RESEARCH PAPER

Pharmacological Evaluation Of *Passiflora incarnata* For Its Anti-Inflammatory, And Analgesic Efficacy In Experimental Animal

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ABSTRACT

The present study evaluated the analgesic and anti-inflammatory activities of the hydroalcoholic extract of *Passiflora incarnata* in experimental animal models. Acute toxicity studies indicated that the extract is safe up to 2000 mg/kg. Analgesic activity was assessed using Haffner's tail immersion and hot plate methods, while anti-inflammatory activity was evaluated using carrageenan- and formalin-induced paw edema models in Wistar rats. The extract showed significant dose-dependent analgesic effects, with increased reaction time observed at 250 and 500 mg/kg doses. In anti-inflammatory models, the extract significantly reduced paw edema, particularly during the late phase of inflammation. Although the effects were slightly less potent than standard drugs like aspirin and diclofenac, the extract demonstrated considerable pharmacological activity, possibly due to flavonoids and phenolic compounds.

Keywords: *Passiflora incarnata*, Analgesic activity, Anti-inflammatory activity, Carrageenan-induced edema, Hot plate method

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INTRODUCTION

Inflammation and pain are fundamental biological responses to tissue injury and infection, yet their persistence can contribute to a wide range of chronic disorders, including arthritis, neuropathic pain, and autoimmune diseases. [1, 2] Conventional pharmacological treatments such as nonsteroidal anti-inflammatory drugs (NSAIDs) and opioids are widely used for managing these conditions; however, their long-term use is often associated with adverse effects such as gastrointestinal irritation, renal dysfunction, and risk of dependency. [3] These limitations have driven increasing interest in the exploration of plant-based therapeutics, which may offer safer and more sustainable alternatives with fewer side effects. [4]

Because of its calming, anxiolytic, and analgesic qualities, Passiflora incarnata, or passionflower, is a medicinal plant that has long been utilised in many medical systems. It is thought to have medicinal promise since it contains a wide variety of bioactive substances, such as flavonoids, alkaloids, and glycosides. [5, 6] According to preliminary research, these phytoconstituents may have anti-inflammatory and pain-relieving actions via modulating central nervous system activity and inhibiting pro-inflammatory mediators. [7] However, rigorous pharmacological study of its analgesic and anti-inflammatory effects is still lacking despite its historic usage. Thus, the current work uses well-established experimental animal models to examine the analgesic and anti-inflammatory properties of Passiflora incarnata. This study aims to give a better knowledge of its pharmacological potential and probable mechanisms of action by assessing its effects using scientifically recognised methodologies. These results might aid in the creation of innovative plant-based medicines for the treatment of pain and inflammation.

MATERIALS AND METHODS

Collection and Authentication of Plant: The *Passiflora incarnata* plant was collected from the Local region of Bareilly during the month of January-February 2023. The collected herbs were dried under shade and crushed to coarse powder with mechanical grinder. Plant authentication is the process of identifying a plant species. The plant was authenticated by Dr. Alok Srivastav, Associate Professor, Department of Plant Science, MJP Rohilkhand University, Bareilly. The voucher specimen of plant was RU/PS/2023/01.

Hydro-alcoholic extraction: Because the hot continuous percolation method is a continuous process that continuously replaces the saturated solvent with new solvent, it is more efficient than maceration. Repercolation was used to extract 50.0 grams of raw material using 100 millilitres of Hydro-alcoholic (70% ethanol). [8, 9]

Experimental Animals: The IVRI at Bareilly, Uttar Pradesh, India, and BIU College of Pharmacy, Bareilly International University, Bareilly, U.P. provided healthy adult Wistar rats of either sex (160–220g) for analgesic

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activity and anti-inflammatory activity. Every rat was given commercial pellets and provided unlimited access to water. To reduce stress, the rats were acclimated to all procedures for one week prior to the start of the trial. The globally recognised standard criteria for the use of laboratory animals were followed in the handling of all the rats used in this investigation. [10]

Acute oral toxicity study: For this investigation, six animals were employed in accordance with OECD 423 criteria. Three animals from each group were given oral hydro-alcoholic (70% ethanolic) extract of *Passiflora incarnata* at doses of 50, 300, and 2000 mg/kg of body weight; none of these animals died. For a period of 14 days, all of the test animals were monitored for different parameters. [11]

Animal Grouping and Dosing of Analgesic activity: Four sets of six Wistar rats each, weighing between 160 and 220 grams, were randomly selected. Group I was given cars and designated as usual control. As a positive control, Group II received normal medication treatments, including aspirin (70 mg/kg) for the Haffner's tail clip technique and diclofenac (10 mg/kg) for the carrageenan test and formalin-induced paw oedema. As test groups, Groups III and IV received 250 and 500 mg/kg of the extract, respectively. Based on a prior acute toxicity investigation, doses were chosen. [12, 13]

Evaluation of Analgesic Activities of the Extract

Haffner's tail immersion test: The tail immersion test was used to differentiate between analgesics that act centrally and peripherally [14]. The animals were put in separate restraining cages with their tails unrestricted. The lower 5 cm of the tail was marked, and it was submerged in a cup of precisely 55° water. The rat responded in a matter of seconds by pulling back its tail. A stopwatch was used to measure this reaction time 0, 30, 60, and 90 minutes after the standard and test medications were administered.

Hot Plate Method: For this study, Franzotti *et al.,* (2000) adapted the approach outlined by Shethy and Anika (1982). Each rat was put on a hot plate that was kept at 55 ± 1°C. The latency period, also known as the pain reaction time (PRT), was measured using a stopwatch to show how long it took the rats to respond to the pain stimuli. Jumping, lifting, and licking the rear foot were among the reactions to pain stimuli taken into consideration. Twenty seconds was the set cutoff time. This was used to regulate the reaction time to pain. [15, 16]

Anti-Inflammatory Activity

Carrageenan-Induced Paw Edema: Rats that had fasted for the whole night and had unrestricted access to water were used to create acute inflammation in their paws. Carrageenan (1% w/v in normal saline, 0.05 mL) was injected into the plantar side of the left hind paw of the rats. Each rat's leg was tagged on the skin over the lateral malleolus just before to inflammation induction so that it may be equally submerged in the plethysmometer's measuring chamber. An hour after the extract, vehicle, and standard medication were administered to the corresponding groups of rats, carrageenan was injected. Using a digital plethysmometer, inflammation was measured in millilitres (mL), or the amount of water displaced by oedema, at times 0, 1, 2, 3, and 4 following carrageenan injection. The following formula was used to determine the percentage inhibition of oedema in relation to the control rats: [17]

$$\% \text{ Inhibition} = \frac{(V_c - V_t)}{V_c} \times 100$$

where V_c is the paw volume of the control group and V_t is the paw volume of the treated group. Results are expressed as mean ± standard error or standard deviation, and statistical analysis is performed to determine the significance of anti-inflammatory activity.

Formalin-Induced Paw Edema: In this process, sub-acute inflammation was induced using 1% v/v formalin. On the first and third days of observations, freshly made 1% v/v formalin with "distilled water (0.01mL)" was injected subplantarly into the right hind paw of rats that had fasted overnight. Before formalin induction, each rat's right hind paw was marked at the level of the lateral malleolus so that it could be consistently submerged to the same degree in the plethysmometer's measuring chamber during the observation days. For seven days in a row, the extract, the conventional medication, and the vehicle were administered according to their respective groups one hour before formalin injection. After one hour after extract, medication, and vehicle administration, the rats' paw volume was measured every day using a plethysmometer until the seventh day. The aforementioned formula was then used to determine the percentage of oedema inhibition. [18] The following formula is used to represent the anti-inflammatory efficacy as the percentage inhibition of paw oedema in treated groups relative to the control group:

$$\% \text{ Inhibition} = \frac{(V_c - V_t)}{V_c} \times 100$$

Where V_c represents the mean paw volume of the control group and V_t represents that of the treated group. Results are expressed as mean ± standard error or standard deviation, and statistical analysis is performed to determine significance.

Statistical Analysis: Version 21 of the Statistical Package for Social Sciences (SPSS) program was used to analyse the data. The mean ± standard error of mean (SEM) of responses was used to express all of the results. One-way Analysis of Variance (ANOVA) and a Tukey post hoc test were used to compare group variations and evaluate statistical significance. The findings were deemed significant at $p < 0.05$. When needed, tables and graphs were used to display the data that had been analysed. [19, 20]

RESULTS AND DISCUSSION

Acute toxicity test (AOT): The hydroalcoholic extract of *Passiflora incarnata* did not cause any behavioural abnormalities or death when administered at doses up to 2000 mg/kg, p.o. (Table 1). Therefore, 250 and 500 mg/kg, p.o. for extract was the dose chosen for more research.

Table 1: Acute Toxicity Test of HEPI

Sr. No.	Extracts	Dose (2000 mg/kg, p.o.)	No. of Animals Dead/Survived
1	HEPI	5 mg/kg	0/3
2	HEPI	50 mg/kg	0/3
3	HEPI	300 mg/kg	0/3
4	HEPI	2000 mg/kg	0/3

HEPI: Hydroalcoholic Extract of Passiflora incarnata

Analgesic Activity

1. Haffner's Tail Clip Method: The tail immersion method was used to assess the impact of the hydroalcoholic extract of Passiflora incarnata on nociceptive response; the findings are shown in Table 2. After the medication was administered, reaction times were recorded at 0, 30, 60, 90, and 120 minutes. Over the course of the observation period, there was no discernible change in the normal control group's (Group I) response time, suggesting consistent pain sensitivity. When compared to the normal control, the conventional medication aspirin (70 mg/kg, Group II) caused a noticeable and time-dependent increase in reaction time. Strong analgesic action was demonstrated by a substantial rise at 60 minutes ($p < 0.05$), which became extremely significant at 90 minutes (*$p < 0.01$) and continued to grow until 120 minutes. The response time of the hydroalcoholic extract of *Passiflora incarnata* at 250 mg/kg (Test-I, Group III) increased noticeably from the first measurement and continued to grow with time. When compared to the usual control, a statistically significant increase ($p < 0.05$) was seen at 90 minutes, and the impact lasted until 120 minutes. Similarly, a dosage-dependent increase in reaction time was seen with the greater extract dose of 500 mg/kg (Test-II, Group V). In comparison to the normal control, there was a highly significant rise (*$p < 0.01$) at 90 minutes, and the analgesic effect peaked at 120 minutes. Overall, Passiflora incarnata extract showed notable analgesic action at both dosages, with the 500 mg/kg dose having a stronger impact than the 250 mg/kg dose.

Table 2: Effect of hydroalcoholic Extract of *Passiflora incarnata* on rats

Group	Treatment / Dose	Mean reaction time (in Sec) by tail immersion method				
		0 min (sec)	30 min (sec)	60 min (sec)	90 min (sec)	120 min (sec)
I	Normal Control	3.1 ± 0.02	3.1 ± 0.04	2.8 ± 0.03	2.8 ± 0.10	2.8 ± 0.33
II	Aspirin (70 mg/kg)	4.1 ± 0.02	8.1 ± 0.32	14.1 ± 0.04**	16.3 ± 0.06***	22.3 ± 0.03
III	Test-I (250 mg/kg)	8.1 ± 0.12	14.8 ± 0.07	15.9 ± 0.11	19.7 ± 0.32**	21.8 ± 0.51
V	Test-II (500 mg/kg)	6.7 ± 0.11	12.5 ± 0.34	13.8 ± 0.11	15.6 ± 0.45***	20.1 ± 0.32

Values are expressed as mean ± S.E.M.; n=6 rats per group. one-way ANOVA followed by Dunnett's test when compared with Normal control **p<0.05, ***p<0.01. $p < 0.05$ = significant; $p < 0.01$ = highly significant

2. Hot Plate Method: The hot plate technique was used to assess the impact of the hydroalcoholic extract of Passiflora incarnata on thermal nociception in rats. Table 3 summarises the findings. Paw withdrawal delay gradually decreased in the normal control group over time, suggesting that there was no analgesic protection against heat pain stimuli. When compared to the normal control group, the administration of aspirin (70 mg/kg) resulted in a substantial increase in paw withdrawal latency at 60 minutes (8.01 ± 0.48 sec; ***$p < 0.01$) and 90 minutes (6.65 ± 0.92 sec; ***$p < 0.01$), showing its centrally mediated analgesic effect. Paw withdrawal latency was significantly increased at all time points in Test-I (250 mg/kg) of the hydroalcoholic extract, peaking at 60 minutes (10.66 ± 0.33 sec). When compared to the normal control group, the rise did not approach statistical significance, although being more than that of the conventional medication. A dose-dependent increase in latency time was seen in Test-II (500 mg/kg), with a significant impact at 90 minutes (7.15 ± 0.45 sec; **$p < 0.05$). At this dosage, the analgesic impact was similar to aspirin's, especially at later time periods.

Table 3: Effect of hydroalcoholic Extract of *Passiflora incarnata* on Hot plate in Rats

Treatment Group	0 min	30 min	60 min	90 min
Normal Control	**7.33 ± 1.45**	**3.66 ± 0.33**	**3.33 ± 0.33**	**3.00 ± 0.58**
Aspirin (70 mg/kg)	5.87 ± 0.39	5.95 ± 0.41	8.01 ± 0.48***	6.65 ± 0.92***
Test-I (250 mg/kg)	**8.00 ± 1.00**	**9.66 ± 1.20**	**10.66 ± 0.33**	**9.53 ± 0.33**
Test-II (500 mg/kg)	6.04 ± 0.47	6.98 ± 0.51	9.63 ± 0.46	7.15 ± 0.45**

Values are expressed as mean ± S.E.M.; n=6 rats per group. one-way ANOVA followed by Dunnett's test when compared with Normal control **p<0.05, ***p<0.01. $p < 0.05$ = significant; $p < 0.01$ = highly significant

Anti-inflammatory Activity

1. Carrageenan method: Using the carrageenan-induced paw oedema paradigm in rats, the hydroalcoholic extract of Passiflora incarnata was tested for its anti-inflammatory properties. The findings are shown in Table 4. After receiving a carrageenan injection, the normal control group's paw volume gradually increased, peaking at 120 minutes, suggesting the onset of acute inflammation. When compared to the normal control group, the usual medication, diclofenac (10 mg/kg), significantly reduced paw oedema at 60 minutes (1.36 ± 0.04 ml; ***p < 0.01) and 120 minutes (1.28 ± 0.06 ml; ***p < 0.01). Diclofenac's strong anti-inflammatory effects were confirmed by a time-dependent rise in the percentage inhibition of oedema. At subsequent time periods, Test-I (250 mg/kg) caused a modest decrease in paw oedema. At 60 minutes (2.55 ± 0.14 ml; *p < 0.05) and 120 minutes (2.63 ± 0.08 ml; *p < 0.05), there was a statistically significant inhibition. In contrast to the conventional medication, the degree of inhibition was less. A significant and dose-dependent anti-inflammatory impact was shown by Test-II (500 mg/kg). At 60 minutes (1.76 ± 0.04 ml; **p < 0.05) and 120 minutes (1.48 ± 0.05 ml; **p < 0.05), there was a significant reduction in paw oedema. Strong anti-inflammatory efficacy at the higher dose was demonstrated by the percentage inhibition at these time points, which was similar to that of diclofenac. One popular experimental paradigm for assessing acute inflammation and testing anti-inflammatory drugs is carrageenan-induced paw oedema. In this paradigm, the inflammatory response is biphasic: prostaglandins and other inflammatory mediators are released during the late phase (after 60 minutes), but histamine and serotonin are mostly released during the early phase (0–60 minutes).

Table 4: Effect of hydroalcoholic Extract of *Passiflora incarnata* on carrageenan induced paw edema in rats

Treatment	0 min	15 min	30 min	60 min	120 min
Normal control	1.40±0.01	1.43 ± 0.24	2.59±0.03	2.56 ± 0.10	2.78 ± 0.07
Diclofenac (10 mg/kg)	1.24±0.01	1.10 ± 0.11 (23.14)	1.40±0.32 (32.94)	1.36 ± 0.04*** (46.84)	1.28 ± 0.06*** (57.67)
Test-I (250 mg/kg)	1.11±0.02	1.23 ± 0.06 (14.30)	2.45±0.03 (8.87)	2.55 ± 0.14* (15.54)	2.63 ± 0.08* (21.28)
Test-II (500 mg/kg)	1.24±0.02	1.12 ± 0.06 (21.63)	1.50±0.01 (23.55)	1.76 ± 0.04** (39.11)	1.48 ± 0.05** (50.54)

Values are expressed as mean ± S.E.M.; n=6 rats per group. one-way ANOVA followed by Dunnett's test when compared with Normal control **p<0.05, ***p<0.01. $p < 0.05$ = significant; $p < 0.01$ = highly significant

2. Formalin induced: Using the formalin-induced paw oedema paradigm in rats, the hydroalcoholic extract of Passiflora incarnata was tested for its anti-inflammatory properties. The findings are shown in Table 5. Following formalin injection, the normal control group's paw volume steadily increased, with oedema starting at 15 minutes and peaking at 120 minutes, showing that inflammation had been successfully induced. When compared to the normal control group, the usual anti-inflammatory medication, diclofenac sodium (10 mg/kg), significantly reduced paw oedema. At 30, 60, and 120 minutes, there was a highly significant (***p < 0.01) reduction in paw volume, indicating strong and long-lasting anti-inflammatory action. When compared to the usual control, the hydroalcoholic extract of Passiflora incarnata at a dosage of 250 mg/kg (Test-I) resulted in a moderate decrease in paw oedema. The moderate anti-inflammatory efficacy at this dosage is shown by the fact that the decrease in paw volume at various time intervals was not statistically significant. When compared to the normal control group, the extract's greater dose of 500 mg/kg (Test-II) significantly reduced paw oedema. At 30, 60, and 120 minutes, a statistically significant reduction (**p < 0.05) was noted, indicating a dose-dependent anti-inflammatory effect. Formalin-induced inflammation was significantly inhibited by the extract; however this impact was not as strong as that of diclofenac. An established experimental paradigm for assessing the neurogenic and inflammatory aspects of acute inflammation is formalin-induced paw oedema. Prostaglandins and other inflammatory mediators are released during the late phase of inflammation, whereas histamine, serotonin, and bradykinin are the main mediators during the early phase.

Table 5: Effect of hydroalcoholic Extract of *Passiflora incarnata* on formalin induced paw edema in rats

Treatment	0 min	15 min	30 min	60 min	120 min
Normal control	1.32 ± 0.02	1.58 ± 0.04	1.89 ± 0.05	2.12 ± 0.06	2.35 ± 0.08
Diclofenac (10 mg/kg)	1.36 ± 0.01	1.32 ± 0.03	1.29 ± 0.04***	1.25 ± 0.03***	1.21 ± 0.02***
Test-I (250 mg/kg)	1.31 ± 0.02	1.44 ± 0.05	1.62 ± 0.3	1.78 ± 0.04	1.85 ± 0.04
Test-II (500 mg/kg)	1.33 ± 0.02	1.31 ± 0.04	1.30 ± 0.03**	1.27 ± 0.04**	1.22 ± 0.03**

Values are expressed as mean ± S.E.M.; n=6 rats per group. one-way ANOVA followed by Dunnett's test when compared with Normal control **p<0.05, ***p<0.01. $p < 0.05$ = significant; $p < 0.01$ = highly significant

Discussion

The present study evaluated the analgesic and anti-inflammatory activities of the hydroalcoholic extract of *Passiflora incarnata* using established experimental models in Wistar rats. The findings clearly demonstrate that the extract possesses significant pharmacological activity, which appears to be dose-dependent. The acute oral toxicity study, conducted according to OECD 423 guidelines, revealed that the extract was safe up to a dose of 2000 mg/kg, with no mortality or observable behavioral changes. This indicates a wide safety margin and supports the selection of 250 mg/kg and 500 mg/kg doses for further pharmacological evaluation.

In the assessment of analgesic activity, both the tail immersion and hot plate methods were employed to evaluate centrally mediated nociception. In the tail immersion test, the extract significantly increased reaction time, particularly at the 500 mg/kg dose, indicating strong analgesic activity. The effect was comparable to the standard drug aspirin and showed a clear time-dependent pattern, with peak activity observed at 90–120 minutes. Similarly, in the hot plate method, which specifically evaluates central pain mechanisms, the extract produced an increase in latency time. Although the lower dose (250 mg/kg) showed higher latency values, the higher dose (500 mg/kg) demonstrated statistically significant effects at later time points, suggesting a centrally mediated analgesic mechanism. The observed analgesic effects may be attributed to the presence of bioactive constituents such as flavonoids, alkaloids, and glycosides reported in Passiflora incarnata. These compounds are known to modulate pain pathways, possibly through interaction with opioid receptors or inhibition of prostaglandin synthesis.

The anti-inflammatory activity of the extract was evaluated using carrageenan-induced and formalin-induced paw edema models, representing acute and sub-acute inflammation, respectively. In the carrageenan-induced model, the extract significantly reduced paw edema, especially at the higher dose (500 mg/kg), with effects comparable to diclofenac at later time points. Since the late phase of carrageenan-induced inflammation is mediated by prostaglandins, the extract's inhibitory effect suggests interference with cyclooxygenase pathways and suppression of prostaglandin synthesis.

In the formalin-induced paw edema model, which involves both neurogenic and inflammatory phases, the extract exhibited moderate to significant anti-inflammatory activity. The higher dose (500 mg/kg) produced statistically significant inhibition of edema, although the effect was less potent than diclofenac. This indicates that the extract may act on both early and late phases of inflammation, possibly by inhibiting the release of mediators such as histamine, serotonin, and prostaglandins. Overall, the results demonstrate that the hydroalcoholic extract of Passiflora incarnata possesses significant analgesic and anti-inflammatory properties, with greater efficacy observed at higher doses. The effects are likely mediated through both central and peripheral mechanisms involving inhibition of inflammatory mediators and modulation of pain pathways.

CONCLUSION

The results of this investigation show that *Passiflora incarnata* hydroalcoholic extract has important analgesic and anti-inflammatory qualities. The results were dosage-dependent, and in both analgesic and anti-inflammatory models, the 500 mg/kg dose was more effective. The presence of bioactive phytoconstituents that are known to influence pain and inflammatory pathways, such as flavonoids, alkaloids, and phenolic chemicals, may be responsible for the action. The extract demonstrated excellent therapeutic potential with a positive safety profile, albeit having somewhat less potency than conventional medications. The traditional usage of Passiflora incarnata to treat pain and inflammation is supported by these findings. To identify the active ingredients and clarify the exact mechanisms of action, more investigation is advised.

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