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ORIGINAL ARTICLE

Evaluation of Anti-anxiety Activity of Passiflora Incarnata F ruit in Experimental Animal Models

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ABSTRACT

Objectives: Anxiety disorders are the most widespread mental health issues worldwide. Anxiety symptoms are prevalent in society, affecting not only the youth but also the elderly. While normal anxiety aids in dealing with challenges, excessive anxiety can be detrimental. This study aimed to assess the anxiolytic effects of aqueous *Passiflora incarnata* fruit extract in animal models.

Methods: Phytochemical screening of *Passiflora incarnata* was conducted. The effects of *Passiflora incarnata* on reducing anxiety were examined using various well-established animal models, including the elevated plus maze, staircase, hole board, and light/dark model. Each group consisted of six animals and different doses of *Passiflora incarnata* (100, 200, and 400 mg/kg) were administered. Distilled water served as the control group, and diazepam (2 mg/kg) was used as the standard reference.

Findings: Preliminary phytochemical screening revealed the presence of flavonoids, alkaloids and tannin and carbohydrates, proteins, steroids and terpenoids were found to be absent. These findings led to the conclusion that the medium (200 mg/kg) and high (400 mg/kg) doses exhibited notable anti-anxiety activity in comparison to the control group. Conversely, the low dose did not show any significant anxiolytic activity when compared to the control group.

Novelty: *Passiflora incarnata* has the potential to serve as a natural psychotherapeutic agent for treating different anxiety-related disorders compared to existing therapies. This study shows encouraging results for the application of herbs in therapies for mental disorders, such as depression, anxiety, and insomnia.

Keywords: Passiflora incarnata; Phytochemical screening; Antianxiety

INTRODUCTION

Anxiety has affected 1/8th of the world's total population. Researchers in the advanced world are still examining traditional remedies to uncover a suitable cure for these 'mind affecting diseases' which have been the outcome of man's zest to win the nature. Passiflora incarnata Linn. (Passifloraceae), commonly referred to as Maypop, Maracuja, or Passion flower, has been utilized as an anxiolytic and sedative worldwide for many years[1,2]. Flavonoids are the major phytoconstituent of *P. incarnata*. Apart from flavonoids, *P. incarnata* is also reported to contain several indole alkaloids that are based on the β-carboline ring system, including harman, harmine, harmalol, and harmaline[3]. The presence of several other phytoconstituents in *P. incarnata* has been reported, including carbohydrates[4], essential oils[5], amino acids[4], and the cyanogenic glycoside gyanocardin.

Despite a long history of use, supported by well-documented phytochemical reports on *P. incarnata*, the precise mechanism underlying its anti-anxiety effects and the active plant components responsible for the much-acclaimed central nervous system (CNS) effects have not been clearly described. Recent studies have shown that *P. incarnata* possesses sedative and anxiolytic properties, which are thought to be driven by biochemical processes involving the benzodiazepine and GABA receptors in the body[6]. Despite these reports, extensive pharmacological research on *P. incarnata* by Soulimani et al. has shown that none of the recognised phytoconstituents is responsible for the well-established anxiolytic and sedative effects of *P. incarnata*[7]. The present study aimed to evaluate the anxiolytic activity of *P. incarnata* fruit in mice models.

METHODOLOGY

Collection and authentication of P. incarnata

The fruit of *P. incarnata* was purchased from Mr. Chelladurai, Chennai, in its dried form. The plant material was identified and authenticated by Dr. P.E. Rajasekharan, Principal Scientist, Division of Plant Genetic Resources.

Method of extraction of P. incarnata fruit extraction

The fruits were subjected to maceration extraction. Fifty grams of the dry fruit powder was added to 100 ml of distilled water and soaked overnight. The extract was then filtered through a muslin cloth.

Phytochemical analysis of the extract

The prepared extracts were subjected to phytochemical analysis. The remaining extract was stored in a refrigerator until further use.

Test for carbohydrates

Benedict's test: Equal volumes of Benedict's reagent and test solution were mixed in a test tube and heated for 5 min in a boiling water bath. The yellow, green, or red solutions indicate the presence of carbohydrates.

Test for proteins

Biuret test: 3mL of extract was taken in 4% sodium hydroxide, and a few drops of 1% $CuSO_4$ solution were added. A pink or violet colour indicates the presence of a protein.

Test for steroids and Terpenoids

Libermann Burchard t est: Few drops of acetic anhydride, was added to crude extract, then boiled and cooled. Concentrated sulphuric acid was then added to the sides of the test tube. Formation of a brown ring at the junction of the two layers was observed. The green colouration of the upper layer and the formation of a deep red colour in the lower layer indicate a positive test for steroids and triterpenoids, respectively.

Test for glycosides

Liebermann's test: 2 ml of acetic acid and 2 ml of chloroform were mixed with the entire plant crude extract. The mixture was then cooled and con. H_2SO_4 was added, and the green colour indicates the entity of the aglycone steroidal part of the glycosides.

Test for flavonoids

Shinoda test: To 3 ml of the extract, 5 ml 95% ethanol, a few drops of conc. HCl and 0.5g of magnesium turnings were added. The pink colour indicated the presence of flavonoids.

Lead acetate t est: The extract was treated with a lead acetate solution, and the formation of yellowish-white precipitates indicated the presence of flavonoids.

Test for alkaloids

The aqueous alcoholic extract was evaporated and to the residue, dilute HCl was added and properly mixed and filtered. The filtrate was tested for the presence of alkaloids.

Hager's Test – The extract was treated with few drops of Hager's reagent (saturated picric acid solution). Formation of yellow precipitate shows a positive result for the presence of alkaloids.

Test for tannins

Ferrric chloride test: A 5% $FeCl_3$ solution was added to 2-3 mL of aqueous or alcoholic extract, and the formation of a deep blue-black colour indicated the presence of tannins.

Acute toxicity study

Healthy Swiss albino male mice weighing of 18-22 g, 4-week-old were procured from Krupanidhi College of Pharmacy. The animals were acclimated to the laboratory conditions for 15 days. They were housed in standard glass cages and kept under standard conditions at a temperature of 23°C (±2°C) with a 12-hour light/12 hrs dark cycle. In each assay model, 30 male mice weighing 25-30 g, were randomly assigned into five groups, one control group, and four treatment groups, and housed in five different cages. The mice were provided ad libitum access to food and water. The study was approved by the Ethics Committee of the Council for Scientific Animal Ethical Committee clearance 2016/PCOL/01 for the procurement of animals. The study was conducted according to OECD guidelines 423[8].

Overnight fasted mice were then weighed. The aqueous fruit extract of *P. incarnata* was dosed in a stepwise procedure, with the initial dose selected as the dose expected to produce some signs of toxicity and was observed for a period of two weeks. The Swiss albino mice were divided into 5 groups, each group having 6 mice. The animals in each group were subjected to acute toxicity studies at doses of 100, 200, and 400 mg/kg orally. The animals were observed for signs of toxicity such as hyperactivity, convulsions, and sedation, continuously for 2 h, and for mortality up to 24 h, after administration of the doses, and daily thereafter, for a total of 14 days.

Experimental groups

- **Group 1**: Negative control (Vehicle 5ml/kg b.w, i.p)
- **Group 2**: Diazepam (2mg/kg i.p)[9]
- **Group 3**: Low dose of aqueous fruit extract of P. incarnata, 100 mg/kg, p.o
- **Group 4**: Intermediate dose of aqueous fruit extract of P. incarnata, 200 mg/kg p.o

- **Group 5**: High dose of aqueous fruit extract of P. incarnata, 400 mg/kg p.o

Evaluation of Anti-Anxiety activity using elevated plus maze model[10]

An established animal model for evaluating anxiolytic medications is the elevated plus-maze model. The elevated plus maze device consists of an open ceiling with the entire labyrinth elevated (25 cm for mice and 50 cm for rats) from the floor, two closed arms (16 × 5 × 12 cm for mice and 50 × 10 × 40 cm for rats), and two open arms (16 × 5 cm for mice and 50 × 10 cm for rats)[11]. The following parameters were recorded for three minutes after each animal was put separately in the middle of the maze with its head facing open arms. a) Mice favour closed and open arms first. b) The sum of entries in both closed and open arms (each arm entry is defined as the entry of four paws into the arm). c) The average amount of time each animal spends in each arm (average time = total duration in the arm/number of entries)

Procedure

The mice had access to all arms and could move freely around them. The animal was placed on the maze's centre platform, facing the open arm (Figure 1). Damps and dry towels were used to clean the equipment during testing. The frequency of entries and duration spent in the open arms were used as indicators of open space-induced anxiety in mice[12].

Fig. 1: Elevated plus maze model

Evaluation of Anti-Anxiety activity using Hole –Board Model[13]

The apparatus consisted of a wooden box (40 cm × 40 cm × 25 cm) with 16 holes (each 3 cm in diameter) evenly placed on the floor (Figure 2). The fruit extract and vehicle were administered once daily for 5 days p. o., with the last dosage administered on the fifth day, 60 min before the trial began. The reference medication was administered orally at a dose of 2 mg/kg p.o. 60 min before the trial began. The number of line crossings and head dipping were determined throughout a three-minute period.

Fig. 2: Hole board apparatus

Procedure

Each mouse was placed in the apparatus for three minutes of observation before removal. Three common actions were observed, namely, head dipping, raising, and mobility.

Evaluation of Anti-Anxiety activity using Light - dark model transition test in mice

The light-dark apparatus was made up of two compartment chambers (40 × 60 × 20 cm): a brightly lighted section (40 × 40 cm) and a dark area (40 × 20 cm) divided by a wall with a circular hole (7 cm diameter) (Figure 3). During the three-minute test session, each mouse was placed individually in the illuminated region of the cage, and the following parameters were noted: the total number of crossings, the number of crossings between the bright and dark areas, the total amount of time spent in the illuminated area, the total amount of time spent in the dark area, the number of rearings in the illuminated area, and the number of rearings in the dark area[14].

Procedure

Each mouse was placed in the light chamber of the apparatus and allowed to roam around. Next, the number of entries was determined. To be deemed an entrance, all four paws must be inserted into the opposing chamber[15].

Fig. 3: Dark and light chamber

Fig. 4: Staircase

Evaluation of Anti-Anxiety activity using Staircase test in mice[16]

Five identical steps that are 2.5 cm high and 10 cm deep make up the staircase (Figure 4). All the way up the stairs, the interior height of the walls is the same. Each animal was used only once. After the trial was completed, each mouse was placed on the floor of the box, with its back facing the stairwell. Over the course of three minutes, the total number of stairs ascended and rearings were noted. A mouse can only be said to have climbed a step if all four paws are on it.

Procedure

For three minutes, each mouse was left alone at the foot of the stairwell for observation. Anxiety indices were measured by counting the number of climbed steps and readings.

Statistical analysis

All results were presented as mean ± SEM. The significance of the differences compared to the positive control groups was determined using one-way analysis of variance (ANOVA) followed by Dunnett's test using GraphPad Prism version 5. Statistical significance was set at p value < 0.05.

RESULTS

Preliminary phytochemical screening revealed the presence of flavonoids, alkaloids and tannin and carbohydrates, proteins, steroids and terpenoids were found to be absent.

Acute Toxicity

No mortality or noticeable behavioural changes were observed in any of the groups tested. The extract was found to be safe up to a dose level of 2000 mg/kg body weight. The final doses were 100, 200, and 400 mg/kg.

Anti-anxiety activity of P. incarnata in elevated plus maze model

The results showed that *P. incarnata* at doses of 200 and 400 mg/kg significantly increased the time spent in the open arms and the number of entries in the open arm, while the time spent in the closed arms decreased significantly, similar to the effects observed after administration of the reference anxiolytic drug diazepam (2 mg/kg), indicating that the plant may possess antianxiety activity. However, at a low dose (100 mg/kg), there was no significant increase in the time spent and number of entries in the open arm compared with the control group (Table 1).

Anti-anxiety activity of P. incarnata in light and dark model

The results showed that medium and high doses of *P. incarnata* increased the time spent and number of entries into the light compartment. The anxiolytic activity of *P. incarnata* was similar to that of the diazepam (Table 2).

Anti-anxiety activity of P. incarnata in hole board model

It was observed that *P. incarnata* at doses of 200 and 400 mg/kg significantly increased the number of head dips, indicating an anxiolytic-like effect, whereas at a low dose of the aqueous extract of *P. incarnata*, there was no significant increase in the number of head dips as compared with control animals (Table 3).

Table 1: Effect of various doses of Aqueous extract of P. incarnata on elevated plus-maze model of anxiety

Sr. No.	Experimental groups	No. of entries in open arm	Time spent in open arm (sec)	No. of entries in closed arm	Time spent in closed arm (sec)
1.	Normal control (5ml/kg b.w, i.p)	1.83 ± 0.31	13.33 ±2.25	4.67 ± 0.62	166.7 ± 2.25
2.	Standard (Diazepam; 2 mg/kg, i.p.)	8.33 ± 0.67[a]	140.8 ±8.09[a]	2.17 ± 0.31[a]	39.17 ± 8.09[a]
3.	Test Group- 1 (Low dose, 100 mg/kg, p.o.)	2.50 ± 0.92	31.67 ±6.31	3.67 ± 0.67	148.3 ± 6.31
4.	Test Group- 2 (Medium dose, 200 mg/kg, p.o.)	5.00 ± 0.58[b]	46.00 ±5.45[b]	2.33 ± 0.33[b]	124.0 ± 5.66[b]
5.	Test Group- 3 (High dose, 400 mg/kg, p.o.)	7.50 ± 0.72[c]	143.3 ±5.34[c]	1.83 ± 0.31[c]	36.67 ± 5.34[c]

Values are expressed as (Mean ± SEM), n= 6. Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Dunnett's test. All groups were compared with Normal control group. [a] P<0.001; [b] P<0.01; [c] P<0.05

Table 2: Effect of various doses Aqueous extract of P. incarnata on Dark/light box test in mice

Sr. No.	Experimental groups	Time spent in each chamber (in seconds)		No. of Crossings
		Light	Dark	
1.	Normal control (5ml/kg b.w ,i.p)	30.83 ± 5.38	149.2 ± 5.39	4.33 ± 0.49
2.	Standard (Diazepam; 2 mg/kg, i.p.)	133.3±6.18[a]	46.67 ± 6.18[a]	9.17 ± 0.60[a]
3.	Test Group- 1 (Low dose, 100 mg/kg, p.o.)	37.17±7.57	142.8 ± 7.57	3.50 ± 0.43
4.	Test Group- 2 (Medium dose, 200 mg/kg, p.o.)	59.67±8.05[b]	120.3 ± 8.05[b]	6.33 ± 0.49[b]
5.	Test Group- 3 (High dose, 400 mg/kg, p.o.)	122.2±5.78[c]	57.83 ± 5.78[c]	7.33 ± 0.49[c]

Values are expressed as (Mean ± SEM), n= 6. Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Dunnett's test. All groups were compared with Normal control group. [a] P<0.001; [b] P<0.01; [c] P<0.05

Table 3: Effect of various doses of Aqueous extract P. incarnata in Hole board model in mice

Sr. No.	Experimental groups	Number of head dips	Number of rearing
1.	Normal control (5ml/kg b.w, i.p)	6.17 ± 0.60	16.50 ± 0.76
2.	Standard (Diazepam; 2 mg/kg, i.p.)	27.67 ± 1.49[a]	30.17 ± 1.58 [a]
3.	Test Group- 1 (Low dose, 100 mg/kg, p.o.)	7.67 ± 0.49	14.83 ± 0.79
4.	Test Group- 2 (Medium dose, 200 mg/kg, p.o.)	12.17 ± 1.42[b]	22.00 ± 1.16 [b]
5.	Test Group- 3 (High dose, 400 mg/kg, p.o.)	24.00 ± 1.67[c]	25.67 ± 1.52 [c]

Values are expressed as (Mean ± SEM), n= 6. Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Dunnett's test. All groups were compared with Normal control group. [a] P<0.001; [b] P<0.01; [c] P<0.05

Table 4: Effect of various doses of Aqueous extract P. incarnata on stair case model in mice

Sr. No.	Experimental groups	Number of climbing	Number of rearing
1.	Normal control (5ml/kg b.w ,i.p)	10.00 ± 1.06	27.33 ± 2.58
2.	Standard (Diazepam; 2 mg/kg, i.p.)	27.17 ± 3.95[a]	13.67 ± 0.80 [a]
3.	Test Group- 1 (Low dose, 100 mg/kg, p.o.)	11.50 ± 1.31	22.83 ± 1.78
4.	Test Group- 2 (Medium dose, 200 mg/kg, p.o.)	21.33 ± 3.06[b]	18.50 ± 1.68 [b]
5.	Test Group- 3 (High dose, 400 mg/kg, p.o.)	24.67 ± 3.36[c]	11.33 ± 1.28 [c]

Values are expressed as (Mean ± SEM), n= 6. Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Dunnett's test. All groups were compared with Normal control group. [a] P<0.001; [b] P<0.01; [c] P<0.05

Anti-anxiety activity of P incarnata in stair case model

The results showed that the aqueous extract at doses of 200 and 400 mg/kg increased the number of steps taken and reduced the number of rearing steps. At the low dose of 100 mg/kg, no anxiolytic activity was observed (Table 4).

DISCUSSION

In light of the findings and observations of earlier studies, this strongly affirms that certain important factors must be considered before starting any pharmacological work on *P. incarnata*. In the therapy of anxiety disorders or acute anxiety symptoms, a combination of therapeutic

interventions is mostly indicated. In addition to psychotherapeutic approaches, anxiolytics have become a part of anxiety treatment[17]. The self-administration of herbal medicines has become the most popular alternative therapy for anxiety. There has also been a considerable increase in the interest in the development of new anxiolytics. As it is already known, new therapies for the treatment of anxiety disorders are necessary, and the study of medicinal plants could always provide new therapeutic options[18]. It is clear that secondary metabolites of several plants that are used in psychiatric disorder treatments, especially for anxiety in traditional systems of medicine, directly or indirectly facilitate the effect of CNS neurotransmitters, especially noradrenalin, γ-aminobutyric acid (GABA), dopamine, and 5-hydroxytryptamine[19].

P. incarnata has been traditionally used to alleviate anxiety and insomnia. The possibility of a phytoconstituent with a benzoflavone moiety (BZF) being the fundamental element responsible for the bioactivity of *P. incarnata* is greatly anticipated. The anxiolytic effects of chrysin, derived from Passiflora, and its influence on the benzodiazepine receptor in relation to gamma-aminobutyric acid (A) (GABA A) receptors in laboratory rats were investigated. According to the proposed theory, chrysin is thought to reduce anxiety levels in laboratory rats by interacting with the GABA (A) receptor. This hypothesis was supported by the findings from the elevated plus maze, corticosterone, and catecholamine assays. Phytochemical analysis of the dried fruit of *P. incarnata* revealed the presence of flavonoids, tannins, and alkaloids. Our current investigation uncovered the presence of flavonoids, alkaloids, and tannins through preliminary phytochemical screening. *P. incarnata* was reported to possess anxiolytic activity at a dose of 400 mg/kg and sedative activity at a very high dose of 400 g/kg. According to their findings, the two forms of tranquillizer activity depend on the solvent used to prepare the extract. In their study, the hydroalcoholic extract (70:30) was reported to possess anxiolytic activity at a dose of 400 mg/kg in mice. A team of Italian scientists[20] investigating *P. incarnata* suggested that chrysin, a flavonoid, may contribute to the anxiolytic effects of the plant. In view of the observations and findings by Dhawan et al., it was concluded that a fraction derived from the methanol extract of *P. incarnata* showed significant anti-anxiety activity at a dose of 10 mg/kg p.o. in mice using the elevated plus-maze model of anxiety[21].

The present study investigated the phytochemical composition and anxiolytic activity of an aqueous extract of *P. incarnata* using various behavioural models in mice. These findings revealed the presence of flavonoids, alkaloids, and tannins in the extract, supporting its traditional use as an anxiolytic agent. Additionally, acute toxicity evaluation demonstrated the safety of the extract at doses of up to 2000 mg/kg body weight, further supporting its potential therapeutic use.

The anxiolytic activity of *P. incarnata* was assessed using the elevated plus-maze, dark/light box, hole board, and staircase models, which are well-established paradigms for evaluating anxiolytic effects in rodents. In the elevated plus-maze model, the extract exhibited dose-dependent increases in the number of entries and time spent in the open arms, indicating reduced anxiety-like behaviour. These effects were comparable to those of the standard anxiolytic drug diazepam, suggesting the efficacy of the extract in mitigating anxiety-related behaviours.

In the present study, the anxiolytic effects of *P. incarnata* were investigated in classic animal models, such as the elevated plus maze, staircase, hole board, and light/dark tests. The elevated plus-maze results and the dark/light box model also demonstrated the anxiolytic effects of the extract, as evidenced by the increased time spent in the light chamber and number of crossings. Moreover, in the hole-board model, the extract significantly increased the number of head dips and rearing, further supporting its anxiolytic properties. Similarly, in the staircase model, the extract increased climbing behaviour and rearing, indicating reduced anxiety levels.

In the present study, *P. incarnata* significantly increased the time spent in the open arms and the number of entries into the open arm at doses of 200 and 400 mg/kg. Additionally, there was a significant decrease in the time spent in the closed arms, which was similar to the effects observed with diazepam (2 mg/kg), a reference anxiolytic drug. These findings suggest that *P. incarnata* possesses anxiolytic activity. However, at a low dose of 100 mg/kg, there was no significant increase in the time spent or number of entries into the open arm compared to the control group. The results also indicated that medium and high doses of *P. incarnata* increased the time spent and number of entries into the light compartment. The anxiolytic activity of *P. incarnata* is comparable to that of diazepam. Diazepam has been shown to significantly increase the number of entries into the open arm and produce anxiolytic effects in various anxiolytic screening procedures, including EPM and light-dark models. Furthermore, *P. incarnata* significantly increased the number of head dips at doses of 200 mg/kg and 400 mg/kg, indicating an anxiolytic-like effect. However, at a low dose of the aqueous extract of *P. incarnata* (100 mg/kg), there was no significant increase in the number of head dips compared with the control animals. In addition, the aqueous extract at doses of 200 and 400 mg/kg increased the number of steps taken and reduced the number of rearing steps. However, at the low dose of 100 mg/kg, no anxiolytic activity was observed.

The anxiolytic effects of *P. incarnata* extract can be attributed to its phytochemical constituents, particularly flavonoids, alkaloids, and tannins, which have been reported to possess anxiolytic and sedative properties. Flavonoids, in particular, have been shown to modulate neurotransmitter

systems involved in anxiety regulation, such as gamma-aminobutyric acid (GABA) and serotonin receptors. Alkaloids may also contribute to anxiolytic effects by interacting with the neurotransmitter systems implicated in anxiety disorders.

The dose-dependent response observed in this study suggested that higher doses of the extract may produce more pronounced anxiolytic effects. However, further investigations are warranted to elucidate the underlying mechanisms of action and to determine the optimal dosage for therapeutic use. Additionally, future studies should explore the long-term effects and safety profile of *P. incarnata* extract as well as its potential synergistic interactions with conventional anxiolytic medications.

CONCLUSION

The findings of this study provide scientific evidence supporting the traditional use of *P. incarnata* as an anxiolytic agent. The extract demonstrated significant anxiolytic activity in animal behavioural models, possibly mediated by its phytochemical constituents. These findings underscore the therapeutic potential of *P. incarnata* as a natural remedy for anxiety disorders, warranting further clinical investigation to validate its efficacy and safety in humans.

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