Original research article

Phytochemical & pharmacological investigation of stems of *Passiflora foetida L*

ABSTRACT

Aims: To carry out extraction, preliminary phytochemical analysis and invivo analgesic screening of extract of the stem of *Passiflora foetida L.*
Methodology: *Passiflora foetida* L; Family: Passifloraceae, is an exotic fast-growing perennial and medicinal vine occurring in Germany, France and other European countries and USA and grown in different parts of India. Dried stems of Passiflora foetida L was coarsely powdered and maceration was done using Soxhlet apparatus. The ethanolic extract of stems of Passiflora foetida L was subjected to preliminary phytochemical tests. Then subjected to in vivo analgesic activity.
Results: Phytochemical investigation of the stem of *Passiflora foetida L* preliminary test showed the presence of carbohydrates, glycosides, flavonoids and steroids. Acute toxicity study of ethanolic extract of stems of *Passiflora foetida L* was carried out and extracts were found to be safe up to 2000 mg/kg body weight. Pharmacological activities of stems of *Passiflora foetida L* was carried out from ethanolic extract.
Conclusion: Phytochemical investigation of ethanolic extract of stems were carried out and Analgesic activity by tail flick method in rats and acetic acid induced writhing method in mice, showed statistically significant activity (P=.05) when compared to control. The ethanolic stem extract of Passiflora foetida L proved to have significant pain relieving action in a dose dependent manner.

Keywords: Passiflora foetida.L, Passifloraceae, acute toxicity, tail flick, writhing.

1. INTRODUCTION

Medicinal plants which form the strength of traditional medicine is being explored extensively for the treatment of various health issues by subjecting to intense pharmacological studies. Number of traditional plants have been used in the treatment of various ailments which has been acknowledged and has made known the value of medicinal plants as potential sources of new compounds of therapeutic value and as sources of lead entities in the drug development. In developing countries and now in developed countries, it is estimated that about majority of the population rely on/or turning to traditional medicine for their primary healthcare. There arises therefore a need to screen medicinal plants for bioactive compounds as a basis for further pharmacological studies[1]. Passiflora foetida is a biennial herb, commonly referred to as red fruit passion flower of the class Dicotyledonea. It is a evergreen climber with twisted slender structures on the stems that help in climbing. Stems sometimes angular to 15 cm height. The stems have a disagreeable odour[2].

A review article on Passiflora reported that different parts of the plant either in fresh or dried form can be extracted for medicinal use in European countries as anxiolytics. A decoction prepared from the leaves and fruits is used to treat psychological disorders and asthmatic conditions. Paste prepared from the leaves of *Passiflora foetida L* is useful for the treatment of skin infections and headaches.It is believed to have antispasmodic, sedative and hypotensive activity[3]as well as effective antidepressant properties[4]. Three polyketides, alpha-pyrones named Passifloricins from Passiflora foetida resin were isolated [5], several natural and hemisynthetic passifloricins showed Leishmanicidal activity [6].

Passiflora edulis commonly known as passion fruit expressed free radical scavenging properties [7], antibacterial and cytotoxic properties [8], anti-helmenthic activity of Passiflora edulis Linn, using ethanol and water extracts of leaves [9], roots of *Passiflora foetida* Linn showed significant anti bacterial properties against different bacterial strains [10].

It was learnt that no substantial work on the stems of *Passiflora foetida* L was carried out both in the chemical investigation & screening for analgesic activity, hence the aim of present work is to carry out screening for analgesic activity.

2. MATERIAL AND METHODS

Toshniwal apparatus was used for recording of melting points. For the present investigation the plant stem were collected from local area of Mangaluru, The plant stem was authenticated by Dr. Noeline J. Pinto, Professor and Head, Dept. of Botany, St.Agnes College, Mangaluru,Karnataka State.

2.1 Experimental procedure for preparation of ethanolic extract

The stems of *Passiflora foetida* L (3 kg) were cleaned, shade dried and broken down into pieces and powdered into a coarse powder by a mechanical grinder. The powder was then passed through sieve no. 40. 750 gms of powder was extracted with ethanol in soxhlet extractor for 72 hrs. A dark brown residue of yield of 12.3% was obtained after concentrating the extract which was done under reduced pressure. Then placed in desiccator for further use.

2.1.1. Pharmacological investigation of stem of *Passiflora foetida L*

Selection of animals: Wistar albino rats of either sex weighing between 100-150g and albino mice of either sex weighing between 20-25g were obtained from KSHEMA, Deralakatte Mangaluru. These animals were used for the acute toxicity and analgesic studies. The animals were stabilized for 1 week; they were maintained in standard condition at room temperature, 60 ± 5% relative humidity and 12 h light and dark cycle. They had been given standard pellet diet supplied by Hindustan Lever Co, Bombay and water ad libitum throughout the course of the study. The animals were handled gently to avoid giving them too much stress, which could result in an increased adrenal output.

All the experiments were carried out as per the rules and regulations of institutional animal ethics committee. (Animal Ethics Committee, K.S. Hedge Medical Academy, Mangaluru-575018, Ref. No. KSHEMA /AEC/13/2011).

Acute toxicity studies: Acute toxicity study was conducted to determine the median lethal dose (LD50) of the ethanolic extract of stems of *Passiflora foetida L*[11]. The acute toxicity study was carried out in adult female albino rats by “up and down”[12] method and OECD guidelines 425 [13] . The animals were divided into 5 groups (n=6). The first group was treated after fasting overnight with a oral dose of 100mg/kg of body weight with the ethanolic extract of the stems of *Passiflora foetida* L suspended in 0.5% sodium carboxy methyl cellulose (NaCMC). The animals were observed continuously for 2-3 hours for general behavioral, neurological, autonomic profiles and finally death after 24 hour [14]. There was no mortality and no signs of toxicity at this dose level. Similarly, the other groups were treated with an oral dose of 2000 mg/kg body weight of ethanolic extract of stems of *Passiflora foetida* L and there was no mortality and no signs of toxicity and the extract were found to be safe at this dose level. The observations were tabulated according to Irwin’s table [15].

Selection of dose: From preliminary toxicity studies of ethanolic extract of stems of *Passiflora foetida* L, it was observed that none of the treated animals died and thus it was concluded that these fractions were nontoxic up to 2000 mg/kg body weight were chosen for the study for analgesic activity, three dose levels were chosen in such a way that, middle dose was approximately one tenth of the maximum dose during acute toxicity studies, and a low dose, which was 50% of the one tenth dose, and a high dose, which was twice that of one tenth dose (200 mg/kg, 100 mg/kg, 400 mg/kg).

Preparation of different doses: The ethanolic extract was prepared in 0.5% Na CMC of dose 100, 200, 400 mg/kg body weight, given to rats and mice by oral route.

Experimental Procedure

Tail flick method: Acute nociception was assessed using a tail flick apparatus (Analgesiometer). Each rat was placed in a restrainer, before treatment the basal reaction time was measured by keeping distal one-third portion of the rats tail. Tail was placed over the nichrome wire without touching it. The extracts were immediately administered by oral route and

reaction time was measured at 30 minute interval after the drug administration for 2 hrs. to prevent tissue damage 10 seconds cut off time kept. In the present study, adult male/female Albino rats (100 150 g, 5-6 weeks old) were selected. They were divided into 5 groups of 6 animals each. The first group served as control and received 2% NaCMC. The second group was treated with pentazocine (10 mg/kg body weight, p.o.). The third, fourth and fifth groups received 100, 200 and 400 mg/kg body weight of ethanolic extract of the stems of *Passiflora foetida* L as a suspension in 0.5%Na CMC. Reaction time was recorded after every 30 minutes for 120 minutes and the average values of reaction time after each time interval are calculated and compared with the pre test value by analysis of significance[16,17]

Evaluation of the analgesic activity of the ethanolic extract of Stem of *Passiflora foetida* L by tail flick method and writhing method are represented in tables 1 & 2 respectively along with group treatment description.

Table 1: Tail flick method

Strain	Albino rat
Sex	Male/female
Body weight	100-150 g
Number of animals in each group	n=6
Number of groups	5
Vehicle	0.5% Na CMC in distilled water
Route of administration	p.o. (Test drug), i.p (Standard drug)
Water and food	ad libitum, standard feed pellets

Groups Treatment

Group I (Control)	vehicle (0.5% w/v CMC in distilled water)
Group II (Standard)	Pentazocine (10 mg/kg body weight)
Group III (Test)	100 mg/kg body weight of extract of stem of Passiflora foetida L in 0.5% w/v NaCMC
Group IV (Test)	200 mg/kg body weight of extract of stem of Passiflora foetida L in 0.5% w/v NaCMC
Group V (Test)	400 mg/kg body weight of extract of stem of Passiflora foetida L in 0.5% w/v NaCMC

Writhing Method[18]: Injection of acetic acid into the peritoneal cavity of the mice produces pain with characteristic stretching behavior referred to as writhing. Male albino mice weighing between 20-25 g body weight were selected for the study. The animals were divided into 5 groups of six animals each. All animals received 0.1 ml acetic acid 0.6% v/v i.p. First group served as control, second group served received aspirin, standard drug. The third, fourth and fifth groups of animals received 100, 200, and 400 mg/kg body weight of ethanolic extract of stems of *Passiflora foetida* L respectively,

30 min prior to the administration of acetic acid injection. The writhing effect was indicated by the stretching of abdomen with simultaneous stretching of at least one hind limb. This was observed for 30min and change in number of writhings in test group compared with standard treated and control treated groups. The percentage inhibition was calculated by using the formula.

Percentage inhibition = [1 - Rt/ Rc] X 100

Where, Rt = Mean number of writhings in treated group

Rc = Mean number of writhings in control group

Table 2:Writhing by acetic acid

Strain	Swiss Albino mice
Sex	Male/female
Body weight	20-25 gm
Number of animals in each group	n=6
Number of groups	5
Vehicle	0.5% Na CMC in distilled water
Route of administration	p.o. (Test drug), i.p (Standard drug)
Water and food	ad libitum, standard feed pellets

GROUPS TREATMENT

Group I (Control)	vehicle (0.5% w/v CMC in distilled water)
Group II (Standard)	Aspirin (100 mg/kg body weight, p.o.)
Group III (Test)	100 mg/kg body weight of extract of stem of Passiflora foetida L in 0.5% w/v NaCMC
Group IV (Test)	200 mg/kg body weight of extract of stem of Passiflora foetida L in 0.5% w/v NaCMC
Group V (Test)	400 mg/kg body weight of extract of stem of Passiflora foetida L in 0.5% w/v NaCMC

3. RESULTS AND DISCUSSION

Acute toxicity studies: The ethanolic extract of stems of *Passiflora foetida* L did not exhibit any toxic effects up to 2000 mg /kg body weight when administered as single oral dose. Selection of dose The LD50 of the ethanol extract was more than 2000 mg/kg body weight in all the cases. Hence a dose of 100, 200 and 400 mg/kg body weight were chosen for the study.

The treatment of rats with the ethanolic stem extract of Passiflora foetida L at doses of 100, 200 and 400 mg/kg body weight respectively exhibited significant (P=.01) increase in reaction time while compared to control in case of tail flick method.

Pretreatment of mice with ethanolic stem extract of Passiflora foetida L at a dose 100, 200, 400 mg/kg body weight respectively produced a significant (P=.01) reduction in writhes induced by acetic acid while compared to control. The % inhibition of writhings or % protection were found to be 48.41%, 59.61% and 63.99% for ethanolic extract of stem at a dose 100, 200 and 400 mg/kg body weight respectively.
The ethanolic extract of the stem of *Passiflora foetida* L exhibited dose dependent analgesic activity in both methods experimented for analgesic activity.
Statistical analysis: All the values reported are expressed as mean ±SEM. Analyzed by One way analysis of variance (ANOVA)followed by Dunnett's multiple comparison test.

The preliminary qualitative analysis was performed for testing the presence phytochemical constituents in ethanolic extract and results are shown in table 3.

Table 3:Results of Qualitative Tests for Phytoconstituents

Sl No.	Tests	Inferences
1.	Alkaloids	
	a) Dragendorff's test	-ve
	b) Hager's test	-ve
	c) Wagner's test	-ve
	d) Mayer's test	-ve
2.	Carbohydrates	+ve
	a) Anthrone test	+ve
	b) Benedict's test	+ve
	c) Fehling's test	+ve
	d) Molisch's test	
3.	Flavonoids	
	Shinoda's test	+ve
4.	Glycosides	
	Legal's test	+ve
5.	Triterpenoids	
	Liebermann – Burchard test	-ve
6.	Resins	-ve
7.	Saponins	-ve
8.	Steroids	
	a) Liebermann -Burchard's test	+ve
	b) Salkowski reaction	+ve

9.	Tannins	-ve
10.	Proteins a) Biuret's test b) Millon's test	 -ve -ve

+ve-positive, -ve- negative

Results of analgesic activity by tail flick method and writhing method are reported in table 4 & 5 respectively.

Table 4: Data for the effect ethanolic stem extract of *Passiflora foetida* L on pain induced by thermal stimulus (tail flick method)

Treatment	Dose (mg/kg)	Reaction time(min)				
		0	30	60	90	120
Control	5ml/kg	2.38±0.006	2.40±0.007	2.35±0.005	2.32±0.006	2.34±0.003
Pentazocine	10	2.51±0.02	4.87±0.21	7.5±0.061	8.37±0.018	5.13±0.045
Ethanolic stem extract of Passiflora foetida L	100	2.61±0.19**	4.88±0.06**	5.37±0.04**	6.15±0.068**	4.64±0.053**
	200	2.43±0.028**	5.19±0.010**	6.19±0.067**	6.73±0.064**	5.24±0.067**
	400	2.74±0.044**	5.61±0.016**	6.85±0.027**	7.84±0.034**	5.43±0.093**

*All the values are expressed as mean± S.E.M. (n=6) ** P=.01 significant compared to control*

Table 5: Effect of ethanolic stem extract of *Passiflora foetida* L on Acetic acid induced writhing

Treatment	Dose (mg/kg)	Writhing	% Inhibition
Control 0.5%	NaCMC	82.20±2.01	-
Aspirin	100	25.50±1.74**	68.97%
Ethanolic stem extract of Passiflora foetida L	100 200 400	42.40±1.03** 33.20±1.08** 29.60±1.56**	48.41% 59.61% 63.99%

*** the mean difference is significant at the .05 level, when compared to the standard*

Discussion: Analgesic action caused visceral pain in acetic acid induced writhing in mice, which may be due to the release of arachidonic acid from tissue phospholipids action that could trigger an inflammatory response via the

cyclooxygenase pathway as well as prostaglandin synthesis. Increased levels of prostaglandins (PGE2 & PGF2α) in the peritoneal region increases pain associated with inflammation by increase in capillary permeability. This method is useful for evaluating peripheral analgesics, as the less number of writhing shows inhibition of prostaglandin synthesis which is a peripheral mechanism of pain inhibition. The crude ethanolic stem extract of *Passiflora foetida* L proved to have significant pain relieving action in a dose dependent manner.

Phytochemical screening showed the presence of carbohydrates, glycosides, flavonoids & steroids which is a probability for its analgesic properties by qualitative tests. Flavonoids bring about their analgesic activity primarily by targeting prostaglandins [19] The tail flick method is used for study of centrally acting nociceptive action. The three groups showed significant increased pain threshold in a dose dependent gradient way[20,21]. The tail flick latency continued to ascend for all three doses till 90 min when compared to the control. There seemed to be no statistically significant difference in mean tail flick latency between the three drug groups at the prefixed time points of 0, 30, 60, and 90 minutes. Therefore, in the tail flick method, the three drug dosages were observed to be equally efficacious in analgesic effect during the experimental time period of 120 minute. This indicates that the stem extract of *Passiflora foetida* L can produce analgesic effect by peripheral central mechanism.

4. CONCLUSION

The Preliminary Phytochemical investigation of ethanolic extract of stems revealed the presence of carbohydrates, glycosides, flavonoids and steroids. But proteins, tannins, saponins, alkaloids, resins were absent. Analgesic activity of stem of *Passiflora foetida* L showed statistically significant and may be attributed to components like flavonoids and steroids. From studies carried on stem it is evident that *Passiflora foetida* L are endowed with significant analgesic activity against animal models, thereby justifying their use in traditional system of medicine.

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

"All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee"- **, REF. NO. KSHEMA /AEC/13/2011)**

REFERENCES

1. Gurneet SC, Kala SK. Vitex agnus castus- an overview. Journal of Natural Remedies .2011;11(2): 90-97.
2. Dhawan K, Dhawan S, Chhabra S. Attenuation of benzodiazepine dependence in mice by a tri-substituted benzoflavone moiety of Passiflora incarnata Linnaeus: a non- habit forming anxiolytic. Journal of Pharmacy and Pharmaceutical Sciences. 2003; 6(3): 215- 222
3. Dhawan K, Dhawan S, Sharma A. Passiflora: a review update. J Ethnopharmacol. 2004 Sep;94(1):1-23. doi: 10.1016/j.jep.2004.02.023
4. Santhosh P, Venugopl R, Nilaksha S, Kunjbihari S, Mangala L .Antidepressant Activity of methanolic extract of Passiflora foetida leaves in mice. International Journal of Pharmacy and Pharmaceutical Sciences. 2011; 3(1): 112-115.
5. Echeverri F, Arango V, Quinones W, Torres F, Escobar G, Rosero Y,Archbold R. Passifloricins, polyketides alpha-pyrones from Passiflora foetida resin. Phytochemistry. 2001; 56(8): 881-885. doi: 10.1016/s0031-9422(00)00478-7.
6. Wilson Cardona G., Winston Quinones F. and Fernando Echeverri L. Leishmanicidal Activity of Passifloricin A and Derivatives .Molecules. 2004; 9(8): 666– 672. doi: 10.3390/90800666.
7. Rasool SN, Jaheerunnisa S, Jayaveera KN, Suresh Kumar C. In vitro callus induction and in vivo antioxidant activity of Passiflora foetida L leaves. International Journal of Applied Research in Natural Products. 2011; 4 (1): 1-10.
8. Farhana AR, Mahmuda H ,Laizuman, Md. MI. Antibacterial, Cytotoxic and Antioxidant Activity of Passiflora Edulis Sims. European Journal of Scientific Research. 2009; 31(4): 592-594.
9. Emin B, Balasubramaniam A, Manivannan R, Joicy J and Senthilkumar. Antibacterial activity of methanolic root extract of Passiflora foetida Linn. Journal of pharmaceutical sciences and research. 2010; 2(1): 38- 40
10. Sangeetha J, Soundarya K, Santhosh K, Sindhura C. Evaluation of In-vitro Anthelmentic Property of Passiflora edulis Linn. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2010; 1(3): 715-718 .

11. Jennifer Fernandes, Michael Antony Noronha, and Ronald Fernandes. 2013. Evaluation of Anti-inflammatory activity of stems of Passiflora foetida Linn. in rats. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 4(2):1236-1241.
12. Ghosh MN. Fundamentals of Experimental Pharmacology. Calcutta.Scientific Book Agency. 1984; 2nd ed: 153-157.
13. OECD/OCED 425 OECD guidelines for testing of chemicals acute oral toxicity up and down procedure. 2001; 26: 1-26.
14. Ghosh MN. Fundamentals of Experimental Pharmacology. Calcutta Scientific Book Agency Calcutta. 2005; 3rd ed: 190-97.
15. Vogel GH. Drug Discovery and evaluation of Pharmacological Assay. 2nded. New York: Springer- Verlag Berlin Heidelberg.2002; 950-951
16. Rang HP, Dale MM, Ritter JM, Moore PK, Text book of pharmacology, Churchill Living stone, 5th ed., Edinburgh. 2003; 560-572.
17. Vogel GH, Vogel WH. Drug Discovery and Evaluation Pharmacological Assay. New York: Springer Berlin Heidelberg. 2002; 694-5
18. Jain NK, Kulkarni SK. J Ethnopharmacol. 1999; 68:251
19. In vivo analgesic activity of ethanolic extracts of two medicinal plants - Scoparia dulcis L. and Ficus racemosa Linn. AHM Zulfiker, M Mahbubur Rahman , M Kamal Hossain, K Hamid , MEH Mazumder , M Sohel Rana. Biology and Medicine, 2010;2 (2): 42-48,
20. Tasleem F, Azhar I ,Ali SN ,Perveen S, Mahmood ZA .Analgesic and anti-inflammatory activities of Piper nigrum L. Asian Pacific Journal of Tropical Medicine,2014,7; S461-S468. DOI10.1016/S1995-7645(14)60275-3
21. Santenna C , Kumar S, Balakrishnan S , Jhaj R , Ahmed SN, A comparative experimental study of analgesic activity of a novel non-steroidal anti-inflammatory molecule – zaltoprofen, and a standard drug – piroxicam, using murine models.journalof experimental pharmacology,2019,11;85-91. doi.org/10.2147/JEP.S212988