ANTIDIARRHOEAL ACTIVITY OF ALCOHOLIC AND AQUEOUS EXTRACTS OF ROOTS OF *TYLOPHORA INDICA* (WIGHT & ARN.) IN RODENTS.

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Summary

The objective of the study is to investigate alcoholic (ALRT) and aqueous (AQRT) extracts of roots of *Tylophora indica (Asclepiadaceae)* for their anti diarrhoeal activity in rodents. Roots were extracted with alcohol and water successively. Preliminary phytochemical investigation was carried out to identify various phytochemical constituents present in the extracts. LD_{50} of ALRT and AQRT extracts were conducted as per as OECD guidelines 425. LD_{50} of ALRT was 3162 while for AQRT was found to be greater than 5000 g/kg. The antidiarrhoeal activity was observed in three experimentally induced diarrhoeal models i.e. castor oil induced diarrhoea and PG-E_2 induced enteropooling in rats and charcoal meal test in mice. ALRT and AQRT showed presence of alkaloids, flavonoids, tannins, amino acids, saponins and carbohydrates. In castor oil induced model, ALRT and AQRT showed significant dose dependent reduction of cumulative wet faecal mass. In PG-E_2 induced enteropooling model, ALRT (200 and 400 mg/kg, po) and AQRT (200 and 400 mg/kg, po) inhibited PG-E_2 induced secretions. Similarly in charcoal meal test, ALRT and AQRT decreased the movement of charcoal indicating its anti-motility activity. It was observed that ALRT and AQRT were found to possess equipotent anti diarrhoeal activity in these models. Moreover, AQRT was safer than ALRT (LD_{50} values). Hence AQRT shall be beneficial in treatment of diarrhoea.

Keywords: Antidiarrhoeal activity, *Tylophora indica,* castor oil, prostaglandin-E_2, charcoal meal test.

Diarrhoea is one of leading cause of mortality in infants and children in the developing countries [1]. Several studies have shown that prior administration of some plant extracts had a protective effect on intestinal tract [2, 3, 4]. Many plants conveniently available in India are used in traditional folklore medicine for the treatment of diarrhoea and dysentery and the indigenous plants like *Andrographis paniculata, Asparagus racemosus, Butea monosperma, Cassia auriculata* and others are widely used for the treatment of diarrhoea [5].

Tylophora indica is a perennial branching climber, shrub up to 1.5 m tall found in eastern and southern regions of India. Root is a long, fleshy and not much branched [6, 7]. The plant has been traditionally used for the treatment of bronchial asthma, dysentery, inflammation, jaundice and to induce emesis [8]. The plant has been scientifically proven for various activities including anti-asthmatic, in allergic rhinitis, as an emetic [6], smooth muscle relaxant, antihistaminic, hypotensive, anti-tumor, anti-inflammatory, analgesic, anticonvulsant and as anti-rheumatic [9]. The root of this plant is widely used locally for treating diarrhoea in northeastern Karnataka. Previously we investigated ALRT and AQRT for hepatoprotective activity (data unpublished). During this study we observed that administration of these extracts produced hard dry stools in rats. Further it was reported that these extracts found to possess anticholinergic, anti histaminic and relaxation of intestinal muscles [9]. Hence we hypothesized that these extracts may possess anti diarrhoeal activity. In addition, there is paucity of scientific evidence in usage of this plant in the diarrhoeal disorders. Hence the present work was aimed to investigate the roots of *Tylophora indica* for anti diarrhoeal activity in rodents.

Methods

Plant material:
Roots of *Tylophora indica* were collected in the month of June from the surrounding fields of Raichur and were authenticated by Prof. Srivasta, Dept. of botany, L.V.D. College, Raichur. The roots were dried under shade and powdered.

Drugs and chemicals:
Castor oil (Yogesh Pharma, Nanded, India), prostaglandin-E_2 (Astra-Zeneca, Bangalore, India), and loperamide (Dr. Reddy's labs, Hyderabad, India) were used in this study. Other chemicals used in the study were of analytical grade.

Experimental animals:
Albino rats (Wistar strain) of either sex weighing between 150-200 g and Swiss albino mice 16-20 g were procured from Shri Venkateswara Enterprises, Bangalore for experimental purpose. After procuring, the animals were acclimatized for seven days under standard husbandry conditions maintained at a room temperature of 24 ± 1^0 C; relative humidity 45-55% and under 12:12 hours light/ dark cycle. The animals had free access to standard rat pellet (Amrut laboratories, Pranava Agro industries ltd., Sangli, India). Water was allowed *ad libitum* under strict hygienic conditions. Each experimental group had separate sets of animals and care was taken to ensure that animals used for one response were not employed elsewhere. Animals were habituated to laboratory conditions for 48 hours prior to beginning of experimental protocol to minimize, if any non-specific stress. All the protocols were approved by the Institutional Animal Ethical Committee (IAEC) of V. L. College of Pharmacy Raichur (Karnataka) and the experiments were conducted in strict compliance with ethical principles and guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Chennai, India.

Preparation of extracts:
100 g of root powder was extracted with alcohol using Soxhlet apparatus for 18 hours and marc was macerated with water for seven days with occasional shaking in a closed vessel. The solvent was evaporated to dryness under reduced pressure.

Preliminary Phytochemical screening:
Preliminary phytochemical screening of ALRT and AQRT were studied as described by Khandelwal, 2000 [10].

Determination of LD_{50}:
The acute toxicity of alcoholic and aqueous extracts of roots of *Tylophora indica* were determined by using female albino mice (18-22 g). The animals were fasted 3 hrs prior to the experiment according to OECD guideline no. 425, up and down procedure [11]. Animals were administered with single dose of extract and observed for its mortality during 48 hours study period (short term) toxicity. Based on the drug short term profile the dose of the next animals were determined as per as OECD guideline 425. All the animals were observed for long term toxicity (14 days). The LD_{50} of the test extracts were calculated using AOT 425 software provided by Environmental protection agency, USA.

Antidiarrhoeal activity:
A. Castor oil-induced diarrhoea:
Albino rats of either sex weighing 160-190 g were used. They were divided into twelve 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 ALRT or AQRT or standard. One hour after drug treatment, each rat received castor oil (1 ml/100 g, po).

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 vehicle treated group.

B. Prostaglandin- E_2 induced diarrhoea :

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 ALRT or AQRT or standard one hour prior to prostaglandin-E_2 administration. All the rats were administered with prostaglandin-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 killed. The whole length of the intestine from the pylorus to the caecum was dissected out and its contents were collected and measured.

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, 1 ml of charcoal meal (3% deactivated charcoal in 2% aqueous tween 80) was administered by oral route to all the animals in each group. After 50 minutes of charcoal treatment, each mouse was sacrificed and distance moved by the charcoal meal from pylorus to caecum was measured to express as a percentage of distance travelled by charcoal meal in ratio to the intestinal length. Percentage inhibition produced by extracts was calculated.

Statistical analysis:

Values are expressed as mean ± SEM. Statistical differences between means were determined by performing one-way ANOVA followed by Dunnett's post hoc test. $P < 0.05$ were considered as significant. All the statistical analysis was performed using demo version of Instat® software (Graph pad Inc., Santabarbara, CA)

Results

Phytochemical investigation:-

The percentage yield of ALRT and AQRT was found to be 10.94 % and 15.37 % respectively. Preliminary phytochemical investigation showed presence of alkaloids, carbohydrates, saponins, flavonoids, proteins and amino acids in both ALRT and AQRT.

Determination of LD_{50}:
AQRT was found to be non toxic even up to a dose of 5000 mg/kg, and LD_{50} of ALRT was found to 3162 mg/kg.

Antidiarrhoeal activity:
Effect of ALRT and AQRT on castor oil induced diarrhoea in rats:
The antidiarrhoeal activity was expressed as percent reduction in faecal weight comparing with control as 100% faecal weight. Loperamide, ALRT and AQRT extracts had significantly decreased the cumulative fecal mass induced by castor oil as compared with vehicle treated group. The percentage reduction in weight of the stool with ALRT and AQRT were represented in Table 1. The percentage of inhibition of standard drug loperamide was 85.02%. Higher dose (800mg/kg) of ALRT and AQRT showed greater anti diarrhoeal activity than loperamide.

Table 1 Effect of ALRT and AQRT on castor oil induced diarrhoea in rats

Treatment	Cumulative fecal mass (g)	% inhibition
Vehicle (2% Tween 80 suspension)	7.613 ± 0.423	--
Loperamide (5 mg/kg)	1.14** ± 0.108	85.02
ALRT (50 mg/kg)	5.22** ± 0.092	31.41
ALRT (100 mg/kg)	3.37** ± 0.306	55.73
ALRT (200 mg/kg)	2.41** ± 0.145	68.36
ALRT (400 mg/kg)	1.48** ± 0.317	80.54
ALRT (800 mg/kg)	0.445** ± 0.199	94.15
AQRT (50 mg/kg)	5.727** ± 0.137	24.78
AQRT (100 mg/kg)	3.89** ± 0.1520	48.94
AQRT (200 mg/kg)	2.79** ± 0.1699	63.37
AQRT (400 mg/kg)	1.77** ± 0.0576	76.77
AQRT (800 mg/kg)	0.66** ± 0.211	91.33

Values are expressed as mean ± SEM from 6 animals.** $P < 0.01$ as compared with control group.

Effect of ALRT and AQRT on prostaglandin- E_2 induced enteropooling in rats:
The antidiarrhoeal activity was expressed as percent reduction in the gastric secretions in prostaglandins-E_2 treated group taking control as 100% secretion. In this model loperamide had reduced gastric secretion by 77.54% where as ALRT (200, 400mg/kg) found to decrease secretions by 58.82% and 70.59% while AQRT (200, 400mg/kg) by 56.5% and 67.38%. (Table 2).

Table 2 Effect of ALRT and AQRT on prostaglandin- E_2 induced enteropooling in rats

Treatment	Volume of intestinal fluid in ml	% inhibition in gastric secretions
Prostaglandin E_2 (100 µg/kg)	3.12±0.12	--
Loperamide (5 mg/kg)	0.70**±0.04	77.54
ALRT (200 mg/kg)	1.28**±0.07	58.82
ALRT (400 mg/kg)	0.92**±0.05	70.59
AQRT (200 mg/kg)	1.37**±0.06	56.15
AQRT (400 mg/kg)	1.01**±0.06	67.38

Values are expressed as mean ± SEM from 6 animals.** $P < 0.01$ as compared with control group.

Effect of ALRT and AQRT on gastrointestinal motility (charcoal meal test) in mice:
The antimotility activity was expressed as percent reduction in movement of the charcoal meal in intestine comparing with control as 100% movement. Administration of atropine, ALRT and AQRT extracts significantly inhibited the distance traveled by charcoal indicating their anti motility effect. In this model atropine had reduced gastric motility by 80.37% where as ALRT (200, 400mg/kg) and AQRT (200, 400mg/kg) extracts reduced motility by 62.24%, 73.10% and 59.72%, 70.42% respectively (Table 3).

Table 3 Effect of ALRT and AQRT on gastrointestinal motility (charcoal meal test) in mice

Treatment	Mean% movement of charcoal (cm)	% inhibition in movement of charcoal
Vehicle	89.35 ± 0.62	--
Atropine (5 mg/kg)	19.62** ± 0.58	80.38
ALRT (200 mg/kg)	37.77** ± 0.33	62.24
ALRT (400 mg/kg)	26.92** ± 0.39	73.10
AQRT (200 mg/kg)	40.28** ± 0.26	59.72
AQRT (400 mg/kg)	29.58** ± 0.51	70.42

Values are expressed as mean ± SEM from 6 animals.** $P < 0.01$ as compared with control group.

Discussion

Alcoholic and aqueous extracts of roots of *Tylophora indica* 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.

ALRT and AQRT exhibited significant anti-diarrhoeal activity against castor oil induced diarrhoea in rats. The extracts at a dose of 400 and 800 mg/kg were found to show similar anti diarrhoeal effect as that of loperamide (5 mg/kg).

It is widely known that castor oil or its active components ricinoleic acid induces permeability changes in mucosal fluid and electrolyte transport that results in a hypersecretory response and diarrhoea [12, 13]. Ricinoleic acid markedly increase the PGE2 content in portal venous and gut lumen and also causes an increase in secretion of the water and electrolytes into the small intestine [14, 15]. Ricinoleic acid also produces irritation and inflammation of the intestinal mucosa, leading to release of prostaglandins, which stimulate motility and secretion [16]. Inhibition of prostaglandin biosynthesis delayed castor oil induced diarrhoea [17]. Earlier studies reported that these extracts exhibited significant analgesic and anti-inflammatory activity through inhibition of prostaglandin [9]. Based on these observations, it seems that the anti-diarrhoeal effect of ALRT and AQRT may be due to the inhibition of prostaglandin biosynthesis or by decreasing the peristaltic movement.

The extracts also significantly inhibited the PGE_2 induced intestinal fluid accumulation (enteropooling). It has been shown that E type of prostaglandins cause diarrhoea in experimental animals as well as human beings [18]. Their mechanism has been associated with dual effects on gastrointestinal motility as well as on water and electrolyte transport [19]. PGE_2 also inhibit the absorption of glucose, a major stimulus to intestinal absorption of water and electrolytes [20]. These observations tend to suggest that both extracts at a dose of 200 and 400 mg/kg reduced diarrhoea by inhibiting PGE_2 induced intestinal accumulation of fluid.

The extracts appear to act on all parts of intestine. Thus, it reduced the intestinal propulsive movement in the charcoal meal treated model; at 200 and 400 mg/kg both extracts showed activity similar to that of atropine. Previous study shows that activated charcoal avidly absorbs drugs and chemicals on the surface of the charcoal particles thereby preventing absorption [21]. Thus, in gastrointestinal motility test with activated charcoal was carried out to find out the effect of ethanol and aqueous extracts on peristaltic movement. The results also show that the ethanol and aqueous extracts suppressed the propulsion of charcoal meal thereby increased the absorption of water and electrolytes. These extracts were reported to possess anti-asthamatic activity through blockade of histamine and acetylcholine [9].The inhibition of peristaltic movement with alcoholic and aqueous extracts of *Tylophora indica* roots may be due to their antihistaminic and anticholinergic actions. From these models we can suggest that ALRT and AQRT non-specifically inhibit diarrhoea either by decreasing the intestinal motility or by decreasing the prostaglandin biosynthesis.

Previous reports have demonstrated the anti-diarrhoeal activity of tannin [22], flavonoids [23], alkaloids [24], saponins, reducing sugars and sterols and/or terpenes [25] containing plant extracts.

Tannins can evoke an antidiarrhoeal effect since these substances may precipitate proteins of the enterocytes; reduce peristaltic movement and intestinal secretions [26, 27]. Quercetin produces an inhibitory effect on gastrointestinal tract mediated through α_2-adrenergic and calcium systems which show the beneficial effects in diarrhoea and other intestinal secretions [28]. The anti-diarrhoeal activity of flavonoids has been ascribed to their ability to inhibit intestinal motility and hydro electrolytic secretion [29, 30] which are known to be altered in this intestinal condition. *In vitro* and *in vivo* experiments have shown that flavonoids are able to inhibit the intestinal secretory response, induced by prostaglandin E_2 [31].In addition, flavonoids possess antioxidant properties [32] which are presumed to be responsible for the inhibitory effects exerted upon several enzymes including those involved in the arachidonic acid metabolism [33]. The phytochemical analysis of the extracts showed the presence of alkaloids, saponins, reducing sugars, tannins and flavonoids. These constituents may responsible for the anti-diarrhoeal activity of *Tylophora indica* extracts.

The results indicate that the ethanol and AQRT possesses significant anti-diarrhoeal activity due to their inhibitory effect both on gastrointestinal propulsion and fluid secretion. The data obtained is consistent with literature reports on anti-diarrhoeal activity of *Tylophora indica* root using gastrointestinal motility test in mice and castor oil induced diarrhoea and intraluminal accumulation of fluids in rats. The inhibitory effect of the plant extracts justified the use of the plant as a non specific anti-diarrhoeal agent in folklore medicine. Further detailed investigations are needed to determine phytoconstituents which are responsible for the anti-diarrhoeal activity.

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