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(Research Article)

Evaluation of Diuretic and Neuropharmacological Properties of the Methanolic Extract of *Avicennia officinalis* L. leaves from Bangladesh

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ABSTRACT

The crude methanolic extract of the leaves of Avicennia officinalis L. (Family: Avicenniaceae) was evaluated for its diuretic and neuropharmacological activities. Phytochemical analysis of the ethanolic extract of the leaves of A. officinalis indicates the presence of alkaloid, steroid, flavonoid, reducing sugars and gums. The extract of A. officinalis leaves also potentiated the pentobarbital induced sleeping time in mice, and decreased the open field score in open field test, decreased the number of hole crossed from one chamber in the hole cross test and decreased the head dip responses in hole board test. Diuretic activity was proved by the electrolyte loss ratio (Na+/K+ excretion ratio was 1.52 and 1.33 at the doses of 200 and 400 mg/kg respectively) as that of the standard diuretic furosemide (1.35). The obtained results provide a support for the use of this plant in traditional medicine and its further investigation.

Key Words: *Avicennia officinalis*, Neuropharmacological, Diuretic, Avicenniaceae

INTRODUCTION

Avicennia officinalis (*A. officinalis*) is a medium-sized tree growing in brackish water. The 15 species in the single genus of Avicenniaceae family are found on tropical coasts as constituents of mangrove vegetation[1]. In Bangladesh, *A. officinalis* is widely distributed in Sundarban and locally it is known as Baen. Previous phytochemical investigations on the different species of *Avicennia* resulted in the isolation of essential oil and sugars like arabinose, glucose and ribose. Among other compounds alkaloids, flavonoids, steroids, terpenoids and iridoids are most considerable components[2]. The earlier studies on this plants resulted in the isolation of C iridoid glucoside, 7-O-trans cinnamoyl-4-epilogenin, geniposidic acid, 2-cinnamoyl-mussaenoside[2]. The anti-inflammatory activity of methanolic extract of *A. officinalis* may be due to the presence of the phytoconstituent, betulinic acid, Mimosol D,taepeenin D, taepeenin L, (E)-7-hydroxy-3-(4-methoxybenzyl)chroman-4-one[3].

Methanol extract of leaves of *A. officinalis* has been shown anti-inflammatory activity in different models such as adjuvant-induced arthritis, carrageenan-, and formalin-induced rat paw oedema model[3]. The fruits are plastered onto tumors in India[4]. Indian mangrove is a folk remedy for boils and tumors[5]. Unripe seeds are poulticed onto abscess, boils, and smallpox sores. Indochinese uses the bark for skin afflictions, especially scabies. A resinous substance exuded from the bark acts as a contraceptive, and apparently can be taken all year long without ill effects[6].

This plant is used for thrush in children. The heartwood is rubbed against a course stone. The tree oils of this plant exhibited cytotoxic activity[2]. It is a folklore medicinal plant used mainly against rheumatism, paralysis, asthma and snake-bites, skin disease, ulcer. A detection of the plant with sugar candy and cumin is used in dyspepsia with acid eructation's [7, 8].

Since no literature is currently available to substantiate diuretic and neropharmacological activities from methanolic extract of *A. officinalis* leaves, therefore the present study is a part of our on-going pharmacological and chemical screening of selected *A. officinalis* leaves and designed to provide scientific evidence for its use as a traditional folk remedy by investigating the diuretic and neropharmacological activities.

MATERIALS AND METHODS

Collection and Identification of Plant Materials

For this present investigation the leaves of *A. officinalis* was collected from Karamjal, Sundarban, Bangladesh and was identified by Bangladesh National Herbarium, Mirpur, Dhaka (Accession No. 30556).

Preparation of Methanolic Extract

The leaves of *A. officinalis* were freed from any of the foreign materials. Then the leaves were air-dried under shed temperature. The dried plant materials were then ground into powder. About 600 g of powdered material was taken in a clean, flat-bottomed glass container and soaked in 1500ml of methanol. The container with its contents was sealed and kept for a period of 10 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 rotary evaporator at bath temperature not exceeding 40˚ to have gummy concentrate of extract (Yield approx. 13.27%).

Test Animals and Drug

Young Swiss-albino mice either sex, 3-4 weeks of age, weighing 20-25 g, were used for in vivo pharmacological screening. Mice were purchased from the Animal Research Branch of the International Centre for Diarrheal Disease and Research, Bangladesh (ICDDR,B). They were housed in standard environmental conditions at animal house of Khulna University animal lab and fed with rodent diet and water ad libitum. All experimental protocols were in compliance with Ethics Committee on Research in Animals as well as internationally accepted principles for laboratory animal use and care.

The standard drug Furosemide was used for this study and purchased from Opsonin Chemical Industries Ltd, Bangladesh. The standard drug Pentobarbital and Diazepam was used for this study and purchased from Square Pharmaceuticals Ltd, Bangladesh.

Phytochemical Screening

The freshly prepared crude extract 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, and steroids with Libermann-Burchard reagent. Reducing sugars with Benedict's reagent[9-11].

Diuretic Activity

Diuretic activity of the extract of leaves was investigated using the method as described by Lipschitz et al.[12].The test animals were randomly chosen and divided into five groups having ten mice in each. Twenty-four hours prior to the experiment, the test animals were placed in to metabolic cages with the withdrawal of food and water. Group-1 or the control group received vehicle (1% Tween 80 in water) at a dose of 10 ml/kg body weight orally. Group-2 was provided with urea solution at a dose of 500 mg/kg. Group-3 was provided with standard diuretic drug furosemide at a dose of 0.5 mg/kg.Group-4 and group-5, the test groups were treated with the methanol extract of *A. officinalis* at the doses of 200 and 400 mg/kg respectively. From the graduated urine chamber of metabolic cage, the urinary output of each group was recorded 5 h after the above treatments. Collected urine was centrifuged and then estimated for sodium and potassium by using digital flame photometer (Elico Pvt. Ltd., model CL 22D). Chloride was estimated by the Schales and Schales method reproduced by Godkar[13].

Neuropharmacological Activity

i) Pentobarbital Induced Hypnosis

Pentobarbital induced hypnosis test was carried out by the method of Williamson et al.[14]. The test animals were divided into three groups consisting of seven mice in each group. Group I was the positive control group and group II and III were the experimental groups. The experimental groups were administered with the methanol extract of *A. officinalis* at dose of 250 and 500 mg/kg body weight intra-peritoneally (i.p.), while the animals of group I (control) were supplied with distilled water containing 0.1% (v/v) tween-80 (i.p.)at the dose of 10 ml/kg of body weight. The total sleeping time were recorded for both controls as well as for treated groups.

ii) Exploratory Behavior

This experiment was performed by (i) Open field test[15] (ii) Hole cross test[16] and (iii) Hole board test[17]. The test animals were divided into four groups consisting of seven mice in each group. Group I was the control group, group II was positive control and group III and IV were the experimental groups. The experimental groups were administered with the methanolic extract of *A. officinalis* (prepared by distilled water and tween-80) at dose of 250 and 500 mg/kg of body weight intra-peritoneally (i.p.), while the animals of group I (control) were supplied with 0.1% (v/v) tween-80 (i.p.) at the dose of 10 ml/kg of body weight and group II was used in positive control as reference standard at the dose of 1 mg/Kg body weight. The observations were made on 0 min before injection and 30, 60, 90, 120, 180 and 240 min after injections of the test samples and control.

RESULTS

Phytochemical Analysis

Results of different chemical tests on the methanolic, crude leaves extract of *A. officinalis* showed the presence of Alkaloid, Steroid, Flavonoid, Reducing sugars and Gums (Table-1).

Diuretic Activity

The effect of the methanolic extract of *A. officinalis* on the urination of mice was observed for 5h which revealed that the extract has a marked diuretic effect in the test animals. This was comparable to that of standard drug furosemide and diuretic agent urea. Electrolyte loss showed similar ratio (Na^+/K^+ excretion ratio was 1.52 and 1.33 at the doses of 200 and 400 mg/kg respectively) as that of the loop diuretic furosemide (1.35) (Table- 2).

Neuropharmacological Activity

i)Pentobarbital Induced Hypnosis Test

Table-3 showed the effect of *A. officinalis* on pentobarbital induced hypnosis in mice. The total sleeping time was about 66 and 82 min at dose of 250 and 500 mg/kg of body weight respectively where as in control group it was about 32 min.

ii)Open Field Test

On open field test with mice were treated with different doses of methanol extract of *A. officinalis*, it was observed that there was a significant decrease in the no. of movements in mice at doses of 250 mg/kg and 500 mg/kg as compared to control. This decrease was more at dose 500 mg/kg than at 250 mg/kg. The effects are given in table -4.

iii)Hole Cross Test

In hole cross test, mice were treated with different doses of methanol extract of *A. officinalis*, it was observed that there

was a significant decrease in the number of hole crossed from one chamber to another chamber by mice at doses of 250 mg/kg and 500 mg/kg as compared to control. This decrease was more at dose 500 mg/kg than at 250 mg/kg. The effects are given in table- 5

iv)Head Dip Test

In head dip test, mice were treated with different doses of methanol extract of *A. officinalis*, it was observed that there was a significant decrease in head dip responses in mice at doses of 250 mg/kg and 500 mg/kg as compared to control. This decrease was more at dose 500 mg/kg than at 250 mg/kg. The effects are given in table-6.

DISCUSSION

Diuretic activity may be very useful in a number of conditions like hypertension, hypercalciuria, cirrhosis of liver. Furosemide, used as the standard drug in this experiment belongs to the loop or high-ceiling diuretics, which act by inhibiting $Na^+/K^+/Cl^-$ co-transport of the luminal membrane in the ascending limb of the loop of Henle and have the highest efficacy in mobilizing Na+ and Cl^- from the body. The extract was able to increase the volume of urine with statistical significance along with a considerable Na^+ and Cl^- load which was comparable to that of furosemide. The diuretic action of the extract may be due to its action on the kidney. The extract may also contain a high proportion of osmotically active compounds or their metabolites that lead to an increased urine volume. There was a decreasing the ratio of concentration of excreted sodium and potassium ions after plant extract treatment. This also indicates that the extract increases sodium ion excretion to a greater extent than potassium, which is a very essential quality of a good diuretic with lesser hyperkalaemic side effect. This observed diuretic effect may be due to the effect of extract on the glomerular filtration rate and the direct inhibitory effect on the reabsorption mechanism of salt. Further studies may be carried out to identify whether these actions are associated with the same agent or a number of agents that are responsible for such activities.

Central depressants elicit their effect by interfering with the functions of the cerebral cortex. A most important method of investigating the probable cortical manifestation of a drug is to check its effect on the pentobarbital narcosis as pentobarbital has multifarious effects on the cerebral cortex[18]. The pentobarbital sleeping time test was performed to find out whether the methanolic extract of the plants have any effect on the cerebral cortex. Pentobarbital shorten the onset of sleep and increases sleep duration. The methanolic extract of *A. officinalis* reduced the onset of sleep and potentiated the pentobarbital induced sleeping time in mice, which suggests its central depressant activity[19]. Thus, suggesting the probable tranquilizing action[20].

Anxiety and sedation are principally mediated in the CNS by the GABAA receptor complex, which is also involved in other physiological functions related to behavior, as well as in various psychological and neurological disorders such as epilepsy, depression, Parkinson syndrome and Alzheimer's disease. The GABA receptor complex comprises a Cl^- channel and binding sites for several compounds, such as benzodiazepines, barbiturates, neuroactive steroids, and a variety of other drugs like loreclezole and propofol[21].

It has been experimentally proven that, in the absence of a special task to perform, the behavior of a given animal tend to maintain that inner activation level that is, at times, inconsistent with the actual level of activation of the animals. In order to get as accurate a picture as possible, on the effect of the drug on exploration, the open field test was performed. The extract also made mice to reduce their behavioral exploration, which further support the central sedative properties of the extract. The overall results tend to predict the CNS depressant action of the extract.

CONCLUSION

Thus, in the present investigation, it could be suggested that the methanol extract of *A. officinalis* leaves has been shown potent neuropharmacological and diuretic activities. These facts indicate the scientific basis of *A. officinalis* being used as a traditional medicine. However, further experiments may help to determine the pharmaceutical potentialities of the plant as a medicine.

Table 1: Preliminary Phytochemical screening of methanolic extract of *A. officinalis* leaves.

Phytoconstituents	Methanolic extract of *A. officinalis*
Alkaloid	+
Reducing sugar	+
Tannins	+
Gums	+
Flavonoids	+
Saponin	-
Steroid	+

+: Positive result; - : Negative result

Table 2: Effect of methanolic extract of *A. officinalis* leaves on urine excretion parameters in miceTreatment.

Treatment	Dose (mg/kg; P.O)	Volume of urine (ml)	Concentration of ions (m.eq.l-1)			
			Na^+	K^+	Cl^-	Na/K^+
Group-1 (Control)	-	1.52 ± 0.21	72.38 ± 2.43	58.16 ± 2.94	71.98 ± 2.55	1.24
Group-2 (Urea)	500	2.81 ± 0.31*	108.99±2.70**	72.38±2.43*	87.08±2.24*	1.51
Group-3 (Furosemide)	0.5	3.29±0.10**	117.50±2.11**	85.08±2.24**	95.79±1.50**	1.35
Group-4 (ME)	200	3.06±0.18	111.79±3.48**	73.78±2.96**	88.06±1.20**	1.52
Group-5 (ME)	400	3.89±0.13**	122.02±2.3**	91.79±1.50**	101.79±1.98**	1.33

*ME: methanolic extract of A. officinalis; Values are expressed as mean ± SEM (Number of animals, n = 10); *indicates P<0.01, **indicates P<0.001 vs. control; bCollected for 5 hours after treatment.*

Table 3: Effect of methanolic extract of *A. officinalis* leaves on pentobarbital induced hypnosis in mice.

Animal group	Treatment	Time of onset of Sleep (min)	Total sleeping time (min)
I (Control)	0.1% Tween 80 solution	8.51±0.16	32.06±1.20
II (Test group-I)	Me. Extract of *A. officinalis* 250 mg/kg.	7.72±0.18*	66.74±2.83**
III (Test group-II)	Me. Extract of *A. officinalis* 500 mg/kg.	6.20±0.58*	82.07±3.57**

*Values are Mean ± SEM; *, P<0.01; **, P<0.001 vs. control, Student's t-test;Me.= Methanol; Values are Mean ± SEM;*

Table 4: Effect of methanolic extract of *A. officinalis* leaves on open field test.

Groups	Movement on open field test before and after drug administration (mean ± SEM)						
	0 min	30 min	60 min	90 min	120 min	180 min	240 min
Control	121.02±2.30	82.08±2.24	59.20±1.19	54.55±0.96	42.8±1.43	33.51±1.41	25.45±1.40
Diazepam 1mg/Kg (i.p.)	90.79±1.50**	35.13±1.07**	20.16±1.23**	15.67±1.38**	14.51±1.33**	11.23±1.38**	6.49±1.13**
A. officinalis (250 mg/kg) (p.o.)	110.50±2.12*	71.38±2.43*	53.28±1.67*	41.85±3.35*	34.46±2.22*	26.48±1.99*	20.87±0.66*
A. officinalis (500 mg/kg) (p.o.)	107.99±2.70*	69.78±2.96*	47.02±2.36**	30.06±2.64**	26.07±1.98**	25.07±1.21**	17.99±1.55*

*Values are Mean ± SEM; *, P<0.01; **, P<0.001 vs. control, Student's t-test; Me. = Methanol*

Table 5: Effect of methanolic extract of *A. officinalis* leaves on hole cross test.

Groups	Movement on hole cross test before and after drug administration (mean ± SEM)						
	0 min	30 min	60 min	90 min	120 min	180 min	240 min
Group-I (Control)	9.55±0.56	8.77±0.78	8.50±0.73	7.64±0.26	7.15±0.22	6.81±0.54	6.18±0.62
Diazepam 1mg/Kg (i.p.)	4.12±0.98**	3.42±0.63**	3.35±0.33**	2.85±0.25**	2.55±0.57**	2.25±0.43**	1.85±0.30**
A.officinalis (250 mg/kg) (p.o.)	7.57±0.18*	6.13±0.43*	5.77±0.32*	5.30±0.69*	4.90±0.46**	4.57±0.50*	4.24±0.28*
A.officinalis (500 mg/kg) (p.o.)	6.61±0.72*	6.06±0.38*	5.66±0.40*	4.90±0.67**	4.77±0.55**	4.20±0.49*	3.77±0.21*

*Values are Mean ± SEM; *, P<0.01; **, P<0.001 vs. control, Student's t-test; Me. = Methanol*

Table 6: Effect of methnolic extract of *A. officinalis* leaves on hole board test. [Mean ± SEM]

Groups	Effect on Hole Board Test (Head dipping) before and after drug administration (mean ± SEM)						
	0 min	30 min	60 min	90 min	120 min	180 min	240 min
Group I (Control)	18.31±0.45	13.71±0.54	12.40±0.61	10.93±1.02	9.80±0.66	8.55±0.56	7.77±0.78
Diazepam 1 (i.p.)	7.90±0.70**	6.79±0.43**	5.10±0.38**	4.30±0.58**	3.66±0.91**	3.12±0.98**	2.42±0.63**
A.officinalis(250 mg /kg) (p.o.)	15.17±0.67**	10.81±0.50**	10.41±0.31*	7.99±0.18*	7.46±0.39*	6.57±0.17*	5.13.66±0.43*
A.officinalis(500 mg /kg) (p.o.)	13.19±0.44**	8.26±0.38**	9.80±0.49**	7.10±0.38*	6.37±0.28**	5.61±0.72*	5.06±0.38*

*Values are Mean ± SEM; *, P<0.01; **, P<0.001 vs. control, Student's t-test; Me. = Methanol*

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