

Evaluation of Antidiarrhoeal Effect of Acetone Extract Fraction from The Stem Bark of *T. populnea*

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ABSTRACT

Thespesia populnea is traditionally used for the treatment of diarrhoea in India. In this study, acetone extract fraction from the stem bark of *T. populnea* (AFTP) was evaluated for its antidiarrhoeal activity. Acute oral toxicity (AOT) of AFTP was conducted as per OECD guidelines 425. AOT results revealed that AFTP is safe up to 2000 mg/kg. The antidiarrhoeal effect was evaluated by three experimentally induced diarrhoea models i.e. Castor oil induced diarrhoea, Prostaglandin E₂ (PG-E₂) induced enteropooling in rats and charcoal meal test in mice. AFTP at the dose level of 2 and 4 mg/kg decreased the mean weight of faeces in castor oil induce diarrhoea. AFTP (1, 2, and 4 mg/kg) significantly inhibited the mean volume of intestinal fluid in a dose dependent manner. AFTP 1, 2, 4 mg/kg decreased the intestinal propulsion of charcoal meal in the mice similar to the standard drug Atropine. These results suggest that AFTP could be developed as a potential antidiarrhoeal agent.

Keywords: *Thespesia populnea*, Diarrhoea, Castor oil, Prostaglandin E₂, Charcoal meal test.

INTRODUCTION

Diarrhoea is responsible for the death of millions of people each year worldwide, especially in developing countries. Acute diarrhoeal disease is one of the principal causes of death in the infants and the symptoms are stomach pain and vomiting. Diarrhoea occurs due to contaminated drinking water, unhygienic conditions, gastro intestinal disorders, plant and animal toxins^{1,2}. *Thespesia populnea* (L.) Soland. is a large tree belongs to the family of Malvaceae, found in coastal forests and tropical regions of India. It is commonly known as 'Indian tulip tree' or 'Portia tree'. The leaves were reported to be employed locally as anti-inflammatory in swollen joints³. It is also used for the treatment of ulcers, psoriasis and urinary tract infections. The flowers and barks have been scientifically proven to possess hepatoprotective, astringent and antioxidant properties⁴. *T. populnea* is traditionally used for the treatment of purgative and antifertility⁵. It is used for the treatment of Alzheimer's disorder and memory enhancing activity⁶. In Ayurveda system of medicine, the fruits are used in the treatment for the control of diabetes. Gossypol was found to be the major component of *T. populnea* producing anti-fertility effects in rats as well as in human beings⁷⁻¹⁰. The fruits contain thespsin and β -sitosterol. The flower part contains gossypetin and kaempferol. Four naturally occurring quinones viz thespsone, thespsone, mansonone-D, and mansonone-H have been extracted from heart wood of the plant¹¹. The infusion of stem bark of *T. populnea* is used in the management of diarrhoea by traditional

medicine practitioners in Tamil Nadu. Already, antidiarrhoeal activity of stem bark of alcoholic and aqueous extract of *T. populnea* was evaluated¹². The aim of the present study was to evaluate the possible antidiarrhoeal activity of acetone extract fraction from the stem bark of *T. Populnea* (AFTP). Currently, used antidiarrhoeal drugs like Loperamide and Racecadotril are playing important role in the management of diarrhoea but having adverse effects and contraindications. They cause fever, bronchospasm, nausea and vomiting¹³.

MATERIALS AND METHODS

Drugs and chemicals

All the solvents used for the extraction process are of Laboratory grade. Castor oil (Medinova Chemicals, Bangalore), Deactivated charcoal (New India chemical enterprises, Kochi), Prostaglandin E₂ (Zydus Alidac, Ahmedabad), Atropine (S. D. Fine Chemicals, Mumbai) and Loperamide (Torrent Pharmaceuticals, Ahmedabad, India) were used for the study.

Experimental animals

Swiss albino mice (18-22g) and Wistar albino rats (150-200 g) of either sex were acclimatized for 7 days under standard husbandry conditions. i.e. room temperature 26 \pm 10°C. relative humidity 45-55% and light: dark cycle 12:12 h. the experimental protocols were approved by the Institutional Animal Ethics Committee of Farooqia College of Pharmacy, Mysore (IAEC/JF-1) and conducted according to the guidelines of the Committee for the

Table 1: Effect of AFTP on Castor oil induced diarrhoea.

Treatment	Mean Weight of Faeces \pm S.E.M after 6 hrs (gm)	Percentage of Inhibition
Control	9.85 \pm 0.90	
Loperamide 2 mg/kg	1.78 \pm 0.62*	81.92
AFTP 1 mg/kg	8.14 \pm 0.45	17.36
AFTP 2 mg/kg	4.34 \pm 0.43*	55.93
AFTP 4 mg/kg	2.38 \pm 0.38	75.83

*P < 0.05 statistically (Mean \pm S.E.M.) significant from control group (n=6).

Table 2: Effect of AFTP on PGE₂ induced diarrhoea.

Treatment	Mean Volume of Intestinal fluid \pm S.E.M after 6 hrs (gm)	Percentage of Inhibition
Control	3.11 \pm 0.33	
Loperamide 2 mg/kg	0.80 \pm 0.10*	74.27
AFTP 1 mg/kg	1.57 \pm 0.18*	15.92
AFTP 2 mg/kg	0.79 \pm 0.07*	74.59
AFTP 4 mg/kg	0.42 \pm 0.03*	86.49

*P < 0.05 statistically (Mean \pm S.E.M.) significant from control group (n=6).

Table 3: Effect of AFTP on Charcoal induced diarrhoea.

Treatment	Mean movement of charcoal (cm)	% of	Percentage of Inhibition
Control	90.24 \pm 3.11		
Atropine 3 mg/kg	41.35 \pm 3.61*		54.17
AFTP 1 mg/kg	58.85 \pm 5.23*		34.78
AFTP 2 mg/kg	40.39 \pm 3.65*		55.24
AFTP 4 mg/kg	27.12 \pm 3.03*		69.94

*P < 0.05 statistically (Mean \pm S.E.M.) significant from control group (n=6).

Purpose of the Control and Supervision on Experiments on Animals (CPCSEA).

Extraction and isolation of the fraction

The collected stem bark of *T. populnea* was shade dried, powdered and sieved in mesh 40. The powdered material was extracted with 5 L of 70% acetone at 60°C for 2 hrs. Extraction was repeated twice with 5 L of acetone. The acetone extracts were combined and evaporated under reduced pressure. The concentrated acetone extract was portioned between n-hexane and methanol. The methanol part was evaporated under reduced pressure to obtain a semi solid extract. This extract was poured into cold diethyl ether to precipitate the crude mixture. This was repeated several times and the precipitate was collected by filtration. The filtered crude mixture (400g) was subjected to column chromatography (Silica gel, 60-120 mesh) and eluted with n-hexane, n-hexane: ethyl acetate mixture, ethyl acetate: methanol mixture, methanol. All the eluted

fractions were monitored by thin layer chromatography using pre coated aluminium plates. Three fractions were obtained. The major fraction (350 g) was taken for further study and other two fractions were not considered as they were negligible amount. The major fraction was subjected for repeated HPLC on a reverse phase C-18 semi preparative column using Acetonitrile: Water (7:3) as mobile phase with flow rate of 10 ml/min. The eluted peak of fraction was collected and concentrated to dry mass. The isolated compound was recrystallized to get pure compound (1.5 g).

Phytochemical investigation

The isolated compound of *T. populnea* was subjected to preliminary qualitative investigations¹⁴.

Acute toxicity studies

The acute toxicity of AFTP was determined in female albino mice (18-22g). After administration with different doses of AFTP, the mortality with each dose was noted at 48 hours (acute) and 14 days (chronic). LD₅₀ was calculated as per OECD guidelines 425 using AOT 425 software¹⁵.

Antidiarrheal Activity

Castor oil induced Diarrhoea

Albino rats of either sex weighing 150-200 g were used. They were divided into five groups of six each as follows: Group I received control (3% Tween 80, p.o.), Group II received standard (Loperamide 2 mg/kg, p.o.) Group III, IV, and V received AFTP at the dose levels of 1, 2 or 4 mg/kg, p.o. respectively. One hour after drug treatment, each rat received castor oil (2ml/100g, p.o). Each rat was then housed separately in cage over clean filter paper. Then diarrhoea episodes were observed for a period of 4 hours. During this period, first defecation time, frequency of defecation and cumulative wet faecal mass were recorded. Antidiarrhoeal activity was determined in terms of percentage reduction in cumulative faecal mass with respect to control group^{16,17}.

Prostaglandin-E₂ induced Diarrhoea

Five groups of rats (150-200 g) consisting of 6 animals in each group were deprived of food and water for 18 hours prior to the experiment. Three different experimental groups of rats received AFTP at various doses of 1, 2 or 4 mg/kg (p.o). The animals in the control group received 3% Tween 80 (p.o) and standard group received Loperamide 2 mg/kg (p.o). All the rats were administered with prostaglandins-E₂ (100 μ g/kg in 2% v/v Tween 80 orally) except normal control group. Thirty minutes after prostaglandin-E₂ all the rats were sacrificed. The whole length of the intestine from the pylorus to the caecum is dissected out and its contents were collected and measured¹⁷. Percentage reduction of intestinal secretion (volume) was calculated.

Charcoal meal test

Albino mice of either sex weighing 20-25 g were fasted for 4 hours before commencing the experiment with free access to water and divided into five groups of six animals each, Group I served as control and were treated orally with 0.5 % w/v sodium carboxymethyl cellulose in distilled water. Group II animals served as standard and treated with atropine (3 mg/kg, i.p.) a positive control.

Animals of group III, IV and V received 1, 2 or 4 mg/kg AFTP. After 1 hour of above treatment, 1 ml of charcoal meal (3% deactivated charcoal in 3% v/v aqueous Tween 80 orally) was administered by oral route to all the animals in each group. After fifty minutes of charcoal treatment each mouse was sacrificed and distance moved by the charcoal meal from the pylorus to caecum was measured to express as a percentage of distance travelled by the charcoal meal in ratio to the intestinal length. Percentage inhibition produced by drug treatment was calculated¹⁷.

Statistical Analysis

Values are expressed as mean \pm SEM from 6 animals. Statistical difference in mean were analysed by using one way ANOVA (analysis of variance) followed by Dunnett's test. $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Phytochemical investigation

It was found that the AFTP contains alkaloids, flavonoids, proteins, tannins and phenolic compounds.

Acute toxicity studies

The acute toxicity study showed that oral administration of acetone extract fraction from stem bark of *T. Populnea* (AFTP) to the mice up to 2000 mg/kg dose neither showed mortality nor any visible clinical signs of general weakness in the animals. In Castor oil induced diarrhoea experiment the AFTP at the dose of 2 mg/kg has significantly decreased ($P < 0.05$) the mean weight of faeces upon administration of castor oil (4.34 ± 0.43) compared to the control group (9.85 ± 0.90). The effect of AFTP 4 mg/kg was similar to the standard drug Loperamide 2 mg/kg (Table-1). In this method the diarrhoea is induced as the normal fluid absorption is reduced because of the inhibition of $\text{Na}^+ \text{K}^+ \text{ATPase}$ activity in the intestine¹⁸. However, it is well documented that the main constituent ricinoleic acid from castor oil induces permeability changes in mucosal fluid and electrolyte transport and enhancing the hyper secretory response¹⁹. Also ricinoleic acid increases the capital PGE_2 in the small intestine which causes more secretion of water and electrolyte. Inhibition of prostaglandin biosynthesis will decrease the castor oil induced diarrhoea^{20,21}. Therefore, here the antidiarrhoeal activity of AFTP was mediated by antisecretory mechanism or by inhibition of prostaglandin biosynthesis. AFTP (1, 2, and 4 mg/kg) and standard drug Loperamide (2mg/kg) significantly inhibited the mean volume of intestinal fluid when compared with the PGE_2 control group. In AFTP treated group the effect was significant and in a dose dependent manner (Table-2). The mechanism behind this model is, PGE_2 inhibits the absorption of Na^+ , Cl^- and glucose which causes the accumulation of intestinal fluid and leads to diarrhoea^{22,23}. AFTP might be enhancing the absorption of water, electrolytes, glucose and also the antisecretory effect causes the antidiarrhoeal effect. The passage of a charcoal meal through the gastrointestinal tract is used as parameter for intestinal motility. Activated charcoal adsorbs drugs and chemicals and prevents their absorption²⁴. AFTP 1, 2, 4 mg/kg decreased the intestinal propulsion of charcoal meal in the mice gastro intestinal

tract compared to the control group (Table-3). A similar result obtained with the standard drug Atropine (1 mg/kg). Several studies shows that phytochemical constituents such as alkaloids, tannins, steroids, flavonoids and saponins are responsible for antidiarrhoeal action through various mechanism^{25,26}. Out of which, tannins and flavonoids play important role for antidiarrhoeal activity by increasing enteropooling and electrolyte reabsorption. It is well documented that tannins decreases the irritability of bowl and there by reduces the peristaltic index²⁷. AFTP also reduced the intestinal transit and observed as decrease in intestinal motility. The antidiarrhoeal activity of AFTP could be due to the anti-histaminic and anti-cholinergic effect. In conclusion, the antidiarrhoeal activity of AFTP upon the diarrhoea models showed a promising result and could be useful for primary medical care. The future work is initiated for the characterization of isolated constituent of AFTP.

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