International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

International Journal of Pharmacology 7 (4): 527-531, 2011
ISSN 1811-7775 / DOI: 10.3923/ijp.2011.527.531

Antinociceptive, Antiinflammatory and Antibacterial Properties of *Tamarix indica* Roots

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Abstract: The present study investigated pharmacological activities to provide scientific basis to traditional usage of *Tamarix indica*. Phytochemical analysis of the dried roots of *Tamarix indica* (Tamaricaceae) indicated the presence of alkaloids, glycosides, flavonoids, saponins and tannins. The pharmacological interest of these compounds, coupled with the use of this plant in traditional medicine prompted the researchers to check for its possible antinociceptive, anti-inflammatory and antibacterial activities in animal models. The antinociceptive activity was studied using acetic acid-induced writhing in mice while anti-inflammatory tests were studied by using carrageenin induced rat paw edema model. Moreover, antibacterial activities were studied by using the disc diffusion method. The extract produced significant writhing inhibition in acetic acid-induced writhing in mice at the oral dose of 500 mg kg^{-1} body weight ($p<0.001$) comparable to the standard drug diclofenac sodium at the dose of 25 mg kg^{-1} of body weight. When given orally to rats at dose of 200 and 400 mg kg^{-1}, the extract showed a significant ($p<0.001$) anti-inflammatory activity against carrageenin induced paw edema in rats comparable to the standard drug aspirin. Moreover, The extract of *Tamarix indica* exhibited significant *in vitro* antibacterial activity against *Staphylococcus saprophyticus*, *Shigella sonnie, Salmonella typhi, Vibrio cholera, Streptococcus epidermidis, Shigella flexneri* and *Staphylococcus aureus* with the zones of inhibition ranging from 10.76 to 16.34 mm. The obtained results provide a support for the use of this plant in traditional medicine and its further investigation.

Key words: Phytochemical, *Tamarix indica*, antinociceptive, anti-inflammatory, antibacterial

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 roots, flower and bark, have been reported in the plant (Ghani, 2003). This plant used for fuel wood and timber in certain areas in the world (Khan *et al*., 2006a). This plant is mainly found in the salty regions and is found between interdunal areas of the desert (Qureshi and Bhatti, 2008). It can be used as prophylactic and therapeutic remedies to cure malaria as folk medicine (Tagarelli *et al*., 2010). The bark is bitter and an astringent, tonic; fruit and roots are useful for dysentery and chronic diarrhoea (Panhwar and Abro, 2007). The major chemical constituents of *Tamarix indica* are tannin (50%), tamarixin, troupin, 4-methylcoumarin and 3,3-di-O-methylellagic acid (Kirtikar and Basu, 1996; Sehrawat and Sultana, 2006). Plants extract are rich by acid compounds that are used as an inhibitor of nephrolithiasis (Bensatal and Ouahrani, 2008). Several types of polyphenols (anthocyanins, tannins, flavonones, isoflavonones, resveratrol and ellagic acid) are currently reported (Sehrawat and Sultana, 2006). Ksouri *et al*. (2009) also showed that the effects of antimicrobial activities and 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 (Balasundram *et al*., 2006; Khan *et al*., 2006b). Literature reviews indicated that no studies combining the antinociceptive, antidiarrhoeal and cytotoxic activities of the roots have so far been undertaken. Taking this in view and part of our ongoing search on Bangladeshi medicinal plants the present study aimed at evaluating the antinociceptive, anti-inflammatory and antibacterial properties of the roots extract of *Tamarix indica*.

MATERIALS AND METHODS

Plant material collection and extraction: The roots of *Tamarix indica* were collected from the Sundarbans' Mangrove Forests, Bangladesh in May 2009 and were

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taxonomically identified by experts at the Bangladesh National Herbarium (accession number: 49566). About 400 g of powdered roots 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 grade 1 and the filtrate thus obtained was concentrated using a rotary evaporator (Bibby RE200, Sterilin Ltd., U.K.) to get the crude extract.

Animals used: 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°C and 12 h light-dark cycle) and fed with standard diets (ICDDR, B formulated) and had free access to tap water

Drugs: Diclofenac (Square Pharmaceuticals Ltd, Bangladesh), Aspirin (Square Pharmaceuticals Ltd., Bangladesh), Gentamycin (Square Pharmaceuticals Ltd., Bangladesh).

Phytochemical tests: The crude extracts of *Tamarix indica* were subjected to preliminary phytochemical screening for the detection of major chemical groups. In each test 10% (w/v) solution of the extract in methanol was used unless otherwise mentioned in individual test (Evans, 1989; Ghani, 2003).

Determination of antinociceptive activity: Antinociceptive activity of the crude extract was tested using the model of acetic acid-induced writhing in mice (Ahmed *et al*., 2007; Roome *et al*., 2008). Analgesic activity of *Tamarix indica* extract was compared to the inhibition of writhing of a standard analgesic agent (diclofenac sodium). Experimental control mice were administered with 10 mL kg^{-1} 1% Tween80 (Alpha labchem) with water; positive control mice were administered with 25 mg kg^{-1} bodyweight diclofenac-sodium (Diclofenac) solution made to 2.5 mL with water and two test concentrations (250 mg kg^{-1} and 500 mg kg^{-1} body weight) of the crude extract of *Tamarix indica* was triturated by addition of small amount of Tween80 and water was slowly added to make the final volume of the test solutions to 2.5 mL. Four groups (Group1, 2, 3 and 4) of experimental animals were randomly selected with 5 animals in each group for each treatment. Mice were carefully administered with Tween80, diclofenac sodium and the test solutions by feeding needle. Thirty minutes interval was given to ensure proper absorption. During the time test mice were noted for any unwanted reactions. Acetic acid (0.7%) at a dose of 0.1 mg/10 g was administered intraperitoneally to induce pain sensation. After an interval of 10 min which was given for the absorption of acetic acid, numbers of writhing were calculated for 10 min.

Anti-inflammatory activity: Anti-inflammatory activity of *Tamarix indica* was tested by using carrageenin induced rat paw edema model (Winter *et al*., 1962; Ahmed *et al*., 2004). Rats were randomly divided into four groups, each consisting of six animals. Group I was kept as 'control' giving 1% (v/v) tween-80 solution in water; group II was kept as 'positive control' and was given the standard drug Aspirin at a dose of 150 mg kg^{-1} of body weight; group III and IV were test groups, treated with extracts at the doses of 200 and 400 mg kg^{-1} of body weight respectively. Control vehicle, standard drug and the extracts were given orally 1 h prior to the injection of 0.1 mL of 1% freshly prepared suspension of carrageenin. The paw volume was measured by using a plethysmometer (Ugo Basile 7140, Italy) just before and 1, 2, 3, 4 and 5 h after the carrageenin injection.

Antibacterial activity: The antibacterial activity of *Tamarix indica* extract was studied against *Staphylococcus saprophyticus*, *Shigella sonnie, Salmonella typhi, Vibrio cholera, Streptococcus epidermidis, Shigella flexneri* and *Staphylococcus aureus* clinical isolates. All bacterial strains were kindly provided by IMTECH, Chandigarh (India). Cultures of these bacteria were grown in a nutrient broth at 37°C and maintained on nutrient agar (Himedia, India) slants at 40°C. The antibacterial property was studied by the disc diffusion method (Chattopadhyay *et al*., 2002) using extract 200 mg/disc. Control disks contained solvents only (50% aqueous methanol).Gentamycin was used as positive controls. Minimum Inhibitory Concentration (MIC) was evaluated by the micro dilution method using 5 mL of liquid broth with different concentrations of extract (Ahmad and Beg, 2001; Bayoud *et al*., 2007).

Statistical analysis: Student's t-test was used to determine significant differences between the control group and test group.

Table 1: Phytochemical properties of *Tamarix indica* crude roots extract

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

+ve: Presence, -ve: Absence

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

Treatment	Dose (mg kg^{-1})	Mean writhing	%Inhibition	SD	p-value (One way Anova)*
Experimental control (1% Tween80)	10	48.0+1.91	-	2.97	-
Positive control (Diclofenac sodium)	25	6.8+0.59	85.95	2.73	p<0.001
Test sample	250	17.7+1.93	47.94	3.85	P<0.001
Test sample	500	17.1+0.94	64.47	2.71	p<0.001

For test group the results were statistically significant (p<0.001). -: *Tamarix indica* Crude Extract. 30 min after treatment, 0.7% acetic acid was injected i.p. 10 min after injection writhing responses was recorded for 10 min. N = 5

Table 3: Effect of methanolic extract of *Tamarix indica* on carrageenin induced rat paw edema

	Time after carrageenin injection#				
Treatment	1 h	2 h	2 h	4 h	5 h
Control (1% Tween 80) 10 mL kg^{-1}; p.o.	15.00±0.47	164.50±1.61	244.50±1.82	272.00±1.92	231.00±1.23
Positive control Aspirin 150 mg kg^{-1}; p.o.	10.54±1.23* (29.71)	100.50±2.34** (38.91)	119.25±1.92** (51.23)	172.80±1.72** (36.47)	155.40±211** (32.72)
Test group^{-1} Methanolic extract 200 mg kg^{-1}; p.o.	12.86±0.52 ** (14.26)	133.80±0.98 ** (18.08)	184.38±1.43 ** (24.59)	224.40±1.66 ** (17.51)	194.7±1.76 ** (15.71)
Test group-2 Methanolic extract 400 mg kg^{-1}; p.o.	11.43±1.04 ** (23.80)	113.3±1.10 ** (31.13)	144.3±2.21 ** (40.28)	192.60±1.65 ** (29.18)	170.50±1.27 ** (26.18)

#Increase in paw edema volume (mL)×1000±SEM. Values are expressed as Mean±SEM. (No. of animals, n = 6); *indicates p<0.05, **indicates p<0.001 vs. control; Test group: *Tamarix indica;* p.o.: Per oral

RESULTS

Phytochemical test: Phytochemical tests were performed with the crude extract of *Tamarix indica* and the results are tabulated in Table 1. The results manifest the presence of alkaloids, glycosides, flavonoids, saponins and tannins from crude extract of the roots.

Antinociceptive activity test: The methanolic extract showed significant inhibition of writhing when compared to the control (Table 2). At dose of 250 and 500 mg kg^{-1} of body weight, the extracts produced 47.94 and 64.47% inhibition in test animals, respectively. The results were found to be statistically significant (p<0.001) and were comparable to the standard drug diclofenac sodium, which showed about 85.95% writhing inhibition at dose of 25 mg kg^{-1} (p<0.001).

Anti-inflammatory activity

Carrageenin-induced rat paw edema: In the carrageenin induced rat paw edema model of anti-inflammatory activity, the methanolic extract of roots of *Tamarix indica* showed a significant inhibitory effect on the edema formation from the first hour to fifth hour. The highest inhibitory effect was found during the third hour where the inhibition was 24.59% (p<0.001) and 40.28% (p<0.001) at the doses of 200 and 400 mg kg^{-1}, respectively. These findings were comparable to standard drug aspirin where the inhibition was 51.23% (Table 3).

Table 4: *In vitro* antibacterial activity of the methanolic extract of *Tamarix indica* Crude roots extract

Bacterial stains	*Tamarix indica* (mm)	Control (mm)	% of inhibition of extract compare to Control (As 100%)
Staphylococcus saprophyticus	12.22	30.00	40.73
Shigella sonnie	16.34	44.88	36.41
Salmonella typhi	14.65	36.88	39.72
Vibrio cholera	14.42	32.44	44.45
Streptococcus epidermidis	12.45	34.28	36.32
Shigella flexneri	10.76	28.90	37.23
Staphylococcus aureus	10.80	30.50	35.41

Control (50% aqueous methanol), Diameter of inhibition zones (mm), Control: Gentamycin

***In vitro* antibacterial activity:** Table 4 showed the extract of *Tamarix indica* exhibited significant *in vitro* antibacterial activity against *Staphylococcus saprophyticus*, *Shigella sonnie, Salmonella typhi, Vibrio cholera, Streptococcus epidermidis, Shigella*

flexneri and *Staphylococcus aureus* with the zones of inhibition ranging from 10.76 to 16.34 mm.

DISCUSSION

Plants are employed as important source of medication in many traditional medications (Grover *et al.*, 2002; Keung and Vallee, 1998; Neves *et al*., 2009). 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 non-polar region. To avoid any solvent effect on the experimental animals, the solvent was evaporated completely to dryness (Ahmed *et al*., 2007).

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 effect, including antinociceptive activity. Roome *et al*. (2008) showed that plants containing flavonoids and pentacyclic triterpenes may cause pain inhibition in mice. Presence of glycosides can cause the antinociceptive activity.

Antonociceptive activity was explored with two different concentrations of 250 and 500 mg kg^{-1} body weight. Antinociceptive activity of *Tamarix indica* was tested by acetic acid-induced writhing model in mice. Acetic acid-induced writhing model causing pain sensation by tiggering localized inflammatory response. Acetic acid, which is used to induce writhing, causes analgesia by liberation of endogeneous substances, which in turn excite the pain nerve endings (Taesotikul *et al*., 2003). Increased levels of PGE_2 and $PGF_{2\alpha}$ in the peritoneal fluid have been reported to be responsible for pain production caused by intraperitoneal administration of acetic acid (Derardt *et al*., 1980). The result of the test showed that the methanolic extract of the roots of *Tamarix indica* at dose 500 mg per kg body weight exhibit significant writhing inhibition ($p<0.001$) as compared with the standard drug diclofenac sodium (Table 2). The polar compounds present in the plant extract may be responsible for the obtained antinociceptive activity. According to the basis of this result it can be concluded that the extract possesses antinociceptive activity.

The most widely used primary test for the screening of new anti-inflammatory agents is the carrageenin-induced rat paw edema model (Winter *et al*., 1962). The edema formation is a biphasic event. The initial phase, observed during the first hour, is attributed to the release of histamine and serotonin (Vinegar *et al*., 1996) and the delayed edema is due to the release of bradykinin and prostaglandins (Di Rosa *et al*., 1971). It has been reported that the second phase of edema is sensitive to steroidal and non-steroidal anti-inflammatory agents (Di Rosa *et al*., 1971). The extract reduced the paw volume significantly from 1 to 5 h in which the highest effects were found at the third hour. These results tend to suggest the probable anti-inflammatory activity of the extract.

In this experiment, methanolic extract of *Tamarix indica* showed moderate sensitivity to the five of the test organisms both gram positive and gram negative type of bacteria. The highest zone on inhibition (16.34 mm) was recorded against *Shigella sonnie*. Moreover, the experiment was only conducted with five species of bacteria as test samples. Therefore further research is essential to evaluate the sensitivity of the plant extract against other species of bacteria, fungi, virus of other microorganisms.

CONCLUSION

Finally, it can be concluded from the study that the antinociceptive, cytotoxic and diuretic effects of the methanolic roots extract of *Tamarix indica* may be presence of different chemical compounds which works through the specific and non-specific mechanisms. However, extensive studies are needed to evaluate the precise mechanism(s), active principles and the safety profile of the plant as a remedy for different therapeutical conditions.

ACKNOWLEDGMENTS

The authors are thankful to Prof. Dr. Samir Kumar Sadhu, Head, Pharmacy Discipline, Khulna University; Ahmed Ayedur Rahman, Assistant professor, Pharmacy Discipline, Khulna University; Dr. Mahiuddin Alamgir, Research Scientist, National Measurement institute (NMI), Australia for their encouragement during the research time. All the informants of the study area are cordially acknowledged for their valuable cooperation.

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