ANTIDIARRHEAL ACTIVITY OF ALCOHOLIC AND AQUEOUS EXTRACTS OF STEM BARK OF *THESPESIA POPULNEA* IN RODENTS.

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Summary

The objective of the study is to investigate the aqueous (AQTP) and alcoholic extracts (ALTP) of stem barks of *Thespesia populnea* (Malvaceae) for their antidiarrheal activity in rodents. Stem bark was extracted with alcohol and water successively. Preliminary phytochemical investigation was carried out to identify various phytochemical constituents present in the extracts. It was found that the AQTP contains alkaloids, carbohydrates, glycosides, saponins, proteins, flavonoids, tannins and phenolic compounds; ALTP contained alkaloids, carbohydrates, glycosides, saponins, proteins, steroids, flavonoids, tannins and phenolic compounds. Acute oral toxicity of ALTP and AQTP were conducted as per OECD guidelines 425. Acute toxicity studies revealed that both the extracts are safe upto 2000mg/kg. The antidiarrheal activity was observed in three experimentally induced diarrhea models i.e. Castor oil induced diarrhea; Prostaglandin E_2 (PG-E_2) induced enteropooling in rats and charcoal meal test in mice. In castor oil induced model ALTP and AQTP showed significant dose dependent reduction of cumulative wet faecal mass. In PG-E_2 induced enteropooling model, ALTP (100, 200 and 400mg/kg, p.o.) and AQTP (50,100 and 200mg/kg, p.o) inhibit PG-E_2 induced secretions. Similarly in charcoal meal test ALTP and AQTP decreased the movement of charcoal indicating its antimotility activity. It was observed that AQTP is having more potent anti-diarrheal activity than ALTP in these models.

Keywords: Antidiarrheal activity**,** *Thespesia populnea*, castor oil, Prostaglandin E_2, Charcoal meal test.

Introduction

Diarrhea, an important health problem worldwide, especially in developing countries, accounts for more than 5-8 million deaths in infants and children under 5 years, each year [1]. In recent years there has been a great interest in herbal remedies for the treatment of a number of ailments .Medicinal plants are promising source of antidiarrheal drugs [2]. Indigenous plants such as *Andrographalis paniculata, Asparagus racemosus, Butea monosperma, Cassia auriculata, Ficus hispida, Hemidesmus indicus, Guiera senegalensis* etc are widely used for treatment of diarrhea [3].

Thespesia populnea Soland ex Correa, family Malvaceae is a large avenue tree found in the tropical regions and coastal forests in India. The bark, leaves, flowers and fruits are useful in cutaneous infections, such as scabies, psoriasis, eczema, ringworm and guinea worm.

A decoction of the bark is commonly used for the treatment of skin and liver diseases. Oil of the bark mixed with vegetable oil is useful in urethritis and gonorrhea. The astringent bark, roots and fruits were used in dysentery, cholera and hemarrhoids [4]. The leaves were reported to be employed locally as anti-inflammatory in swollen joints. The infusion of the bark powder is traditionally used in the treatment of diarrhea and dysentery. However; the plant has not been experimentally tested for its antidiarrheal activity. Hence the present study has been undertaken to investigate the antidiarrheal activity of bark extracts of *T. populnea* in experimentally induced diarrhea in rodents.

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 E_2 (Zidus Alidac. Ahmedabad.), Atropine (S.D.Fine chemicals. Mumbai.) and Loperamide (Torrent Pharmaceuticals. Ahmedabad, India) were used for the study.

Plant extraction

The bark of the plant was collected in the month of May – June 2007 and authentified by Dr.K.P.Sreenath, Reader and Taxonomist, Botany Department from Bangalore University. A sample specimen was deposited, bearing voucher number **Coll.no.I.** The shade dried plant material was powdered. The coarse powder was subjected to successive extraction with petroleum ether, alcohol (70%) in soxhlet apparatus and the marc obtained after alcoholic extraction was macerated with distilled water to obtain an aqueous extract. The % yield of alcoholic and aqueous extracts was found to be 5.33% and 2.5% respectively.

Phytochemical investigation

The aqueous (AQTP) and alcoholic (AQTP) extracts of *T.populnea* were subjected to preliminary qualitative investigations [5].

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 ± 1^0c. relative humidity 45-55% and light: dark cycle 12:12 h. the experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) of PES College of Pharmacy, Bangalore and conducted according to the guidelines of the Committee for the Purpose of the Control and Supervision on Experiments on Animals (CPCSEA).

Acute toxicity studies

The acute toxicity of AQTP and ALTP was determined in female albino mice (18-22g). After administration with different doses of these extracts, the mortality with each dose was noted at 48 hours

(acute) and 14 days (chronic). LD_{50} was calculated as per OECD guidelines 425[6] using AOT 425 software.

Antidiarrheal activity

A.Castor oil induced Diarrhea

The method described by Awouters *et al* [7] as followed. Albino rats of either sex weighing 150-200 g were used. They were divided into 8 groups each group containing six animals. Rats were fasted 24 hrs before the test with free access to water. Rats were treated orally with vehicle or aqueous extract or ethanolic extract or standard. 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 diarrhea 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. Antidiarrheal activity was determined in terms of percentage reduction in cumulative faecal mass with respect to vehicle treated group [8].

B.Prostaglandin-E_2 induced Diarrhea

Six 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. Rats were treated orally with vehicle or aqueous extract or ethanolic extract or loperamide one hour prior to prostaglandin-E_2 administration. All the rats were administered with prostaglandins-E_2 (100 µg/kg in 2% v/v Tween 80 orally) except normal control group. Thirty minutes after prostaglandin-E_2 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 measured [8].Percentage reduction of intestinal secretion (volume) was calculated.

C.Charcoal meal test

Albino mice of either sex weighing 20-25 g were used. Mice were fasted for 4 hours before commencing the experiment with free access to water. After 1 hour of extracts treatment, 1ml of charcoal meal [3% deactivated charcoal in 2% 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 extracts was calculated [8].

Statistical analysis

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

Results

Phytochemical investigation

It was found that the aqueous extract of *T.populnea* contains alkaloids, carbohydrates, glycosides, saponins, proteins, flavonoids, tannins and phenolic compounds; alcoholic extract of *T.populnea* extract contained alkaloids, carbohydrates, glycosides, saponins, proteins, steroids, flavonoids, and tannins and phenolic compounds .

Acute toxicity studies

In acute toxicity there was no mortality recorded in all the groups, i.e. AQTP and ALTP up to maximum dose of 2000mg/kg. Hence the extracts were found to be safe till 2000 mg/kg.

Antidiarrheal activity

Castor oil induced diarrhea

The standard drug Loperamide (1mg/kg), ALTP (100, 200 & 400mg/kg) and AQTP (50,100 & 200mg/kg) of *Thespesia populnea* bark significantly reduced the mean weight of the faeces when compared to untreated control rats. The aqueous extract has shown more significant activity than alcoholic extract, the results are shown in Table No. 1.

Table.1.Effect of *Thespesia populnea* bark extracts on Castor oil induced diarrhoea

Group	Treatment	Dose mg/kg	Mean weight of faeces ± S.E.M. after 6 hrs. (gm)	Percentage of inhibition
I	Control	–	7.595 ± 0.55	–
II	Lopermide	1	1.35 ± 0.21***	82.22
III	ALTP	100	5.19 ± 0.35*	31.65
IV	ALTP	200	2.98 ± 0.84***	60.65
V	ALTP	400	2.44 ± 0.19***	67.34
VI	AQTP	50	4.96 ± 0.45*	34.69
VII	AQTP	100	3.45 ± 0.48***	54.53
VIII	AQTP	200	2.76 ± 0.55***	63.64

Values are Mean ± S.E.M. (n=6); Significance vs. Control group: ***P < 0.001, **P < 0.01 and *P < 0.05

Prostaglandin E_2 induced diarrhea

The standard drug loperamide (3mg/kg), ALTP (100, 200 & 400mg/kg) and AQTP (50,100 & 200mg/kg) extracts of *Thespesia populnea* bark significantly inhibited PGE_2 induced enteropooling in rats compared with PGE2 control animals. PGE_2 induced a significant increase in fluid volume of the rat intestine when compared with the vehicle control animals. Results are shown in Table No. 2.

Table.2.Effect of *Thespesia populnea* bark extracts on PG-E_2 induced diarrhea

Group	Treatment	Dose mg/kg	Mean volume of intestinal fluid ± S.E.M. (ml)	Percentage of inhibition
I	Control	–	2.98 ± 0.07	–
II	Lopermide	3	0.83 ± 0.09***	72.07
III	ALTP	100	1.95 ± 0.11***	34.63
IV	ALTP	200	1.23 ± 0.18***	58.77
V	ALTP	400	0.80 ± 0.10***	73.18
VI	AQTP	50	1.93 ± 0.16***	35.19
VII	AQTP	100	1.10 ± 0.12***	63.12
VIII	AQTP	200	0.73 ± 0.09***	75.41

Values are Mean ± S.E.M. (n=6); Significance vs. Control group: ***P < 0.001, **P < 0.01 and *P < 0.05.

Charcoal meal test

The standard drug atropine (1mg/kg), ALTP (100, 200 & 400mg/kg) and AQTP (50,100 & 200mg/kg) extracts of *Thespesia populnea* (Linn) bark significantly decreased the propulsion of charcoal meal through the gastrointestinal tract, as compared with the control group.
Results are shown in Table No. 3.

Table.3.Effect of *Thespesia populnea* bark extracts on Charcoal meal test

Group	Treatment	Dose mg/kg	Mean % Movement of charcoal (cm)	Percentage of inhibition
I	Control	–	86.17 ± 1.89	–
II	Atropine	1	24.07 ± 1.84***	72.06
III	ALTP	100	63.04 ± 1.84***	26.84
IV	ALTP	200	46.95 ± 1.59***	45.51
V	ALTP	400	32.85 ± 1.36***	61.87
VI	AQTP	50	62.41 ± 1.80***	27.57
VII	AQTP	100	46.16 ± 1.59***	46.42
VIII	AQTP	200	30.35 ± 1.82***	64.77

Values are Mean ± S.E.M. (n=6); Significance vs. Control group: ***P < 0.001, **P < 0.01 and *P < 0.05.

Discussion

Alcoholic and aqueous extracts of stem bark of *Thespesia populnea* that have not been studied so far, were evaluated for their antidiarrhoeal potential against castor oil induced diarrhoea and prostaglandin-E_2 induced enteropooling in albino Wistar rats and antimotility effect in charcoal meal test in Swiss albino mice.

ALTP and AQTP exhibited significant antidiarheal activity against castor oil induced diarrhea in rats. The AQTP (100 & 200mg/kg) and AQTP (200 & 400mg/kg) extracts showed almost similar antidiarrheal activity as that of loperamide (1mg/kg).

It is widely known that castor oil or its active component ricinoleic acid induces permeability changes in mucosal fluid and electrolyte transport that results in hypher secretory response and diarrhea [9, 10].

Ricinoleic acid markedly increases the PG-E_2 in portal venous and gut lumen and also causes an increase in secretion of water and electrolytes in to the small intestine [11, 12]. Ricinoleic acid also produces irritation and inflammation of the intestinal mucosa, leading to release of prostaglandins, which stimulate motility and secretion [13]. Inhibition of prostaglandin biosynthesis delayed castor oil induced diarrhea [7]. Based on these observations, it seems that the antidiarrheal effect of ALTP and AQTP may be due to the inhibition of prostaglandin biosynthesis or by decreasing the peristaltic movement.

To ensure that ALTP and AQTP modify the action of prostaglandin, effect of ALTP and AQTP on PG-E_2 induced diarrhoea was studied in rats. AQTP and ALTP significantly inhibited the PG-E_2 induced intestinal fluid accumulation (enteropooling). It has been shown that E type of prostaglandins cause diarrhea in experimental animals as well as human beings [14]. Their mechanism has been associated with dual effects on gastrointestinal motility as well as on water and electrolyte transport [15]. These observations tend to suggest that ALTP (100, 200 & 400mg/kg) and AQTP (50,100 & 200mg/kg) reduced diarrhea by inhbiting PG-E_2 induced intestinal accumulation of fluid.

Studies showed that activated charcoal readily adsorbs drugs and chemical on the surface of the charcoal particles and their by preventing absorption [16]. Hence gastrointestinal motility test with deactivated charcoal was carried out to find out the effect of ALTP and AQTP on peristaltic movement. The extracts appear to act on all parts of intestine. Thus, it reduced the intestinal propulsive movement in the charcoal meal treated model; at the doses 100, 200 and 400mg/kg of ALTP and 50, 100 and 200mg/kg of AQTP. The results also showed that ALTP and AQTP suppressed the propulsion of charcoal meal there by increased the absorption of water and electrolytes. The inhibition of peristaltic movement with alcoholic and aqueous extracts of stem bark of *Thespesia populnea* may be due to the anti histaminic and anticholinergic actions. From these models we can suggest that ALTP and AQTP non-specifically inhibit diarrhea either by decreasing intestinal motility or by decreasing the prostaglandin biosynthesis.
The result indicates that ALTP and AQTP possess significant antidiarrheal activity due to their inhibitory effect both on gastrointestinal propulsion and fluid secretion.

The data obtained is consistent with literature reports on antidiarrheal activity of *Thespesia Populnea* stem bark using gastrointestinal motility test in mice and castor oil induced diarrhea and intraluminal accumulation of fluids in rats.

The inhibitory effect of the plant extracts justified the use of plant as a non-specific antidiarrheal agent in folklore medicine. Further detailed investigations are needed to determine the phytoconstituents which are responsible for antidiarrheal activity.

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