ANTINOCICEPTIVE, ANTIDIARRHOEAL AND CYTOTOXIC ACTIVITY OF *TAMARIX INDICA* LEAF

U. Habiba, U. Bose* and A.A. Rahman

Pharmacy Discipline, Khulna University,Khulna-9208, Bangladesh.

Corresponding Author:
Utpal Bose*
Present address: s107, School of Biotechnology and Biomolecular Sciences, University of New South Wales, NSW- 2052, Australia. Phone: +61420841383. E-mail: utpal.bose@unsw.edu.au

Summary

The methanol extract of the dried leafs of *Tamarix indica* (Tamaricaceae) was investigated for its possible antinociceptive, antidiarrhoeal and cytotoxic activities in animal models. The extract produced significant writhing inhibition in acetic acid-induced writhing in mice at the oral dose of 500 mg/kg body weight ($p<0.001$) comparable to the standard drug diclofenac sodium at the dose of 25 mg/kg of body weight. The extract showed antidiarrhoeal activity on castor oil induced diarrhoea in mice, it increased mean latent period and decreased the frequency of defecation significantly at the oral dose of 500 mg/kg body weight ($P<0.001$) comparable to the standard drug Loperamide at the dose of 50 mg/kg of body weight. The crude extract produced the most prominent cytotoxic activity against brine shrimp *Artemia salina* (LC_{50} = 20 µg/ml). The obtained results provide a support for the use of this plant in traditional medicine and its further investigation.

Key words: *Tamarix indica*; Antinociceptive activity; Antidiarrhoeal activity; Cytotoxic activity

Introduction

Tamarix indica (Family- Tamaricaceae), locally known in Bangladesh as 'Nona jhau', is mainly growing up gregariously on newly formed alluvial land rivers and by the coastal areas. These plants are mainly found as green, branchlets shrub or small tree. It is distributed in the coast forests of Bengal, Pakistan, Ceylon, Burma, Malay and Andamans. Different chemical constituents, particularly from the leaf, flower and bark, have been reported in the plant[1]. This plant used for fuel wood and timber in certain areas in the world[2]. This plant is mainly found in the salty regions and is found between interdunal areas of the desert[3]. The bark is bitter and an astringent, tonic; fruit and leaves are useful for dysentery and chronic diarrhoea[4]. The major chemical constituents of *Tamarix indica* are tannin (50%), tamarixin, troupin, 4-methylcoumarin and 3,3-di-O-methylellagic acid[5,6]. Several types of polyphenols (anthocyanins, tannins, flavonones, isoflavonones, resveratrol and ellagic acid) are currently reported[6]. Ksouri *et al*[7] also showed that the presence of some antioxidant compound i.e. terpenoids (carotenoids and essential oils). Presence of these compounds exhibit a wide spectrum of medicinal properties, such as anti-allergic, anti-inflammatory, anti-thrombotic,

cardio-protective and vasodilatory effects[8,9]. Literature reviews indicated that no studies combining the antinociceptive, antidiarrhoeal and cytotoxic activities of the leafs have so far be undertaken. Taking this in view and part of our ongoing search on Bangladeshi medicinal plants the present study aimed at evaluating the antinociceptive, antidiarrhoeal and cytotoxic properties of the leafs extract of Tamarix *indica*.

Materials and Methods

Plant material collection and extraction

The leaves of *Tamarix indica* were collected from the Sundarbans' Mangrove Forests, Bangladesh in June 2007, and were taxonomically identified by experts at the Bangladesh National Herbarium (accession number: 38556). About 400 g of powdered leaves were taken in a clean, flat-bottomed glass container and soaked in 1,300 ml of 80% methanol. The container with its contents was sealed and kept for a period of 7 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of cotton followed by a filtration through Whatmann filter paper and the filtrate thus obtained was concentrated using a rotary evaporator (Bibby RE200, Sterilin Ltd., U.K.) to get the crude extract.

Drugs

Diclofenac sodium (Opsonin Chemical Industries Ltd, Bangladesh), Loperamide (Square Pharmaceuticals Ltd., Bangladesh).

Preliminary phytochemical analysis

The crude extracts were subjected to preliminary phytochemical screening for the detection of major chemical groups. In each test 10% (w/v) solution of the extract in ethanol was used unless otherwise mentioned in individual test[1,10].

Tests for reducing sugar

Benedict's Test: 0.5 ml of the extract was placed in a test tube and then 5 ml Benedict's solution was added to it, boiled for 5 min and allowed to cool spontaneously.

Fehling's Test (Standard Test): 2 ml of the extract was added in 1 ml of a mixture of equal volumes of Fehling's solutions A and B, and was boiled for few min.

Combined Reducing Sugar test: 1 ml of the extract was boiled with 2 ml of diluted hydrochloric acid for 5 min. After cooling the mixture was neutralized with sodium hydroxide solution and then Fehling's test was performed as described above.

Tests for tannins

Ferric Chloride Test: 5 ml of the extract was placed in a test tube and then 1 ml of 5% Ferric chloride solution was added to it.

Potassium dichromate test: 5 ml of the extract was placed in a test tube and then 1 ml of 10% potassium dichromate solution was added.

Test for flavonoids

A few drops of concentrated hydrochloric were added to 5 ml of the extract.

Test for saponins
1 ml of the extract was placed in a graduated cylinder and was diluted to 20 ml with distilled water and shaken gently for 15 min.

Test for gums
5 ml of the extract was placed in a test tube and then Molish's reagent and sulphuric acid were added to it.

Tests for steroids
Libermann-Burchard test: 1 ml of the extract was placed in a test tube and then 2 ml Libermann-Burchard reagent was added to it.

Sulphuric acid test: 1 ml of the extract was placed in a test tube and 1 ml sulphuric acid was added to it.

Tests for alkaloids

Mayer's test: 2 ml of the extract and 0.2 ml of dilute hydrochloric acid were taken in a test tube and 1 ml of Mayer's reagent was added to it.

Dragendroff's test: 2 ml of the extract and 0.2 ml of dilute hydrochloric acid were placed in a test tube and then 1 ml Dragendroff's reagent was added.

Wagner's test: 2 ml of the extract and 0.2 ml of dilute hydrochloric acid were placed in a test tube. Then 1 ml of iodine solution (Wagner's reagent) was added.

Hager's test: 2 ml solution of the extract and 0.2 ml of dilute hydrochloric acid were placed in a test tube. Then 1 ml of picric acid solution (Hager's reagent) was added.

Animals
Young Swiss-albino mice of either sex, weighing 20 - 25 g, purchased from the Animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B) were used for the test. The animals were kept at animal house (Pharmacy Discipline, Khulna University) for adaptation after their purchase under standard laboratory conditions (relative humidity 55 - 65%, room temperature 25.0 ± 2.0°C and 12 h light-dark cycle) and fed with standard diets (ICDDR, B formulated) and had free access to tap water. The experimental met the national guidelines on the proper care and use of animals. The Institutional Animal Ethics Committee (IAEC) approved the experimental protocol.

Pharmacological studies

Antinociceptive activity
Antinociceptive activity of the crude extract was tested using the model of acetic acid-induced writhing in mice[11,12].The experimental animals were randomly divided into four groups, each consisting of ten animals. Group I was treated as 'control group' which received 1% (v/v) Tween-80 in water at the dose of 10 ml/kg of body weight; group II was treated as 'positive control' and was given the standard drug diclofenac sodium at dose of 25 mg/kg of body weight; group III and group IV were test groups and were treated with the extracts at dose of 500 mg/kg of body weight respectively. Control vehicle, standard drug and extracts were administered orally, 30 min prior to acetic acid (0.7%) injection. Then after an interval of 15 min, the number of writhes (squirms) was counted for 5 min.

Cytotoxicity test

The brine shrimps used for cytotoxicity test were obtained by hatching 5 mg of eggs of *Artemia salina* in natural seawater after incubation at about 29°C for 48h. The larvae (nauplii) were allowed another 48 h in seawater to ensure survival and maturity before use. Five doses of plant extract (1, 2, 4, 6, 8 and 10 µg/ml) in 5% DMSO and/or seawater were tested. Each extract preparation was dispensed into clean test tubes in 10 ml volumes and tested in duplicates. The concentration of DMSO in the vials was kept below 10 µl/ml. For control, same procedure was followed except test samples. After marking the test tubes properly, 10 living shrimps were added to each of the 20 vials with the help of a Pasteur pipette[13] .The test tube containing the sample and control were then incubated at 29°C for 24 h in a water bath, after which each tube was examined and the surviving nauplii counted. From this, the percentage of mortality was calculated at each concentration.

Antidiarrhoeal activity

Antidiarrhoeal activity of the methanol extract of leaves of *Tamarix indica* was tested using the model of castor oil-induced diarrhoea in mice[14]. The mice were all screened initially by giving 0.5 ml of castor oil and only those showing diarrhoea were selected for the final experiment. The test animals were randomly chosen and divided into three groups having five mice in each. Group-I was kept as control and received 1% Tween-80 at the dose of 10 ml/kg of body weight; group II was treated as 'positive control' and was given the standard drug loperamide at a dose of 50 mg/kg of body weight; group III was test group and was treated with the extract at a dose of 500 mg/kg of body weight. Control vehicle, standard drug and the extract were administered orally, 1 h prior to the oral administration of castor oil at a dose of 0.5 ml per mouse. Individual animals of each group were placed in separate cages having adsorbent paper beneath and examined for the presence of diarrhoea every hour in five hours study after the castor oil administration. Number of stools or any fluid material that stained the adsorbent paper was counted at each successive hour during the experiment. The latent period of each mouse was also counted. At the beginning of each hour old papers were replaced by the new ones.

Statistical analysis

Student's *t*-test was used to determine a significant difference between the control group and experimental groups.

Results

Preliminary phytochemical analysis

Results of different chemical tests on the methanol extract of *Tamarix indica* showed the presence of alkaloids, glycosides, flavonoids, saponins and tannins (Table 1).

Table 1. Phytochemical properties of *Tamarix indica* crude leaf extract

Compound	Alkaloids	Glycosides	Steroids	Gums	Flavonoids	Saponins	Reducing sugars	Tannins
Observation	+ve	+ve	-ve	- ve	+ ve	+ ve	-ve	+ve

Key: +ve = Presence -ve = Absence

Antinociceptive activity

Table 2 showed the effect of the methanol extract of *Tamarix indica* on acetic acid-induced writhing in mice. At dose of 500 mg/kg of body weight, the extract produced about 64.67% writhing inhibition in test animals. The results were statistically significant ($P < 0.001$) and were comparable to the standard drug diclofenac sodium, which showed about 85.95% writhing inhibition at the dose of 25 mg/kg ($P < 0.001$).

Table 2. Effects of *Tamarix indica* crude leaf extract on writhing effect on acetic acid induced mice

Treatment	Dose (mg/kg)	Mean writhing	% Inhibition	SD	P value (One way Anova)*
Experimental control (1% Tween80)	10	48.0 ± 1.91	-	2.97	P<0.001
Positive control (Diclofenac sodium)	25	6.8 ± 0.59	85.95	2.73	P<0.001
Test sample	500	17.1 ± 0.94	64.67	2.71	P<0.001

Key: *- (VassarStats, 2009); Test sample- *Tamarix indica*. Crude Extract. 30 minutes after treatment, 0.7% acetic acid was injected i.p. 10 minutes after injection writhing responses was recorded for 10 minutes. N=5.

Cytotoxic activity

In brine shrimp lethality bioassay (table 3), the extract showed lethality against the brine shrimp nauplii. It showed different mortality rate at different concentrations. From the plot of percent mortality versus log concentration on the graph paper LC_{50} was deduced (LC_{50}: 20 µg/ml) (Table 3).

Table 3: Brine shrimp lethality bioassay of *Tamarix indica* leaf extract

Test sample	Conc. (µg/ml)	No. of alive shrimp			% mortality	LC_{50} (µg/ml)	LC_{90} (µg/ml)
		Test 1	Test 2	Average			
Ethanolic extract	1	10	9	9.5	5	5.5	9.3
	2	8	8	8	20		
	4	7	6	6.5	35		
	6	4	5	4.5	55		
	8	2	3	2.5	75		
	10	0	0	0	100		

Antidiarrhoeal activity

Antidiarrhoeal activity of the methanol extract of *Tamarix indica* extract was tested by castor oil-induced diarrhoea in mice. Diarrhoeal initiation time and the number of stools excreted by the animals in 4 hours were collected. The extract caused an increase in latent period (0.7h) and (0.9h) i.e. delayed the onset of diarrhoeal episode of 500 mg/kg body of weight significantly (P<.01) which was comparable to the standard drug loperamide at the dose of 50 mg/kg body weight in which the resulted value was 1.5h (P<.001) (Table 4). The selected concentration of the extract also showed a good diarrheal inhibition with 44.8%. Loperamide, standard antidiarrhoeal agent showed an inhibition of 71.4%.

Table 4: Effects of *Tamarix indica* crude leaf extract on inhibition of castor oil diarrhoea

Treatment	Dose (mg/kg)	Latent Period (Hrs)	Mean number of stools*	% Inhibition	SD	P value (One way Anova)*
Experimental control (1% Tween80)	10	0.98 ± 0.15	24.6	-	0.39	
Positive control (Loperamide)	25	1.97 ± 0.13	13.2	71.4	0.26	P < 0.001
Test sample	500	1.68 ± 0.09	15.2	44.8	0.18	P < 0.001

Key: *- (VassarStats, 2009); Test sample- *Aegiceras corniculatum* Crude Extract. 40 minutes after treatment, 0.3mL castor oil was administered orally. Latent period of castor oil induced diarrhea was noted. Number of stools excreted for the next 4 hours were noted. *1 – Mean number of stools was an average number of stools for 4 hours for each treatment. % inhibition, SD and P value was also calculated with respect to the number of stools. N=5.

Discussion

Plants are employed as important source of medication in many traditional medications [15,16,17]. Since *Tamarix indica* belongs to the coastal forests, part of the plant constituents may be polar in nature. Methanol was used which has a wide range of solubility in both polar and nonpolar region. To avoid any solvent effect on the experimental animals, the solvent was evaporated completely to dryness[18].

Preliminary phytochemical screening of the extract showed the presence of alkaloids, glycosides, flavonoids, saponins and tannins. Polyphenolic compounds, like flavonoids and tannins, commonly present in mangrove plants have been reported to have multiple pharmacological effects, including antinociceptive and antidiarrhoeal activities. Roome *et al.*[19] (2008), showed that plant contains flavonoids and pentacyclic triterpenes may caused the inhibition pain mice. This study also revealed that the presence of benzoquinones also can inhibit the lipooxygenase pathways which support the uses of *Tamarix indica* in folk medicine against diarrhoea. Presence of saponins and tannins also involved in the antidiarrhoeal activities. Another study conducted by Ahmed *et al.*[18] (2007) with the leaves of

Tamarix indica showed the presence of steroids, alkaloids and glycosides can caused the antinociceptive and antidiarrhoeal acvities.

Antinociceptive activity of the methanol extract of *Tamarix indica* was tested by acetic acid-induced writhing model in mice. Acetic acid-induced writhing model represents pain sensation by triggering localized inflammatory response. Acetic acid, which is used to induce writhing, causes algesia by liberation of endogenous substances, which in turn excite the pain nerve endings [20]. Increased levels of PGE2 and PGF2α in the peritoneal fluid have been reported to be responsible for pain sensation caused by intraperitoneal administration of acetic acid[21]. The extract produced significant writhing inhibition comparable to the standard drug diclofenac sodium (Table 2). The polar compounds present in the plant extract may be responsible for the obtained antinociceptive activity. Based on this result it can be concluded that the ethanol extract of *Tamarix indica* might possess antinociceptive activity.

The cytotoxic activity of the ethanol extract of *Tamarix indica* was tested by using brine shrimp lethality bioassay. It is a recent development in the bioassay for the bioactive compounds. The plant is reported to contain saponins[1]. There is growing interest in natural saponins caused as much by the scientific aspects extraction and structural analysis of these compounds, as by the fact of their wide spectrum of pharmacological activities; for instance, bactericidal, antiviral, cytotoxic, analgesic, anti-inflammatory, anti-cancer and antiallergic[7,8]. Brine shrimp lethality bioassay indicates cytotoxicity as well as a wide range of pharmacological activities such as antimicrobial, pesticidal, antitumor, etc.[22]. The extract was found to show potent activity against the brine shrimp nauplii. Therefore the positive response obtained in this assay suggests that the extract may contain antitumor, antibacterial or pesticidal compounds.

Antidiarrhoeal activity of the extract of *Tamarix indica* was tested by using the model of castor oil-induced diarrhoea in mice[23]. Number of mechanisms have been previously proposed to explain the diarrhoeal effect of castor oil including inhibition of intestinal Na+, K+- ATPase activity to reduce normal fluid absorption[24], activation of adenylate cyclase or mucosal cAMP mediated active secretion[25], stimulation of prostaglandin formation[26], platelet activating factor and recently nitric oxide has been claimed to contribute to the diarrhoeal effect of castor oil[27.]

However, castor oil induced diarrhoea when it mixes with bile and pancreatic enzymes and liberates ricinoleic acid from the triglycerides upon oral administration. Most of the ricinolic acid remains in the intestine and produces its absorptive or secretory effect. The ricinolic acid thus liberated readily forms of ricinoleate salts with sodium and potassium in the lumen of the intestine. The salt formed as such behaves like a soap or surfactant within the gut and at the mucosal surface. Generally ricinoleate salts stimulates the intestinal epithelial cells adenyl cyclase[28] or released prostaglandin[29]. The extract caused and increased in latent period and decreased the frequency of defecation as well as the number of total stool count.

Generally the methanol bark extract of *Tamarix indica* experimentally inhibited the castor oil-induced diarrhoea. Furthermore, flavonoids present in the plant extract are reported to inhibit release of autacoids and prostaglandins, as a result it can inhibit motility and secretion induced by castor oil. The antidiarrhoeal activity of the extract may also be due to denature proteins forming protein tannates which make intestinal mucosa more resistant and reduce secretion.

Conclusion

Finally, it could be suggested that the methanol extract of *Tamarix indica* leaf possesses antinociceptive, cytotoxic and antidiarrhoeal activities. These facts indicate the scientific basis of *Tamarix indica* being used as a traditional medicine. However, further experiments may help to determine the pharmaceutical potentialities of the plant as a medicine.

Acknowledgements

The authors are thankful to Prof. Dr. Samir Kumar Sadhu, Head, Pharmacy Discipline, Khulna University; Dr. Mahiuddin Alamgir, Research Scientist, National Measurement institute (NMI), Australia for their encouragement during the research time. Thanks are also due to Mr. Samuel Kutty, Researcher, School of Chemistry, University of New South Wales, Australia for his valuable advice. All the informants of the study area are cordially acknowledged for their valuable cooperation.

References

1. Ghani A. Medicinal Plants of Bangladesh-Chemical constituents and uses, 2nd edition, The Asiatic Society of Bangladesh, Dhaka. 2003; pp.323-5,505-7.
2. Khan MA, Ansari R, Gul B, Qadir M. Crop diversification through halophyte production on salt-prone land resources. 2006;1(048):1-9.
3. Qureshi R, Bhatti GR. Diversity of micro-habitats and their plant resources in Nara desert, Pakistan. Pak J Bot. 2008;40(3):979-992.
4. Panwar AQ, Abro H. Ethnobotanical studies of Mahal Kohistan (Khirthar National Park). Pak.J.Bot. 2007;39(7):2301-15.
5. Kirthikar KR, Basu BD. Indian Medicinal Plants, 2nd ed. Allahabad;1996b;pp 376-471.
6. Sehrawat A, Sultana S. *Tamarix gallica* ameliorates thioaceamide-induced hepatic oxidative stress and hyperproliferative response in Wistar rats. J Enzyme Ihibit Med Chem. 2006;21(2):215-223.
7. Ksouri R, Falleh H, Megdiche W, Trabelsi N, Mhamdi B, Chaieb K, Bakrouf A, Magne C, Abdelly C. Antioxidant and antimicrobial acivities of the edible medicinal halophyte Tamarix gallica L. and related polyphenolic constituents. Food Chem Toxicol. 2009;47:2083-2091.
8. Balasundaram N, Sundaram K, Sammar S. Phenolic compounds in plants and agri-industrial by-products. Antioxidant activity, occurance and potential uses. Food Chem. 2006;1:191-203.
9. Khan MA, Kust GS, Barth H-J & Boer B. Sabkha Ecosystems. 2006; Vol:*II*: pp 129-153.
10. Evans WC. Trease and Evan's Textbook of Pharmacognosy. 1989 13th ed, Cambidge University Press, London,pp 546.
11. Ahmed F., Selim MST., Das AK, Choudhuri MSK. Anti-inflammatory and antinociceptive activities of *Lipia nodiflora* Linn. Pharmazie 2004;59:329-330.
12. Whittle BA. The use of changes in capillary permeability in mice to distinguish between narcotic and non-narcotic analgesics. Br. J. Pharmacol. Chemother.1964; 22: 246-253.

13. Meyer BN, Ferrigni NR, Putnam JB, Jacobsen LB, Nichols DE, McLaughlin JL. Brine shrimp: a convenient general bioassay for the active plant constituents. Planta Med. 1982; 45:31-34.
14. Chatterjee TK" *Handbook of laboratory Mice and Rats*" First Edition, Jadavpur University, India. 1993; pp. 133-139.
15. Grover JK, S Yadav, V Vats. Medicinal plants of India with anti-diabetic potential. J. Ethnopharmacol. 2002; 81(1): 81-100.
16. Keung WM, BL Vallee. Kudzu root: An ancient chinese source of modern antidipsotropic agents. Phytochemistry. 1998; 47 (4): 499-506.
17. Neves JM, C Matos, C Moutinho, G Queiroz, LR Gomes. Ethnopharmacological notes about ancient uses of medicinal plants in Trás-os-Montes (northern of Portugal). J. Ethnopharmacol. 2009; 124(2): 270-283.
18. Ahmed F, Al Mamun AH, Shahid IZ, Rahman AA, Sadhu SK. Antinociceptive, antidiarrhoeal and cytotoxic activity of *Aegiceras corniculatum.* Orient Pharm Exp Med. 2007; 7(2):191-196.
19. Roome T, Dar A, Naqvi S, Ali S, Choudhary MI. A study on antioxidant, free radical scavenging, anti-inflammatory and hepatoprotective actions of *Aegiceras corniculatum* (stem) extracts. J Ethnopharmacol. 2008; 118: 514-521.
20. Taesotikul T, Panthong A, Kanjanapothi D, Verpoorte R, Scheffer JJC. Anti-inflammatory, antipyretic and antinociceptive activities of *Tabernaemontana pandacaqui* Poir. J. Ethnopharmacol. 2003; 84: 31-33.
21. Derardt, R, Jougney S, Delevalcee F, Falhout M. Release of prostaglandins E and F in an algogenic reaction and its inhibition. Eur. J. Pharmacol. 1980; 51: 17-24.
22. Anderson JE, Chang CJ, McLaughlin JL. Bioactive components of *Allamanda nodiflora* Linn. J.Nat. Prod**.** 1988; 51: 307-308.
23. Chatterjee TK, (1993) "Handbook *of laboratory Mice and Rats*" First Edition, Jadavpur University, India, pp. 133-139.
24. Ganinella TS, P Bas. Laxatives: an update on mechanism of action. Life Sci. 1978;23:1001-10.
25. Capasso F, N Mascolo, AA Izzo, TS Ganginella. Dissociation of castor oil-induced diarrhoea and intestinal mucosal injury in rat: effect of NG-nitro-L-arginine methyl ester. British J. Pharmacol. 1994; 113: 1127-30.
26. Galvez A, ME Zarzuelo, MD Crespo, M Lorente, A Ocete, J Jimenez. Anti-diarrhoeal activity of *Euphorbia hirta* extract and isolation of an active flavonoidal constituent. Planta Med.1993; 59:333-6.
27. Mascolo N, AA Izzo, G Autore, F Barbato, F Capasso. Nitric oxide and castor oil-induced diarrhoea. J. Pharmacol. Exp. Ther. 1994; 268:291-5.
28. Racusen LC, H.J. Binder. Ricinolic acid stimulation of active anion secretion in colonic mucosa of the rat. J. Clin. Invest. 1979; 63: 743-749.
29. Beubler E, H. Juan. Effect of Ricinolic acid other Laxatives in Net Water Flux and Prostaglandin E release by the Rat colon. J. Pharm. Pharmacol. 1979;31: 681-685.