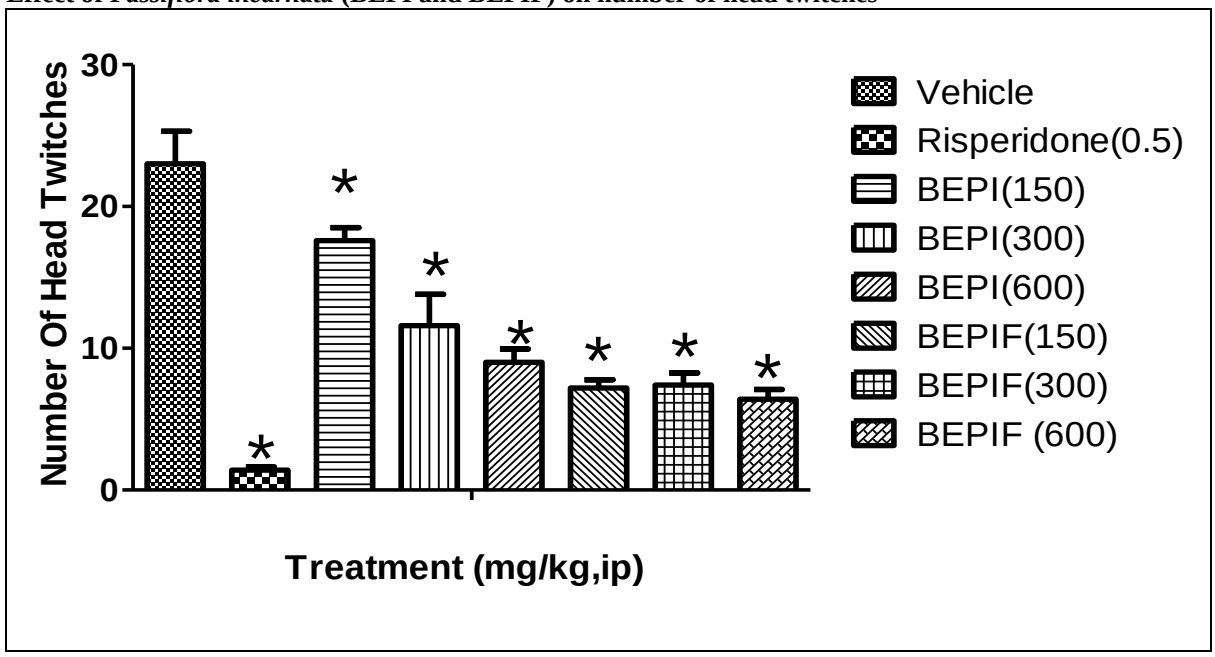
*P≤0.001, n=6 (One way ANOVA followed by Dunnett's test) compared to vehicle treated group

Fig 2. Effect of *Passiflora incarnata* (BEPI and BEPIF) on swim induced grooming behavior in mice



*P≤0.001, n=6 (One way ANOVA followed by Dunnett's test) compared to vehicle treated group

Fig 3. Effect of *Passiflora incarnata* (BEPI and BEPIF) on number of head twitches



*P≤0.001, n=6 (One way ANOVA followed by Dunnett's test) compared to vehicle treated group

DISCUSSION

In the present work, antipsychotic activity of BEPI and BEPIF was investigated using amphetamine induced stereotyped behavior and swim induced grooming behavior. Amphetamine, an indirectly acting sympathomimetic agent releases dopamine and induces characteristic stereotyped behavior. Amphetamine-induced stereotyped behavior is a measure of dopamine D_2 receptor reactivity. It is known that amphetamine-induced stereotyped behavior is mediated by the hyperactivity of dopaminergic mechanism in the nigrostriatal and mesolimbic pathway. Inhibition of this behavior is regarded as a very selective test for screening of antipsychotic activity. Amphetamine induced

stereotyped behavior (SB) in rats can be blocked by the drugs that are useful in the treatment of schizophrenia (Yadav and Nade, 2008; Rio and Fuentes, 1969). In present study, BEPI and BEPIF inhibited amphetamine induced stereotyped behavior dose dependently. This revealed antipsychotic/ neuroleptic potential of BEPI and BEPIF.

It has been reported that the swim induced grooming behavior in mice also involves a dopaminergic mechanism since it was inhibited in a dose-dependent manner by dopamine receptor antagonists such as spiroperidol, haloperidol, chlorpromazine (Gregory and David,1980). In present study, BEPI and BEPIF reduced swim induced grooming behavior dose dependently. This confirmed that BEPI and BEPIF have significant antipsychotic activity which is mediated through its dopamine D_2 receptor blocking actions.

All clinically effective antipsychotics acts as dopamine D_2 receptor blockers and antipsychotic potency is correlated with their capacity of binding to D_2 receptor. Blockade of dopamine receptor in corpus striatum is responsible for the extrapyramidal symptoms like Parkinsonism due to antipsychotic agents. Besides dopaminergic receptor blockade, some (atypical) antipsychotics like risperidone and clozapine also block 5-HT system, which helps to lessen extrapyramidal reactions and is related to their usefulness in improving negative symptoms (Kaur et la.,2010) . Ceulemans *et al* provided the first supporting clinical evidence by demonstrating that administration of a 5-HT antagonist resulted in a significant improvement in patients suffering from schizophrenia, a condition which is similar in symptoms to amphetamine induced psychoses (Ceulemans *et al*., 1985).

Very recently, Kapur and Remington (Kapur and Remington, 1996) reviewed the interaction between 5-HT and DA and its relevance to schizophrenia and have suggested that serotonergic modulation of dopaminergic function may provide a viable mechanism for enhancing therapeutics in schizophrenia (Kandi *et al*., 1998).

An abundance of evidence suggests that a dysfunction of serotonin neurotransmitter systems also contributes to the pathogenesis of schizophrenia and related psychoses. It has long been recognised that blockade of the 5-HT_{2a} serotonin receptor subtype is an important part of atypical antipsychotic drug action. It is observed that 5- HT_{2a} receptors are highly enriched on the pyramidal neurons in the cortex. Thus, it is now believed that blockade of the 5-HT_{2a} receptor normalizes the firing of pyramidal neurons, thereby stabilizing perception of reality. Thus, hallucinogens, such as lysergic acid diethylamide (LSD) activate 5-HT_{2a} receptors, whereas all approved atypical antipsychotic drugs are potent 5-HT_{2a} receptor antagonists. It is believed that long-term treatment with atypical antipsychotic drugs normalizes the receptor mediated signaling in cortical pyramidal neurons, thereby improving positive and negative symptoms of schizophrenia (Bryan, 2003). Previous study in our laboratory has reported antiparkinsonian activity of BEPI and BEPIF (unpublished data). These evidences made us to identify the involvement of 5-HT receptors in mechanism of action of BEPI and BEPIF as antipsychotics.

Possible involvement of 5-HT receptor in antipsychotic action of BEPI and BEPIF was studied using lithium induced head twitches, a serotonin mediated behavior in animals. Lithium sulphate administered to animals releases serotonin from serotonergic neurons which stimulate the 5-HT_2 receptors and produced head twitches. These head twitches are antagonized by drugs that block 5-HT_2 receptors (Wielosz and Kleinrok, 1979).

In present study, BEPI and BEPIF decreased number of head twitches induced by lithium sulphate significantly similar to risperidone (atypical antipsychotic with 5- HT_2 receptor antagonist action). This concludes that, BEPI and BEPIF have 5- HT_2 antagonist action besides dopaminergic receptor blockade like risperidone and clozapine which helps to lessen extrapyramidal reactions.

Flavonoids are present in all vascular plants. Several biological properties have been attributed to flavonoids. Among them the anti-inflammatory, antioxidant, antihepatotoxic, antiviral activities are well known, together with the vasculoprotector and spasmolytic properties (Zanoli *et al*., 2000). *P. incarnata* is known to contain higher concentration of flavonoids. This shows that flavonoid content of *P. incarnata* may be responsible for the antipsychotic activity of BEPI and BEPIF. Furthermore, it may act synergistically or other minor constituents may also contribute to the observed activity.

CONCLUSION

The results of present investigation exhibited significant antipsychotic activity of BEPI and BEPIF for the first time. Thus, it can be concluded that BEPI and BEPIF possess significant antipsychotic activity which is mediated through its dopamine D_2 receptor and 5-HT_2 receptor antagonistic actions with mechanism of action similar to atypical antipsychotics. Furthermore, the presence of flavonoids in *P. incarnata* may be considered as partially responsible of antipsychotic action.

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