ANTIDIARRHOEAL ACTIVITY OF LEAF EXTRACTS OF *TYLOPHORA INDICA (ASCLEPIADACEAE) IN RODENTS*

Nilesh J. Patel[1]*, Vipul B. Gujarati[2], T.S. Gouda[2], N. Venkat Rao[2], K. Nandakumar[3]

1. Department of Pharmacology, S.K. Patel College of Pharmaceutical Education & Research, Ganpat Vidyanagar, Kherva, Gujarat-382711, India
2. Department of Pharmacology, V.L. College of Pharmacy, Raichur, Karnataka-584103, India
3. Department of Pharmacology, Manipal College of Pharmaceutical Sciences, Manipal, Karnataka-576 104, India

Summary

Tylophora indica is widely used locally for treating diarrhoea in northeastern Karnataka. The objective of this study is to investigate alcoholic (ALLT) and aqueous (AQLT) extracts of leaves of *Tylophora indica (Asclepiadaceae)* for their anti diarrheal activity in rodents. Leaves 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 ALLT and AQLT were conducted as per as OECD guidelines 425. The antidiarrheal activity was observed in three experimentally induced diarrhoeal models i.e. castor oil induced diarrhoea and PG-E_2 induced enteropooling in rats and gastrointestinal motility test in mice. ALLT and AQLT showed presence of alkaloids, flavonoids, tannins, amino acids, saponins and carbohydrates. LD_{50} of ALLT was found to be greater than 5000 g/kg while AQLT was 3162. In castor oil induced model, ALLT and AQLT showed significant dose dependent reduction of cumulative wet faecal mass. In PG-E_2 induced enteropooling model, ALLT (200 and 400 mg/kg, p.o.) and AQLT (200 and 400 mg/kg, p.o.) inhibited PG-E_2 induced secretions significantly. Similarly in gastrointestinal motility test, ALLT and AQLT decreased the movement of charcoal indicating its anti-motility activity.
It was observed that ALLT and AQLT were found to possess equipotent anti diarrheal activity in these models. Moreover, ALLT was safer than AQLT (LD50 values). Hence ALRT shall be beneficial in treatment of diarrhoea.

Keywords: Antidiarrhoeal activity, *Tylophora indica,* Castor oil, Prostaglandin-E_2, Gastrointestinal motility test.

Address of Corresponding Author:
Dr. Nilesh J. Patel
Assistant Professor,
Dept. of Pharmacology,
S.K. Patel College of Pharmaceutical Education & Research,
Ganpat Vidyanagar, Mehsana- Gozariya Highway Road,
Kherva, Gujarat-382711, India
Cell no: 91- 09427684312
Fax No.: 02762- 286082
E-mail ID: nileshcology127@yahoo.co.in

1. INTRODUCTION

Tylophora indica wright (Asclepiadaceae) is a branching climber, shrub up to 1.5 m tall with a ovate-oblong to elliptic-oblong, base rounded or subcordate, apex acute or obtuse but apiculate, 3-10 cm long and 1.5-7 cm wide, thick, glabrous and dark green above, pale green, often pubescent beneath leaves. It grows wild in forests and in sandy localities in Bengal, Eastern India, Assam, Orissa, Konkan and in Tamilnadu up to 1000m[1,2,3]. The plant has been traditionally used for the treatment of bronchial asthma, dysentery, inflammation, jaundice and to induce emesis. The plant has been scientifically proven for various activities including anti-asthmatic, in allergic rhinitis, as an emetic[3], smooth muscle relaxant, antihistaminic, hypotensive, anti-tumor, anti-inflammatory and immunopathological reactions, analgesic, anticonvulsant, anti-rheumatic and for increasing lochia in parturient women[4,5,6,7,8]. The root paste is used for treating jaundice in northeastern Karnataka[9]. Previously we investigated hepatoprotective and antidiarrhoeal activity of root extracts of *Tylophora indica*[10,11]. The aim of the present investigation was to test the anti-inflammatory and antinociceptive activity of leaf extracts of *Tylophora indica.*

2. MATERIALS AND METHODS

2.1 Plant material:

Leaves of *Tylophora indica* were collected in the month of June from the surrounding fields of Raichur and were authenticated by Prof. B. Nagraj, Dept. of botany, L.V.D. College, Raichur. The leaves were dried under shade and size reduced to coarse powder.

2.2 Drugs and chemicals:

Castor oil (Yogesh Pharma, Nanded, India), prostaglandin-E2 (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.

2.3 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, India for experimental purpose. After procuring, the animals were acclimatized for seven days under standard husbandry condition 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., Sangali, 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.

2.4 Preparation of extracts:
100 g of powdered leaves was extracted with alcohol using Soxhlet apparatus for 18 hours and marc was macerated with water for seven days with shaking in a closed vessel. The solvent was evaporated to dryness in under vacuum.

2.5 Preliminary Phytochemical screening:
Preliminary phytochemical screening of ALLT and AQLT were studied as described by standard book[12].

2.6 Determination of LD_{50}:
The acute toxicity of ALLT and AQLT 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 (OECD -guideline on Acute Oral Toxicity (AOT-425)[13]. 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.

2.7 Antidiarrhoeal activity:

A. Castor oil- induced diarrhea[14]:
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 ALLT or AQLT 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[15]:
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 ALLT or AQLT 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. Gastrointestinal motility test[15]:
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.

2.8 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)

3 Results

3.1 Phytochemical investigation:-

Preliminary phytochemical showed presence of alkaloids, carbohydrates, saponins, steroids and triterpines in ALLT whereas alkaloids, carbohydrates and saponins in AQLT.

3.2 Determination of LD_{50}:

ALLT was found to be non toxic even up to a dose of 5000 mg/kg, and LD_{50} of AQLT was found to be 3162 mg/kg.

3.3 Antidiarrhoeal activity:

A. Effect of ALLT and AQLT on castor oil- induced diarrhoeal model:

The antidiarrhoeal activity was expressed as percent reduction in faecal weight comparing with control as 100% faecal weight. Loperamide, ALLT and AQLT 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 ALLT and AQLT is represented in Figure 1. The percentage of inhibition of standard drug loperamide was 85.02%. Higher dose (800mg/kg) of ALLT and AQLT showed greater anti diarrhoeal activity than loperamide.

B. Effect of ALLT and AQLT on prostaglandin- E_2 induced enteropooling in rats:

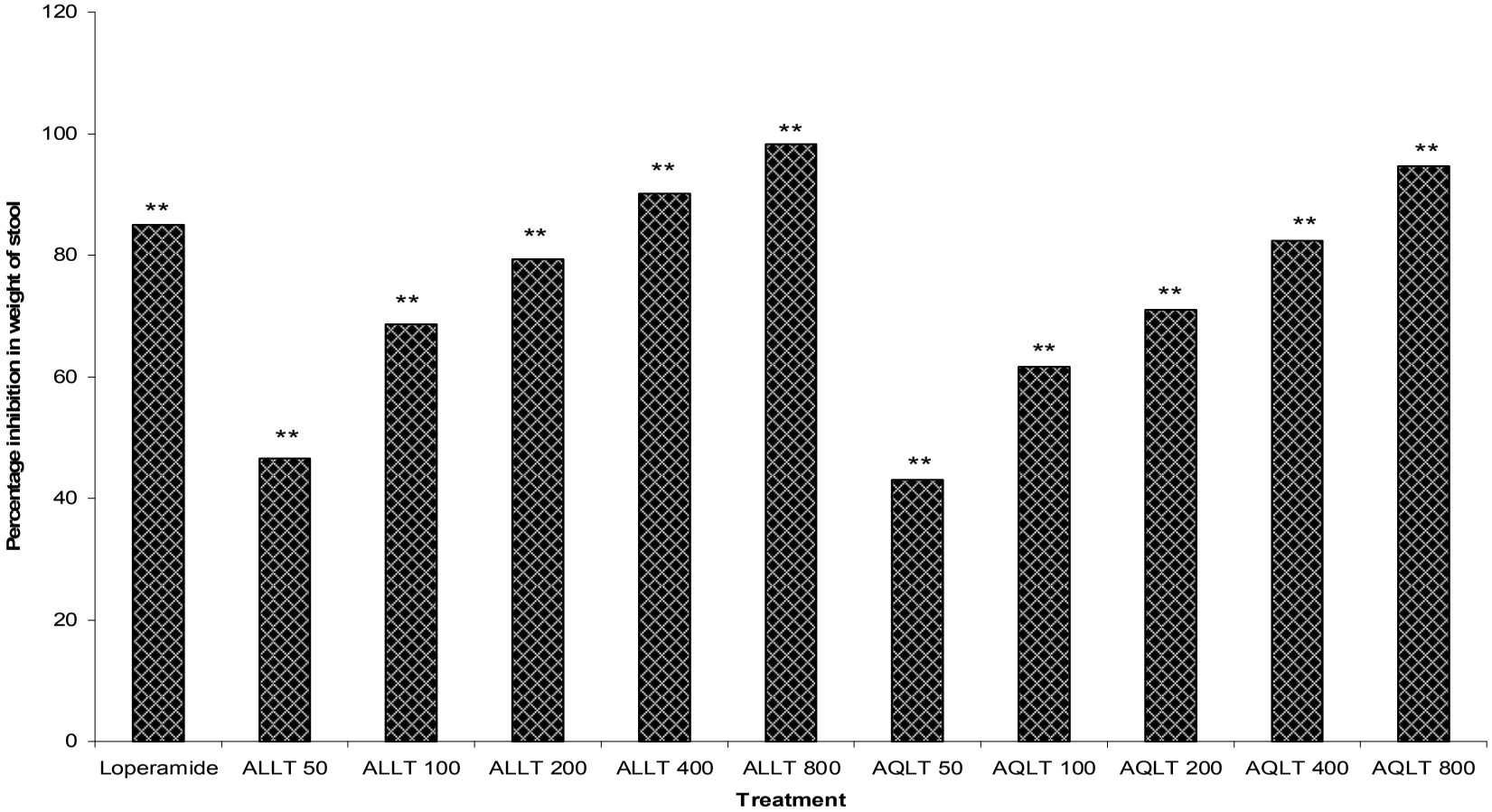
The antidiarrhoeal activity was expressed as percent reduction in the gastric secretions in prostaglandins-E_2s treated group taking control as 100% secretion. In this model loperamide had reduced gastric secretion by 77.54% where as ALLT (200, 400mg/kg) found to decrease secretions by 59.38% and 69.52% while AQLT (200, 400mg/kg) by 58.82% and 68.39 %. (Figure 2).

C. Effect of ALLT and AQLT 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, ALLT and AQLT 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 ALLT (200, 400mg/kg) and AQLT (200, 400mg/kg) extracts reduced motility by 61.76%, 72.81% and 60.39%, 69.94% respectively (Figure 3).

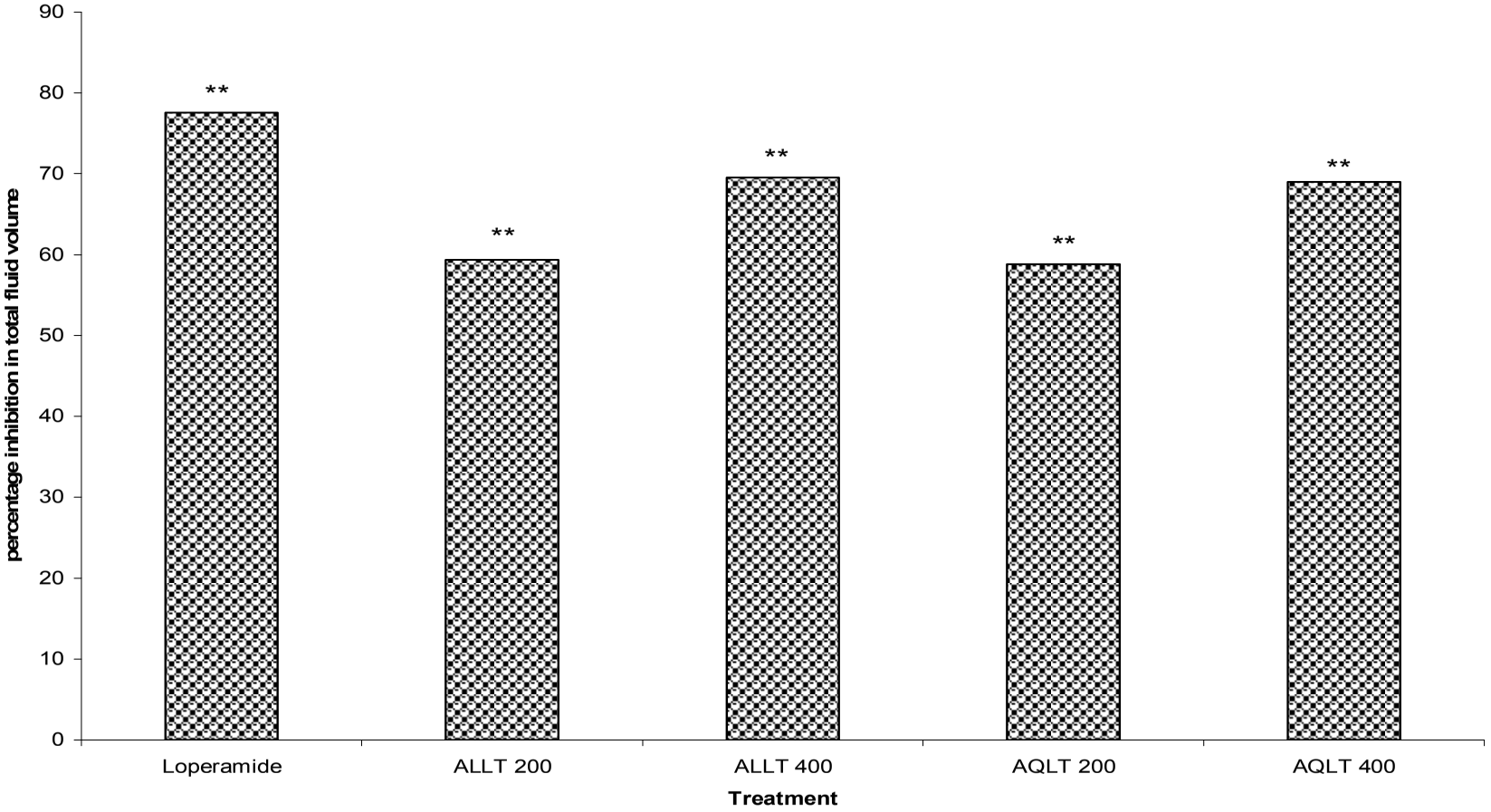
Fig No. 01

Histogram showing effect of leaf extracts of *Tylophora indica* on castor oil induced diarrhoea in rats



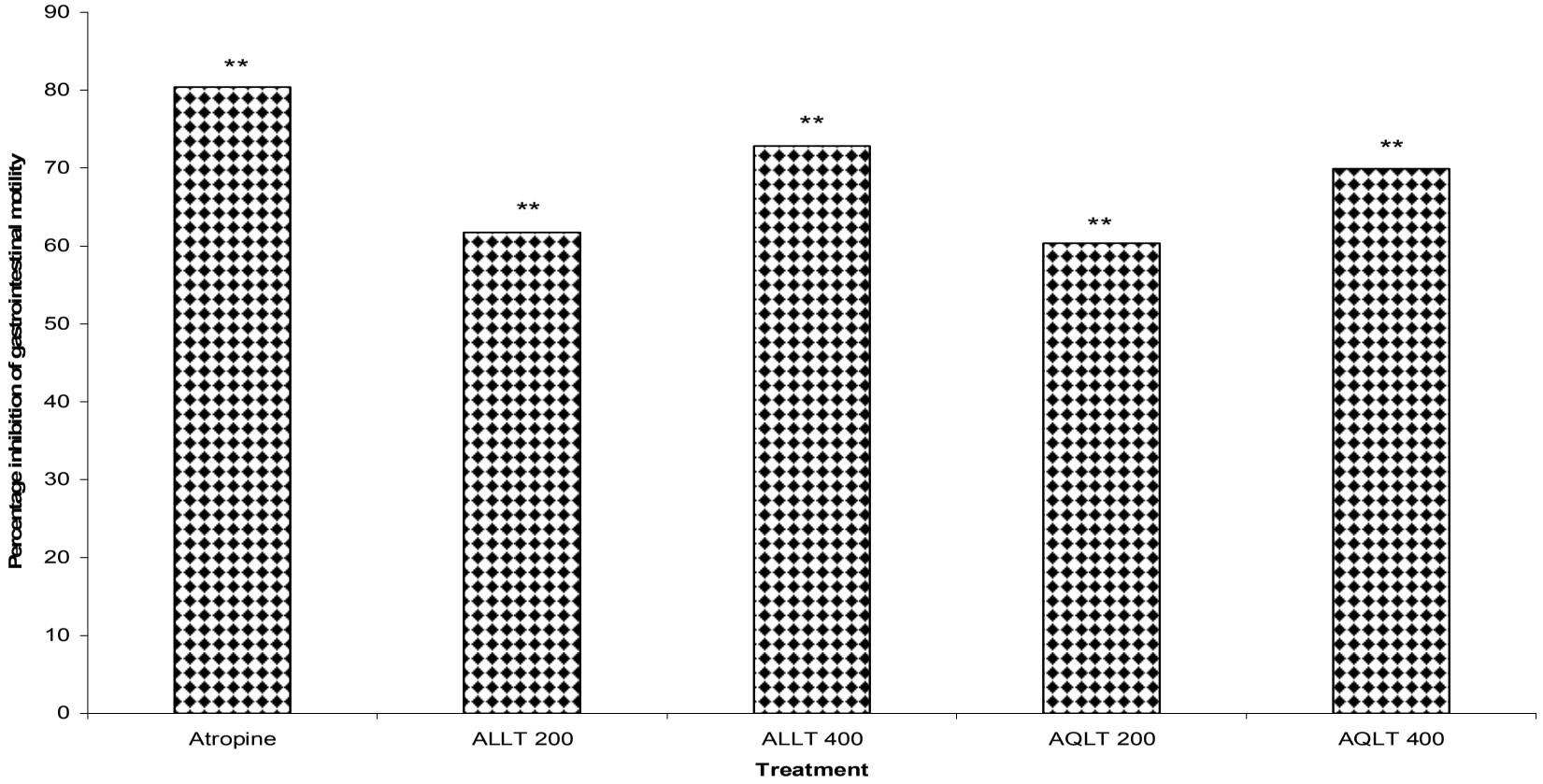
**- Indicates significant reduction in fecal output at $p<0.01$

Fig No. 02
Histogram showing effect of leaf extracts of *Tylophora indica* on prostaglandin-E_2 induced enteropooling in rats



** - Indicates significant reduction in intestinal fluid volume at $p < 0.01$

Fig No. 03
Histogram showing effect of leaf extracts of *Tylophora indica* on gastrointestinal motility (charcoal meal test) in mice



** - Indicates significant reduction in gastrointestinal motility at $p < 0.01$

DISCUSSION:

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[16,17]. Ricinoleic acid markedly increase the PGE_2 content in portal venous and gut lumen and also causes an increase in secretion of the water and electrolytes into the small intestine[18,19]. Ricinoleic acid also produces irritation and inflammation of the intestinal mucosa, leading to release of prostaglandins, which stimulate motility and secretion[20]. Inhibition of prostaglandin biosynthesis delayed castor oil induced diarrhoea[21]. Earlier studies reported that these extracts exhibited significant analgesic and anti-inflammatory activity through inhibition of prostaglandin[8]. Based on these observations, it seems that the anti-diarrhoeal effect of ALLT and AQLT 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[22]. Their mechanism has been associated with dual effects on gastrointestinal motility as well as on water and electrolyte transport[23]. PGE_2 also inhibit the absorption of glucose, a major stimulus to intestinal absorption of water and electrolytes[24]. 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[25]. 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[8].The inhibition of peristaltic movement with alcoholic and aqueous extracts of *Tylophora indica* leaf may be due to their antihistaminic and anticholinergic actions. From these models we can suggest that ALLT and AQLT 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[26], flavonoids[27], alkaloids[28], saponins, reducing sugars and sterols and/or terpenes[29] containing plant extracts.

Tannins can evoke an antidiarrhoeal effect since these substances may precipitate proteins of the enterocytes; reduce peristaltic movement and intestinal secretions[30,31] Quercetin produces an inhibitory effect on gastrointestinal tract mediated through α2-adrenergic and calcium systems which show the beneficial effects in diarrhoea and other intestinal secretions[32]. The anti-diarrhoeal activity of flavonoids has been ascribed to their ability to inhibit intestinal motility and hydro electrolytic secretion[33, 34] 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 [35]. In addition, flavonoids possess antioxidant properties[36] which are presumed to be responsible for the inhibitory effects exerted upon several enzymes including those involved in the arachidonic acid metabolism[37]. 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.

CONCLUSION

The results indicate that the ALLT and AQLT possess 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* leaf 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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