

ANTINOCICEPTIVE, ANTIDIARRHOEAL AND CYTOTOXIC ACTIVITIES OF *PASSIFLORA FOETIDA* LINN.

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Summary

The ethanol extract of dried whole plants of *Passiflora foetida* Linn. (Family–Passifloraceae) was investigated for its possible antinociceptive, antidiarrhoeal and cytotoxic activities in animal models. The extract produced significant ($P < 0.001$) writhing inhibition in acetic acid-induced writhing in mice at the oral dose of 250 and 500 mg/kg of body weight comparable to the standard drug diclofenac sodium at the dose of 25 mg/kg of body weight. The extract showed antidiarrhoeal activity on castor oil induced diarrhoea in mice, it increased mean latent period and decreased the frequency of defecation significantly ($P < 0.001$, $P < 0.01$) at the oral dose of 500 mg/kg of body weight comparable to the standard drug Loperamide at the dose of 50 mg/kg of body weight. The crude ethanolic extract also produced the most prominent cytotoxic activity against brine shrimp *Artemia salina* ($LC_{50} = 40 \mu\text{g/ml}$ and $LC_{90} = 80 \mu\text{g/ml}$). The obtained results provide a support for the use of this plant in traditional medicine and its further investigation.

Key Words: antinociceptive activity, antidiarrhoeal activity, cytotoxic activity, *Passiflora foetida* Linn.

Introduction

Passiflora foetida Linn. (English Name: Passionflower, Love-in-a-mist; Family: Passifloraceae; Synonym: *Passiflora incarnate*, *Passiflora ciliata* Dryand.) locally known as 'Mukkopeera' in Bangladesh. It is also known as Bedoca (Spanish-Galapagos Islands), Bombom (Chuuk), Dulce (Saipan), Grenadier Marron (French-Reunion (La Réunion)), Kinahulo' Atdao (Saipan), Kudamono (Palau), Lani Wai (Hawaii (Ni'ihau)), Loliloli Ni Kalavo (Fiji), Pasio Vao (Samoa), Passiflore (French), Pohapoha (Hawaii), Pompom (Pohnpei), Tea Biku (Kiribati) and Vaine 'Initia (Tonga). It is a fetid, herbaceous, hairy, perennial vine, scrambling or climbing to 5 m or more by axillary, unbranched, coiling tendrils with soft to hard, yellow to brown hairs, distributed and naturally grown throughout Bangladesh. It is native to the Southwestern United States (Southern Texas and Arizona), Mexico, the Caribbean, Central America, and much of South America. It has been introduced to tropical regions around the world, such as Southeast Asia and Hawaii¹⁻².

Passiflora foetida Linn. is stated to possess sedative, hypnotic, antispasmodic and anodyne properties¹. Tea of its leaves is used as an expectorant and for nervous disorders. Traditionally it is used for diarrhoea, intestinal tract, throat, ear infections, fever and skin diseases². It is also used in vomiting, eczema and chronic ulcer³.

Literature study shows that the fruits of *Passiflora foetida* Linn. possess hepatoprotective activity. This property may be attributed to the flavonoids present in the fruits of *Passiflora foetida* Linn.⁴. Another study reveals that the antibacterial properties of leaf and fruit (ethanol and acetone) extracts were screened against four human pathogenic bacteria i.e. *Pseudomonas putida*, *Vibrio cholerae*, *Shigella flexneri* and *Streptococcus pyogenes* by well-in agar method. The results showed the leaf extract having remarkable activity against all bacterial pathogens compared to fruits⁵. On the other hand, the antibacterial activity in methanolic root extract of *Passiflora foetida* by Kirby-Bauer disc diffusion method showed good antibacterial activity against gram negative organism⁶. Besides these, the amount of harmaline in *Passiflora foetida* was estimated by comparing the peak area of standard and that present in the extract. The harmaline content present in the extract was estimated to be 0.75% w/w⁷.

From the existing information it is evident that the plant may possess some important biological activities. The main objective of this study was to evaluate the antinociceptive, antidiarrhoeal and cytotoxic activities of the ethanol extract of dried whole plants of *Passiflora foetida* Linn.

Materials and Methods

Plant Material

Whole plants of *Passiflora foetida* Linn. were collected from Khulna University campus, Khulna, Bangladesh in June 2009 and were authenticated by the experts at National Herbarium (Accession Number: 34404). After collection, the whole plants were sun dried for several days to remove moisture. After drying, the dried whole plants were cut into small pieces by the help of a sharp knife and then were ground into coarse powder by 'Hammer' mill. About 400 gm of powdered whole plants was taken in a clean, flat-bottomed glass container and soaked in 1,300 ml of 80% ethanol. The container with its contents was sealed and kept for a period of 7 days accompanying occasional shaking and stirring. The whole mixture was then underwent a coarse filtration by a piece of cotton followed by a filtration through Whatmann filter paper and the filtrate thus obtained was concentrated using a rotary evaporator (Bibby RE200, Sterilin Ltd., U.K.) to get the crude extract. And this crude ethanolic extract was used for all phytochemical and pharmacological screening.

Animals

For antinociceptive and antidiarrhoeal activity study, young Swiss-albino mice of either sex, weighing 20 - 25 g, purchased from the Animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B), were used. After purchase, the animals were kept at animal house of Pharmacy Discipline, Khulna University, for adaptation under standard laboratory conditions (relative humidity 55-65%, room temperature 25.0±2.0°C and 12h light-dark cycle) and fed with standard diets and had free access to tap water. The experimental met the national guidelines on the proper care and use of animals. The Institutional

Animal Ethics Committee (IAEC) approved the experimental protocol. All the experiments were conducted on an isolated and noiseless condition.

Drugs

Diclofenac sodium (Opsonin Chemical Industries Ltd, Bangladesh), Loperamide (Square Pharmaceuticals Ltd., Bangladesh).

Preliminary Phytochemical Analysis

The ethanol extract of dried whole plants of *Passiflora foetida* Linn. was subjected to a preliminary phytochemical screening for major chemical groups. In each test, 10% (w/v) solution of the extract in ethanol was used unless otherwise specified in individual test^{1,8}.

Tests for Reducing Sugar

Benedict's Test: 0.5 ml of the extract was placed in a test tube and then 5 ml Benedict's solution was added to it, boiled for 5 min and allowed to cool spontaneously.

Fehling's Test (Standard Test): 2 ml of the extract was added in 1 ml of a mixture of equal volumes of Fehling's solutions A and B, and was boiled for few min.

Tests for Tannins

Ferric Chloride Test: 5 ml of the extract was placed in a test tube and then 1 ml of 5% Ferric chloride solution was added to it.

Potassium dichromate test: 5 ml of the extract was placed in a test tube and then 1 ml of 10% potassium dichromate solution was added.

Test for Flavonoids

A few drops of concentrated hydrochloric acid were added to 5 ml of the extract.

Test for Saponins

1 ml of the extract was placed in a graduated cylinder and was diluted to 20 ml with distilled water and shaken gently for 15 min.

Test for Gums

5 ml of the extract was placed in a test tube and then Molish's reagent and sulphuric acid were added to it.

Tests for Steroids

Libermann-Burchard test: 1 ml of the extract was placed in a test tube and then 2 ml Libermann-Burchard reagent was added to it.

Sulphuric acid test: 1 ml of the extract was placed in a test tube and 1 ml sulphuric acid was added to it.

Tests for Alkaloids

Mayer's test: 2 ml of the extract and 0.2 ml of dilute hydrochloric acid were taken in a test tube and 1ml of Mayer's reagent was added to it.

Dragendroff's test: 2 ml of the extract and 0.2 ml of dilute hydrochloric acid were placed in a test tube and then 1 ml Dragendroff's reagent was added.

Wagner's test: 2 ml of the extract and 0.2 ml of dilute hydrochloric acid were placed in a test tube. Then 1 ml of iodine solution (Wagner's reagent) was added.

Hager's test: 2 ml solution of the extract and 0.2 ml of dilute hydrochloric acid were placed in a test tube. Then 1 ml of picric acid solution (Hager's reagent) was added.

Tests for Glycosides

A small amount of extract was taken in 1 ml water. Then few drops of aqueous sodium hydroxide were added. Yellow precipitate is considered as an indication for the presence of glycosides.

In another test, a small amount of extract was taken in 1 ml water and boiled with 5 ml Fehling's solution in a boiling water bath. Brick-red precipitate is considered as an indication for the presence of glycosides.

In another test, a small amount of extract was boiled with few drops of dilute sulfuric acid, neutralized with sodium hydroxide solution and boiled with 5 ml Fehling's solution in a boiling water bath. Brick red precipitate is considered as an indication for the presence of glycosides.

Pharmacological Studies

Antinociceptive Activity

Antinociceptive activity of the ethanolic extract of dried whole plants of *Passiflora foetida* Linn. was tested using the model of acetic acid induced writhing in mice⁹⁻¹⁰. The experimental animals were randomly divided into four groups, each consisting of ten animals. Group I was treated as 'control' which received 1% (v/v) Tween-80 solution in water; group II was treated as 'positive control' and was given the standard drug diclofenac sodium at dose of 25 mg/kg of body weight; group III and group IV were test groups and were treated with ethanolic extracts of dried whole plants of *Passiflora foetida* Linn. at dose of 250 and 500 mg/kg of body weight respectively. Control vehicle, standard drug and the ethanolic extracts were administered orally 30 min prior to the intra-peritoneal injection of 0.7 % acetic acid, then after an interval of 15 min, the number of writhes (squirms) was counted for 5 min.

Antidiarrhoeal Activity

Antidiarrhoeal activity of the ethanolic extract of dried whole plants of *Passiflora foetida* Linn. was tested using the model of castor oil-induced diarrhoea in mice¹¹. The mice were all screened initially by giving 0.5 ml of castor oil and only those showing diarrhoea were selected for the final experiment. The test animals were randomly chosen and divided into three groups having five mice in each. Group-I was kept as control and received 1% Tween-80 at the dose of 10 ml/kg of body weight; group II was treated as 'positive control' and was given the standard drug loperamide at a dose of 50 mg/kg of body weight; group III was test group and was treated with the extract at a dose of 500 mg/kg of body weight. Control vehicle, standard drug and the extract were administered orally, 1 h prior to the oral administration of castor oil at a dose of 0.5 ml per mouse. Individual animals of each group were placed in separate cages having adsorbent paper beneath and examined for the presence of diarrhoea every hour in five hours study after the

castor oil administration. Number of stools or any fluid material that stained the adsorbent paper was counted at each successive hour during the experiment. The latent period of each mouse was also counted. At the beginning of each hour old papers were replaced by the new ones.

Cytotoxicity Test

The brine shrimps used for cytotoxicity test were obtained by hatching 5 mg of eggs of *Artemia salina* in natural seawater after incubation at about 29°C for 48h. The larvae (nauplii) were allowed another 48 h in seawater to ensure survival and maturity before use. Six doses of plant extract (10, 20, 40, 60, 80 and 100 µg/ml) in 5% DMSO and/or seawater were tested. Each extract preparation was dispensed into clean test tubes in 10 ml volumes and tested in duplicates. The concentration of DMSO in the vials was kept below 10 µl/ml. For control, same procedure was followed except test samples. After marking the test tubes properly, 10 living shrimps were added to each of the 20 vials with the help of a Pasteur pipette¹². The test tube containing the sample and control were then incubated at 29°C for 24 h in a water bath, after which each tube was examined and the surviving nauplii counted. From this, the percentage of mortality was calculated at each concentration.

Statistical Analysis

Student's *t*-test was used to determine a significant difference between the control group and experimental groups.

Results

Chemical Group Test

Results of different chemical group tests on the ethanolic extract of dried whole plants of *Passiflora foetida* Linn. showed the presence of steroids, Reducing Sugar, Alkaloids, Gums, Flavonoids and Glycosides (Table 1).

Table 1: Results of different chemical group tests of the extract of dried whole plants of *Passiflora foetida* Linn.

Extract	Reducing Sugar	Steroids	Alkaloids	Tannins	Gums	Flavonoids	Glycosides	Saponins
Ethanolic extract of dried whole plants of <i>Passiflora foetida</i> Linn.	+	+	+	-	+	+	+	-

Key: + = Presence, - = Absence

Antinociceptive Activity

Table 2 showed the effect of dried whole plants of *Passiflora foetida* Linn. on acetic acid-induced writhing model in mice. The extract produced about 41.38% and 71.92% writhing

inhibition at the dose of 250 and 500 mg/kg of body weight respectively, which were comparable to the standard drug diclofenac sodium where the inhibition was about 82.27% at the dose of 25 mg/kg of body weight (Table 2).

Table 2: Effect of ethanolic extract of dried whole plants of *Passiflora foetida* Linn. on acetic acid induced writhing in mice

Animal Group / Treatment	Number of writhes (% writhing)	Inhibition (%)
Control 1% tween-80 in water, p.o.	20.3±1.76 (100)	---
Positive control Diclofenac sodium 25 mg/kg, p.o.	3.6±1.17* (17.73)	82.27
Test group-I Ethanolic extract 250 mg/kg, p.o.	11.9±1.14* (58.62)	41.38
Test group-II Ethanolic extract 500 mg/kg, p.o.	5.7±1.80* (28.08)	71.92

Values are expressed as Mean±S.E.M (n=10), * $P < 0.001$, % = Percentage, p.o. = per oral.

Antidiarrhoeal Activity

Antidiarrhoeal activity of the ethanol extract of dried whole plants of *Passiflora foetida* Linn. was tested by castor oil induced diarrhoea in mice. The extract caused an increase in latent period (1.89 h) i.e. delayed the onset of diarrhoeal episode at the dose of 500 mg/kg of body weight significantly ($P < 0.001$) which was comparable to the standard drug loperamide at the dose of 50 mg/kg of body weight in which the value was 2.20 h ($P < 0.001$) (Table 3a). The extract also decreased the frequency of defecation at the same dose where the mean numbers of stool at the 1st, 2nd, 3rd, 4th and 5th h of study were 1.6, 1.4, 1.2, 1.2 and 1.4 respectively and in standard drug the values were 1.4, 1.2, 1.0, 1.0 and 1.2 respectively (Table 3b).

Table 3a. Effect of the extract of dried whole plants of *Passiflora foetida* Linn. on castor oil induced diarrhoea in mice (latent period)

Animal Group / Treatment	Dose(/kg, p.o)	Latent Period (h)
Group-I (control) 1% tween-80	10 ml	0.92 ± 0.126
Group-II (positive control) Loperamide	50 mg	2.20 ± 0.151*
Group - III Ethanolic extract	500 mg	1.89 ± 0.137*

Values are expressed as Mean±S.E.M (n=5), *P<0.001, p.o. = per oral.

Table 3b. Effect of the ethanolic extract of dried whole plants of *Passiflora foetida* Linn. on castor oil induced diarrhoea in mice (Number of stools)

Animal Group/Treatment	Dose (/kg, p.o.)	Period of study (h)	Total number of stool
Group-I (control) 1% tween-80 solution in water	10 ml	1	3.2 ± 0.327
		2	3.0 ± 0.353
		3	3.2 ± 0.396
		4	3.4 ± 0.418
		5	3.0± 0.377
Group-II (positive control) Loperamide	50 mg	1	1.4 ± 0.229*
		2	1.2 ± 0.223*
		3	1.0 ± 0.287*
		4	1.0 ± 0.273*
		5	1.2 ± 0.245*
Group-III Ethanolic extract	500 mg	1	1.6 ± 0.335*
		2	1.4 ± 0.308*
		3	1.2 ± 0.421*
		4	1.2 ± 0.387*
		5	1.4 ± 0.282*

Values are expressed as Mean±S.E.M (n=5), *P<0.01, p.o. = per oral.

Cytotoxic Activity

In brine shrimp lethality bioassay, the extract showed lethality against the brine shrimp nauplii. It showed different mortality rate at different concentrations. From the plot of percent mortality versus log concentration on the graph paper LC₅₀ and LC₉₀ were deduced (LC₅₀ = 40 µg/ml; LC₉₀ = 80 µg/ml) (Table 4).

Table 4. Brine shrimp lethality bioassay of the ethanolic extract of dried whole plants of *Passiflora foetida* Linn.

Test sample	Concentration (µg/ml)	Log (concentration)	Number of alive shrimp	Mortality (%)	LC ₅₀ (µg/ml)	LC ₉₀ (µg/ml)
Ethanolic Extract	10	1.00	09	10	40	80
	20	1.30	07	30		
	40	1.60	05	50		
	60	1.77	03	70		
	80	1.90	1	90		
	100	2.00	0	100		

Discussion

Antinociceptive activity of the ethanolic extract of dried whole plants of *Passiflora foetida* Linn. tested by acetic acid induced writhing model in mice. Acetic acid, which is used to induce writhing, causes algnesia by liberation of endogenous substances, which then excite the pain nerve endings¹³. The extract produced significant writhing inhibition comparable to standard drug diclofenac sodium. Based on this, it could be concluded that it might possess antinociceptive activity.

Antidiarrhoeal activity of the ethanol extract of dried whole plants of *Passiflora foetida* Linn. was tested using the model of castor oil induced diarrhoea in mice¹¹. Castor oil, which is used to induce diarrhoea in mice, mixes with bile and pancreatic enzymes and liberates ricinoleic acid from the triglycerides upon oral administration. Most of the ricinoleic acid remains in the intestine and produces its anti absorptive or secretory effect. The ricinoleic acid thus liberated readily forms ricinoleate salts with sodium and potassium in the lumen of the intestine. The salt formed as such behaves like a soap or surfactant within the gut and at the mucosal surface. Most agreed view is that ricinoleate salts stimulates the intestinal epithelial cell's adenylyl cyclase¹⁴ or release prostaglandin¹⁵. The extract caused an increase in latent period i.e. delayed the onset of diarrhoeal episode and decreased the frequency of defecation as well as the number of stool. On the basis of the result of castor oil induced diarrhoea, it can be concluded that the ethanol extract of dried whole plants of *Passiflora foetida* Linn. might possess antidiarrhoeal activity.

The cytotoxic activity of the ethanol extract of dried whole plants of *Passiflora foetida* Linn. was tested by using brine shrimp lethality bioassay. It is a recent development in the bioassay for the bioactive compounds. Brine shrimp lethality bioassay indicates cytotoxicity as well as a wide range of pharmacological activities such as antimicrobial, pesticidal, antitumor etc.¹⁶. The extract was found to show potent activity against the brine shrimp nauplii. Therefore the positive response obtained in this assay suggests that the extract may contain antitumor, antibacterial or pesticidal compounds.

In conclusion, it could be suggested that the crude ethanolic extract of dried whole plants of *Passiflora foetida* Linn. might possess antinociceptive, antidiarrhoeal and cytotoxic activities. However, further studies comprising of thorough phytochemical investigations of the used plant to find out the active principles and evaluation for these activities using other models are essential to confirm its pharmacological properties.

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