ANTIDIARRHOEAL AND ANTINOCICEPTIVE ACTIVITIES OF LEAFS AVICENNIA *ALBA*

M.A Rahman[1], S. Biswas[1], V. Bala[1], A. K. Shill[1] and U. Bose [*1,2]

[1]Pharmacy Discipline, Khulna University, Khulna-9208, Bangladesh.

[2]School of Pharmacy, University of Queensland, QLD-4102, Australia.

Corresponding Author:
Utpal Bose (u.bose@uq.edu.au)
Present address:
School of Pharmacy, University of Queensland, Queensland- 4102, Australia. Tel: +61431398923.

Summary

The methanol extract of the dried leafs of *Avicennia alba* (Avicenniceae) was investigated for its possible antidiarrhoeal and antinociceptive 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 considerable 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 antimicrobial activity of the Ethanolic extract of bark of Avicennia alba was investigated, which showed limited antimicrobial activity against specific species of eight types of bacterial culture. The obtained results provide a support for the use of this plant in traditional medicine and its further investigation.

Key words: *Avicennia alba*; Antinocicepti activity; Antidiarrhoeal activity; Cytotoxic activity

Introduction

Avicennia alba (Family- Avicenniceae), locally known in Bangladesh as 'Sada Bain', grows commonly in the mangrove areas through out the country. The *Avicennia alba Linn.* is native and common throughout much of India, Burma and Malacca and dry areas of Ceylon and is often grown in Southern Asia to Southeast Asia, Australia and Oceania.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 [3]. The heartwood is used to make tonics. The bark and seeds are used as a fish poison and birth control[4,7]. The chemical constituents of *Avicennia alba* are tannins, alkaloids, and polyphenols[7],8,9.

Recently three new naphthoquinones and their analogues, named avicequinone-A (1), -B (2), -C (3), and avicenol-A(4), -B (5), -C (6), respectively, were isolated from the stem bark of

Avicennia alba [5]. Deepanjan Banerjee *et al*[6] also showed that the presence of some antioxidant compound. Presence of these compounds exhibit a wide spectrum of medicinal properties, such as anti-cancer, anti-inflammatory, anti-microbial[8,9]. Literature reviews indicated that no studies combining the antidiarrhoeal and analgesic activities of the barks 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 antidiarrhoeal and analgesic properties of the leafs extract of Avicennia alba.

Materials and Methods

Plant material collection and extraction

The leaves of *Avicennia alba* were collected from the Sundarbans' Mangrove Forests, Bangladesh in June 2009, and were taxonomically identified by experts at the Bangladesh National Herbarium (accession number: 40556). 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 (Beximco Pharma 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.

Antidiarrhoeal activity

Antidiarrhoeal activity of the methanol extract of leaves of *Avicennia alba* 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 *Avicennia alba* showed the presence of alkaloids, glycosides, flavonoids, saponins and tannins (Table 1).

Table 1. Phytochemical properties of *Avicennia alba* 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 *Avicennia alba* 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 *Avicennia alba* 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 $\pm$ 1.91	-	2.97	-
Positive control (Diclofenac sodium)	25	6.8 $\pm$ 0.59	85.95	2.73	P<0.01
Test sample	500	18.0 $\pm$ 0.71	76.62	1.41	P<0.01

Key: *- (VassarStats, 2009); Test sample- *Avicennia alba.* 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.

Antidiarrhoeal activity

Antidiarrhoeal activity of the methanol extract of *Avicennia alba* 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 *Avicennia alba* 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	0.97 ± 0.15	24.6	-	0.39	
Positive control (Loperamide)	25	1.96 ± 0.14	13.2	71.4	0.26	P < 0.01
Test sample	500	1.67 ± 0.09	15.2	44.8	0.18	P < 0.001

Key: *- (VassarStats, 2009); Test sample- *Avicennia alba* 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 *Avicennia alba* 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 *Avicennia alba* 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 *Avicennia alba* showed the presence of steroids, alkaloids and glycosides can caused the antinociceptive and antidiarrhoeal acvities.

Antinociceptive activity of the methanol extract of *Avicennia alba* 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 *Avicennia alba* might possess antinociceptive activity.

Antidiarrhoeal activity of the extract of *Avicennia alba* 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 *Avicennia alba* 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 *Avicennia alba* leaf possesses antinociceptive and antidiarrhoeal activities. These facts indicate the scientific basis of *Avicennia alba* 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; Mr. Utpal Bose, Researcher, Queensland University, Australia for their encouragement during the research time. 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. Chihiro,*, Shinya,Yuichi, Hugh T.-W. TAN, and Hiroshi - Chemical Constituents of *Avicennia alba*. Isolation and StructuralElucidation of New Naphthoquinones and Their Analogues Chem. Pharm. Bull.2000;48(3) 339—343.
5. Antioxidant activity and total phenolics of some mangroves in Sundarbans.Deepanjan Banerjee, Shrabana Chakrabarti, Alok K. Hazra, Shivaji Banerjee, Jharna Ray and Biswapati Mukherjee*-African Journal of Biotechnology 2008; 7 (6), pp. 805-810.
6. Wetlands Ecology and Management 10: 421–452, 2002.Bioactivities, bioactive compounds and chemical constituents of mangrove plants W.M. Bandaranayake.
7. Have reported the presence of compounds like tannins,alkaloids, and polyphenols Jamale., B.B., Joshi, G.V., Effect on age of mineral constituents Poly phenoloxides and peroxides in mangrove leaves, In. *J. Exp. Biol*.,1998,16(1),117-120.
8. Ross, S.A., Megalla, S.E., Bisby, D.W., Awad, A.H., Studies for determining someantibiotic substance in some Egyptian plants. Screening of some Antimicrobial activity.*Fitoterpia*, 1980, 51,303-308.
9. Cancer chemopreventive activity of naphthoquinones and theiranalogs from Avicennia plants-Masataka Itoigawaa,b,*, Chihiro Itoa, Hugh T.-W. Tanc, Masato Okudad- Cancer Letters 174 (2001) 135–139.
10. Evans WC. Trease and Evan's Textbook of Pharmacognosy. 1989 13th ed, Cambidge University Press, London.
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.