Turk J Pharm Sci 10 (3), 393-404, 2013

Original article

ANTINOCICEPTIVE AND ANTIDIARRHOEAL ACTIVITIES OF ETHANOLIC LEAF EXTRACT OF *TILIACORA ACUMINATA* (LAM.) MIERS

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Abstract

The crude ethanolic leaf extract of Tiliacora acuminata (ETL) (Lam.) Miers (Menispermaceae) was evaluated for its antinociceptive and antidiarrhoeal activities. The antinociceptive activity of ETL was evaluated using mice model on acetic acid induced writhing response and rat models for antidiarrhoeal activities like the castor oil and magnesium sulfate induced diarrhea, enteropooling induced by the administration of castor oil and magnesium sulfate. At the dose of 250 and 500 mg/kg, the extract showed a significant ($P<0.001$) and dose dependent reduction in the number of writhing episodes compared to the control. The ETL showed a significant protection ($P<0.001$) against experimentally induced diarrhoea by castor oil and magnesium sulfate, as evidenced by a decrease in the number of frequency, weight of stools with respect to control. The enteropooling induced by castor oil and magnesium sulfate was also prevented by all the tested doses. Acute toxicity test showed that the plant might be safe for pharmacological uses. The obtained results confirm the antinociceptive and antidiarrhoeal activity of ETL, thus provide the scientific basis for the traditional uses of this plant as the modality for pain and diarrhea.

Key words: *Tiliacora acuminata, Antinociceptive activity, Antidiarrhoeal activity*

Tiliacora acuminata (ETL) (Lam.) Miers Bitkisinin Etanollü Yaprak Ekstresinin Antinosiseptif ve Antidiyareik Aktivitesi

Tiliacora acuminata (Lam.) Miers (Menispermaceae) bitkisinin yapraklarının etanollü ham ektresi (ETL) antinosiseptif ve antidiyareik aktiviteleri bakımından araştırılmıştır. ETL'nin antinosiseptif aktivitesi farelerde asetik asit ile indüklenen kıvranma modelinde ve antidiyareik aktivitesi ratlarda hint yağı ve magnesyum sülfat ile indüklenen diyare, hint yağı ve magnezyum sülfat uygulanması ile indüklenen enteropooling modellerinde incelenmiştir. Ekstrenin kontrol grubu ile kıyaslandığında 250 ve 500 mg/kg dozda kıvranma sayısını belirgin ($P<0.001$) ve doza bağlı olarak azalttığı görülmüştür. ETL deneysel olarak hint yağı ve magnesyum sülfat ile oluşturulan diyareye karşı belirgin bir koruma ($P<0.001$) sağlamıştır, bu durum kontrol grubuna göre gaita ağırlığı, sıklık sayısında azalma ile kanıtlanmıştır. Ayrıca hint yağı ve magnesyum sülfat ile indüklenen enteropooling test edilen tüm dozlarda önlenmiştir. Akut toksisite testi bitkinin farmakolojik kullanımı için güvenilir olabileceğini göstermiştir. Elde edilen sonuçlar ETL'nin antinosiseptif ve antidiyareik aktivitesini doğrulamaktadır, böylece bitkinin ağrı ve diyareye karşı geleneksel kullanımı için bilimsel dayanak sağlamaktadır.

Anahtar kelimeler: *Tiliacora acuminata, Antinasiseptif aktivite, Antidiyareik aktivite*

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INTRODUCTION

Tiliacora acuminata (Lam.) Miers (Menispermaceae) is a large woody climber, branches cinereous, striate. The leaves are long, ovate, acuminate, chordate, truncate or rounded at the base, flowers yellow in elongate, racemose panicles belonging to the family Menispermaceae. Its other name is *Tiliacora racemosa*. A new lactone (1), two alkaloids tiliaresine (2) and (+) N-methyltiliamosine (3) from leaves and acuminatide (4) from seeds have been isolated and characterized. The diphenylbisbenzyl isoquinoline alkaloids, tiliacorine, tiliacorinine, nortiliacorinine A, tiliarine and tiliamosine have also been isolated from the ethanolic extract of its roots (5,6). The extracts of *T. acuminata* are used in many ayurvedic preparations and regarded as an antidote for snakebite (7). Pain is the most important symptom that brings the patient to a physician. Analgesics relieve pain as a symptom, without affecting its cause (8). Diarrhoea is the world's highest killer disease, contributing substantially to pediatric morbidity and mortality, especially in the malnourished (9). Diarrhea is characterized by an increase in the frequency of bowel movements, wet stool and abdominal pain (10). Antibiotics used as anti diarrhoeal drugs sometimes provoke adverse effects and microorganisms tend to develop resistance towards them (11). A vast majority of the people of developing countries relies on herbal drugs for the management of diarrhoea. Considering this fact the World Health Organization (WHO) has constituted a diarrhoeal disease control programme, which includes studies of traditional medicinal practices, together with the elevation of health education and prevention approaches (12). Since there is no sufficient data currently available to substantiate antinociceptive and antidiarrhoeal activities from ETL, therefore the present study was designed to provide scientific evidence for its use as a traditional folk remedy by investigating the antinociceptive and antidiarrhoeal activities that also confirm its use as a pain killer and modality for diarrhea.

EXPERIMENTAL

Plant materials

The leaves of *T. acuminata* were collected from Khulna, Bangladesh. A specimen copy was deposited in Bangladesh National Herbarium for identification & the accession number was DACB-32706.

Preparation of ethanolic extract

The leaves of *T. acuminata* were freed from any of the foreign materials. Then the leaves were air-dried under shed temperature followed by drying in an electric oven at 40 °C. The dried plant materials were then ground into powder. About 400 g of powdered material was taken in a clean, flat-bottomed glass container and soaked in 1300 mL of 95 % ethanol. The container with its contents was sealed and kept for a period of 05 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then it was filtered through whatman filter paper (Bibby RE200, Sterilin Ltd., UK) which was concentrated with a rotary evaporator at bath temperature not exceeding 40 °C to have gummy concentrate of the extract (yield approx. 2.96 %).

Test animals and drugs

Swiss albino mice (20-25 g) and Wister rats (175-202 g) of both sexes were used for *in vivo* pharmacological screening. The mice and rats were collected from the Animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B). The animals were housed under standard laboratory conditions maintained at 25 ± 2 °C and under 12/12 h light/dark cycle and feed with standard diet and water *ad libitum* acclimatization period. The animals were fasted overnight before the experiments. All experimental protocols were in

compliance with BCSIR Ethics Committee on Research on Animals as well as internationally accepted principles for laboratory animal use and care.

The standard drug diclofenac Na and loperamide were used for this study and purchased from Square Pharmaceuticals Ltd, Bangladesh. Ketamine was purchased from Popular Pharmaceuticals Ltd, Bangladesh.

Chemicals

Folin-Ciocalteu phenol reagent and tannic acid were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Tween 80 and sodium carbonate were of analytical grade and purchased from Merck (Darmstadt, Germany).

Phytochemical screening

The freshly prepared crude extract of leaves of *T. acuminata* was qualitatively tested for the presence of chemical constituents, by using the following reagents and chemicals, for example, alkaloids were identified by the Dragendorff's reagent, flavonoids with the use of Mg and HCl, tannins with ferric chloride and potassium dichromate solutions, steroids with Libermann-Burchard reagent and reducing sugars with Benedict's reagent (13).

Total phenol content determination

The tannins and flavonoids were determined using the Folin-ciocalteu phenol reagent (14). 0.5 mL of the sample extract was added to 3.5 mL of distilled water and then added 1.5 mL of Folin-ciocalteu phenol reagent and 4 mL of 35 % sodium carbonate solution and dilute to 10 ml with distilled water. The mixture was shaken well, kept at room temperature for 30 min and absorbance was measured at 725 nm with a double beam UV/Visible spectrophotometer (Analykjena, Model- 205, and Jena, Germany). Blank was prepared with water instead of the sample. A set of standard solutions of tannic acid is read against a blank. The results of phenols are expressed in terms of gallic acid in mg/g of extract. Total phenol content was determined as mg of gallic acid equivalent per gram using the equation obtained from a standard gallic acid calibration curve $y=4.5692x-0.2538$, $R^2=0.9953$.

Acute toxicity test

The acute toxicity of ETL was determined in rats according to the method of Hilaly et al (15) with slight modifications. Rats fasted for 16 h were randomly divided into groups of five rats per group. Graded doses of the extract (200, 400, 800, 1600 and 3200 mg/kg p.o.) were separately administered to the rats in each of the groups by means of bulbed steel needle. All the animals were then allowed free access to food and water and observed over a period of 72 h for signs of acute toxicity. The number of deaths within this period was recorded.

Analgesic activity test

The Analgesic activity of the ETL was studied using acetic acid induced writhing model in mice (16). The animals were divided into control, positive control and test groups with five mice in each group. The animals of test groups received test substance at the dose of 250 and 500 mg/kg body weight. The positive control group was administered with Diclofenac Na (standard drug) at the dose of 25 mg/kg body weight and vehicle control group was treated with 1% Tween 80 in water at the dose of 10ml/kg body weight. Test samples, standard drug and control vehicle were administered orally 30 min before intraperitoneal administration of 0.7% acetic acid. After an interval of 15 min, the mice were observed writhing (constriction of the abdomen, turning of trunk and extension of the hind legs) for 5 min.

Antidiarrhoeal Activity Tests

Castor oil induced diarrhea

Antidiarrhoeal activity of ETL was tested by using Castor oil induced method in mice (17). Twenty Swiss albino mice were randomly divided in to four groups (n=5). The control group received only 1 % Tween 80 in water (2 mL/mice), the positive control group received loperamide 50 mg/kg body weight as standard and test groups received the extracts at the doses of 250 mg and 500 mg/kg body weight. Mice were housed in separate cages having paper placed below for collection of fecal matters. Diarrhea was induced in the mice by oral administration of castor oil (1.0 mL/mice). Extract and drugs were given orally 1 hr before the administration of castor oil. The time for first excretion of feces and the total number of fecal output by the animals were recorded. Normal stool was considered as numerical value 1 and watery stool as numerical value 2. Percent inhibition of defecation in mice was calculated by using the following equation: % inhibition = {(Mo–M)/Mo} x 100; where, Mo = Mean defecation of control and M = Mean defecation of test the sample.

Castor oil induced enteropooling

Antidiarrhoeal activity of ETL was tested by using Castor oil induced enteropooling method in rats (18). Twenty Wistar rats were fasted for 18 h and rats were randomly divided in to four groups (n=5). Control group received only 1% Tween 80 in water (2 mL/mice), positive control group received loperamide 3 mg/kg body weight as standard and test groups received the extracts at the doses of 250 and 500 mg/kg body weight. 60 min after the administration of drug and extract, 1 mL of castor oil was administered to all animals including the control or vehicle treated group. After 30 min, following administration of castor oil, all rats were sacrificed by overdose of ketamine, whole length of the intestine from pylorus to caecum was dissected out, its content collected in measuring cylinder and volume measured.

Magnesium sulphate induced diarrhea

Antidiarrhoeal activity of ETL was tested by using magnesium sulphate induced diarrhoeal method in rats (19). Twenty Wistar rats were fasted for 18 h and rats were randomly divided in to four groups (n=5). Control group received only distilled water 2 mL/mice, positive control group received loperamide 3 mg/kg body weight as standard and test groups received the extracts at the doses of 250 and 500 mg/kg body weight. After 60 min of drug treatment, the animals in each group received magnesium sulphate (2 g/kg) orally. Again, the faecal material and the frequency of defecation were noted up to 4 hr in the transparent metabolic cages with pre weighed plastic dishes placed at the base. Weight of plastic dish before and after defecation was noted and compared to control.

Magnesium sulphate induced enteropooling

Antidiarrhoeal activity of ETL was tested by using magnesium sulphate induced enteropooling method in rats (20). Twenty Wistar rats were fasted for 18 h and rats were randomly divided in to four groups (n=5). The control group received only distilled water 2 mL/mice, the positive control group received loperamide 3 mg/kg body weight as standard and test groups received the extracts at the doses of 250 and 500 mg/kg body weight. 60 min after the administration of drug and extract, 10 % w/v aqueous solution of Magnesium sulphate was administered to all animals including the control or vehicle treated group. After 30 min, following administration of magnesium sulphate, all rats were sacrificed by an overdose of ketamine, the whole length of the intestine from pylorus to caecum was dissected out, its content collected in measuring cylinder and volume measured.

Statistical analysis

For analgesic and anti-diarrheal determination, data were presented as mean ± standard deviation (SD). Statistical analysis for animal experiment was carried out using one-way

ANOVA followed by Dunnett's multiple comparisons. The results obtained were compared with the control group. P values < 0.05 were considered to be statistically significant

RESULTS

Chemical group test

Results of different chemical tests on the ETL showed the presence of tannin, reducing sugars, flavonoids and alkaloids "Table 1".

Table 1. Results of different group tests of ETL.

Phytoconstituents	ETL
Alkaloid	+
Reducing sugars	+
Tannins	+
Gums	+
Flavonoids	+
Saponin	-
Steroid	-

+ : Positive result; - : Negative result

Total phenol content

The total phenol content was calculated as quite high in ETL and measured as 243.41 ± 0.82 mg/g of gallic acid equivalent that are shown at "Table 2".

Table 2. Total phenol content of ETL.

Extract	Avg. absorbance at 725 nm	Total phenol content
		mg of gallic acid equivalent (GAE) per gm of dry extract
ETL	0.92 ± 0.18	243.41 ± 0.82

Values are expressed as mean ± SEM (Standard Error Mean)

Acute toxicity test

In acute toxicity study, oral administration of graded doses (200, 400, 800, 1600 and 3200 mg/kg, p.o.) of the ETL to rats did not produce any significant changes in behavior, breathing, cutaneous effects, sensory nervous system responses or gastrointestinal effects during the observation period. No mortality or any toxic reaction was recorded in any group after 72 h of administering the extract to the animals. *T. acuminata* was safe up to a dose level of 3200 mg/kg of body weight in rats.

Antinociceptive activity

Table 3 showed the effect of the antinociceptive activity of ETL on acetic acid induced writhing in mice. At the dose of 250 and 500 mg/kg of body weight, the extract produced 38.36 % and 49.32 % writhing inhibition in test animals respectively. The results were statistically significant (P <0.01 and P <0.001) and was comparable to the standard drug diclofenac Na, which showed 75.34% at a dose of 25 mg/kg weight.

Table 3. Effects of antinociceptive activity of ETL on acetic acid induced writhing of mice (n=5).

Group	Treatment and Dose	Number of writhes (% Writhing)	% Writhing Inhibition
Control	1% tween 80 solution 10 ml/kg, p.o.	14.6 ± 1.74 (100)	---
Positive Control	Diclofenac Na 25 mg/kg, p.o.	3.6 ± 0.68[b] (24.66)	75.34
Test Group- 1	ETL 250 mg/kg, p.o.	9.0 ± 0.55[a] (61.64)	38.36
Test group- 2	ETL 500 mg/kg, p.o.	7.4 ± 0.75[a] (50.68)	49.32

Values are expressed as mean±SEM (Standard Error Mean);[a]indicates $P < 0.01$ & [b]indicates $P < 0.001$; one-way ANOVA followed by Dunnet's test as compared to control; n = Number of mice; p.o.: per oral

Antidiarrheal Activity

Castor oil induced diarrhea

Table 4 showed the effect of the ETL on castor oil induced diarrheal method in mice. The result showed that extract reduce the mean number of defecation which were 35.42 % and 50 % ($P < 0.001$) at the doses of 250 and 500 mg/kg respectively. The latent period for the ETL on treated group was ($P < 0.01$ and $P < 0.001$) increased as compared to control group.

Table 4. Antidiarrheal activity of the ETL on castor oil induced diarrheal test method on mice.

Sample	Dose (mg/kg, p.o.)	Mean± SE		% Inhibition
		Latent period	Defecation	
Distilled water	(2 mL/mice)	0.63 ± 0.26	9.6 ± 0.25	--
Loperamide	3	2.32 ± 0.15[b]	4.0 ± 0.32[b]	58.33
Test Group- 1	250	1.56 ±0.17[a]	6.20 ± 0.37[b]	35.42
Test group- 2	500	1.95 ± 0.17[b]	4.80 ± 0.58[b]	50.00

Values are expressed as mean ± SEM (Standard Error Mean); [a]indicates $P < 0.01$; [b]indicates $P < 0.001$, one-way ANOVA followed by Dunnett's test as compared to control; n = Number of mice; p.o.: per oral

Castor oil-induced enteropooling

Castor oil caused accumulation of water and electrolytes in intestinal loop. Castor oil-induced enteropooling is not influenced by loperamide (3 mg/kg, p.o.,) in rats. The ETL at 250 and 500 mg/kg, p.o. dose produced 36.84 % and 58.28 % inhibition of volume of intestinal content respectively with significance ($P < 0.001$). The weight of intestinal content was also reduced significantly at both the doses "Table 5".

Table 5. Antidiarrheal activity of the ethanolic extract of leaves of *T. acuminata* in castor oil induced enteropooling test method on rat.

Sample	Dose	Mean± SE		% Inhibition
		Volume of fluid (mL)	Weight of intestinal content (gm)	
Distilled water	2 mL/mice, p.o.	2.08 ± 0.26	2.91 ± 0.28	
Loperamide	3 mg/kg, p.o.	0.62 ± 0.14[b]	0.77 ± 0.17[b]	73.61
Test Group- 1	ETL 250 mg/kg, p.o.	1.54 ± 0.23[a]	1.84 ± 0.29[b]	36.84
Test group- 2	ETL 500 mg/kg, p.o.	1.46 ± 0.20[a]	1.21 ± 0.19[b]	58.28

Values are expressed as mean ± SEM (Standard Error Mean); [a]indicates $P < 0.05$; [b] indicates $P < 0.001$, one-way ANOVA followed by Dunnett's test as compared to control; n = Number of mice; p.o.: per oral

Magnesium sulphate induced diarrhea

All the rats in control group produced diarrhoea after magnesium sulphate administration during the observation period. Pretreatment of rats with the both doses of ETL caused a significant dose dependent decrease in the frequency of purging (reduction of number of wet stools and total no of stools) and weight of wet stools as shown in Table 6.

Table 6. Antidiarrhoeal activity of the ETL in magnesium sulphate induced diarrheal test method on rat.

Sample	Dose	Mean± SE		% Inhibition
		Latent period	Defecation	
Distilled water	2 mL/mice, p.o.	0.82 ± 0.20	12.20 ± 1.02	--
Loperamide	3 mg/kg, p.o.	3.21 ± 0.16[b]	4.40 ± 0.40[b]	63.93
Test Group- 1	ETL 250 mg/kg, p.o.	1.81 ± 0.19[a]	8.60 ± 0.75[a]	29.51
Test Group- 2	ETL 500 mg/kg, p.o.	2.20 ± 0.24[b]	5.80 ± 0.58[b]	52.46

Values are expressed as mean ± SEM (Standard Error Mean); [a]indicates $P < 0.01$; [b]indicates $P < 0.001$, one-way ANOVA followed by Dunnett's test as compared to control; n = Number of mice; p.o.: per oral

Magnesium sulfate induced enteropooling

The extract reduced the intestinal fluid secretion induced by magnesium sulfate, in a dose dependent fashion. The standard antidiarrheal drug, loperamide (3 mg/kg, p.o.), produced a more marked and significantly greater ($P < 0.05$ and $P < 0.001$) inhibitory effects on magnesium sulfate induced fluid accumulation. The reduction in the intestinal fluid secretion at 500 mg/kg of plant extract treatment was found to be almost comparable with that of treatment by 3 mg/kg dose of loperamide "Table 7".

Table 7. Antidiarrheal activity of the ethanolic extract of leaves of *T. acuminata* in magnesium sulphate induced diarrheal test method on rat.

Sample	Dose	Mean± SE		% Inhibition
		Latent period	Defecation	
Distilled water	2 mL/mice, p.o.	0.82 ± 0.20	12.20± 1.02	--
Loperamide	3 mg/kg, p.o.	3.21 ± 0.16[b]	4.40 ± 0.40[b]	63.93
Test Group- 1	ETL 250 mg/kg, p.o.	1.81 ± 0.19[a]	8.60 ± 0.75[a]	29.51
Test Group- 2	ETL 500 mg/kg, p.o.	2.20 ± 0.24[b]	5.80 ± 0.58[b]	52.46

Values are expressed as mean ± SEM (Standard Error Mean); [a]indicates $P < 0.01$; [b]indicates $P < 0.001$, one-way ANOVA followed by Dunnett's test as compared to control; n = Number of mice; p.o.: per oral.

DISCUSSION

Oral administration of ETL extract produced significant peripheral antinociceptive effect in acetic acid-induced writhing test. Pain sensation in acetic acid induced writhing method is elicited by triggering localized inflammatory response resulting release of free arachidonic acid from tissue phospholipid via cyclooxygenase (COX), and prostaglandin biosynthesis (21). In other words, the acetic acid induced writhing has been associated with increased levels of PGE_2 and $PGF_{2\alpha}$ in peritoneal fluids as well as lipoxygenase product (22). The increase in prostaglandin levels within the peritoneal cavity then enhances inflammatory pain by increasing capillary permeability (23). The acetic acid induced writhing method was found effective to evaluate peripherally active analgesics. The agent reducing the number of writhing will render analgesic effect preferably by inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition (24). The effect of the extracts against the noxious stimulus may be an indication that it depressed the production of irritants and hereby reduction in the number of writhes in the animals. The significant pain reduction of the plant extract might be due to the presence of analgesic principles acting with the prostaglandin pathways.

Phytochemical screening of ETL reveals the presence of tannin, reducing sugars, flavonoids and alkaloids. Flavonoids were reported to have a role in analgesic activity primarily by targeting prostaglandins (25). Tannins also play a role in antinociceptive and anti-inflammatory activities in some studies (26). Because tannins inhibit prostaglandin synthesis by modifying the production of cyclooxygenase (cox-1 and cox-2) and lipooxygenase (lox) involved in the prostaglandin synthesis (27). Besides, alkaloids are well known for their ability to inhibit pain perception (28). So might be these phytoconstituents are responsible for its analgesic activity.

Diarrhea results from an imbalance between the absorptive and secretory mechanisms in the intestinal tract, accompanied by hurry, resulting in an excess loss of fluid in the feces. The ETL showed significant inhibitory activity against castor oil and magnesium sulfate induced diarrhea castor oil and magnesium sulfate induced enteropooling. At doses of 250 and 500 mg/kg, the ETL showed significant antidiarrhoeal activity against castor oil-induced diarrhoea as compared with the control group in a dose dependant manner. Several mechanisms have been supposed to be involved in the diarrhoeal effect of castor oil (18). These include castor oil decreases fluid absorption, increases secretion in the small intestine and colon, and affects smooth muscle contractility in the intestine. Castor oil produces diarrhoeal effect due to its active component of ricinoleic acid (29), inhibition of intestinal Na+,K+-ATPase activity to reduce normal fluid absorption (30), activation of adenylyl cyclase (31), stimulation of prostaglandin formation (31), platelet activating factor and recently nitric oxide was contributed to the diarrhoeal effect of castor oil (32). Prostaglandin contributes to the pathophysiological functions in gastrointestinal tract (33). Inhibitors of prostaglandin biosynthesis delay castor oil induced diarrhea (34). The antidiarrheal activity of the plant extract was closely comparable to the standard drug, loperamide, which at present is one of the most efficacious and widely employed antidiarrheal drugs. Loperamide effectively antagonizes diarrheal activity induced by castor oil (35). Since the ETL successfully inhibited the castor oil induced diarrhoea, it can be assumed that the antidiarrhoeal action was exerted by antisecretory mechanism. This was also evident from the reduction of total number of wet faeces in the test groups in the experiment.

Magnesium sulfate similarly causes an increase in the electrolyte secretion by creating an osmotic imbalance (36). The extract sufficiently counteracted the increase in electrolyte secretion by means of an anti-electrolyte permeability action. ETL may act an above any one of the mechanism. It is also noted that ETL significantly inhibited castor oil induced intestinal fluid accumulation and the volume of intestinal content "Table 2". The secretory diarrhoea is associated with an activation of Cl^- channels, causing Cl^- efflux from the cell, the efflux of Cl^- results in massive secretion of water into the intestinal lumen and profuse watery diarrhea (37). The involvement of muscarinic receptor effect was confirmed by increased production of both gastric secretion and intraluminal fluid accumulation induced by castor oil. The ETL may inhibit the secretion of water into the intestinal lumen and this effect is partly mediated by both

α2-adrenoceptor and muscarinic receptor systems. The significant inhibition of the castor oil-induced enteropooling in mice suggests that the ETL produced relief in diarrhea by spasmolytic activity *in vivo* and antienteropooling effects (17).

Tannic acid present in many of the plant extracts are shown to form a complex with the luminal proteins which then precipitate and form a coat over the intestinal line and reduce secretion in a model of charcoal induced hyper peristalsis (38). The extract possessed anti spasmodic effect and anti enteropooling effect by which the extract produced relief in diarrhoea. Anti-dysentric and antidiarrhoeal properties of medicinal plants were found to be due to tannins, alkaloids, saponins, flavonoids, triterpenoids and reducing sugars (38,39). The phytochemical analysis of ETL revealed the presence of alkaloids, flavonoids, triterpinoids carbohydrates, tannins, phenols, gums and mucilage. These constituents may mediate the antidiarrhoeal property of the ETL.

CONCLUSION

In conclusion, the present study has shown that the ETL has significant antinociceptive and antidiarrhoeal activity in the management of pain and diarrhea, thus justifying its widespread use by the local population for these purposes. Concerted efforts are being made to fully investigate the mechanisms involved in the pharmacological activities of ETL and phytochemical studies are also in progress to isolate and characterize the active constituents of *T. acuminata*. The isolated compounds may serve as useful prototypes of antinociceptive and antidiarrhoeal drugs of natural origin possessing the desired pharmacological activities while lacking certain untoward effects.

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Received: 07.11.2012
Accepted:20.02.2013