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Analgesic and antipyretic activities of the methanolic extract of aerial parts of *Avicennia alba* Blume

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Abstract: The study was designed to investigate the analgesic activity of methanolic extract of *Avicennia alba* Blume (family: Avicenniaceae) aerial parts (100 and 200 mg/kg, p.o.) in radiant heat and tail immersion methods. The antipyretic activity was evaluated by brewer's yeast-induced fever models in rats. Methanolic extract at two different dose levels showed a significant increase in basal reaction time in radiant heat and tail immersion methods. The extract (100 and 200 mg/kg) showed a significant inhibition of elevated body temperature when compared to corresponding control. Our findings indicate that *Avicennia alba* could be a potential source for natural analgesic and antipyretic agent in future.
Key word: *Avicennia alba*, analgesic, antipyretic activity, brewer's yeast-induced.

Introduction

The long historical use of medicinal plants in many traditional medical practices, including experience passed from generation to generation has demonstrated the safety and efficacy of traditional medicine[1]. However, scientific evaluation is needed to provide evidences of their safety and efficacy [2].

Avicennia alba Blume, belonging to the family Avicenniaceae, is known to be a type of mangrove tree, growing in the tidal forests at the mouth of rivers. This species is found far away from salt water, unlike other species *e.g. A. marina*[3].

It is used in Indian system of medicine for the treatment of several type of diseases such as aphrodisiac, scabies, anti-fertlity agent, rheumatism, paralysis, asthma and snake-bites, skin disease and ulcer[4]. Fruits are plastered on to boils and tumours, a poultice of unripe seed and leaves stop inflammation and bitter resin used as contraceptive by women[5]. The plant exhibit a wide spectrum of medicinal properties, such as anti-cancer, anti-inflammatory, anti-microbial, anti-diarrhoeal and analgesic[6,7,8].

The widely occurring mangroov, *Avicennia alba* yielded different secondary metabolites in different regions. The plant is rich source of steroids, triterpenes, saponins, flavonoids, alkaloids and tannins[9]. Recently three naphthoquinones and their analogues, named avicequinone-A, avicequinone-B, avicequinone-C and avicenol-A, avicenol-B, avicenol-C respectively, were isolated from stem bark of *Avicennia alba*[10]. In this study, one new flavonoid, 2-(3-(3-(hydroxymethyl) oxiran-2-yl)-4-(methoxymethyl) phenyl)-4H-chromen-4-one, was isolated from the leaves of *Avicennia alba.* This is the first report of a flavone scaffold with oxirane from *Avicennia alba.* Herein, we report the isolation and structural determination of this new compound.

Materials and Methods:

Plant Materials

The aerial parts of *Avicennia alba* were collected from Sundarban area, South 24 Parganas, West Bengal, India in the month of November, 2011 on the basis of Ethnomedicinal uses. The plants was identified & authenticated by the taxonomist from the botanical survey of India, Botanical Garden, Howrah, West Bengal. A voucher specimen (CNH/128/2011/TECHII/637) has been deposited in the herbarium of the Department of Pharma cognosy, School of Pharmaceutical Sciences, Siksha O Anusandhan University, Odisha, India.

Preparation of extract

The air-dried plant material of *Avicennia alba* were coarsely powdered and extracted exhaustively in a Soxhlet apparatus with petroleum ether (60-80°C) to remove fatty materials. The defatted residue was further extracted with methanol at ambient temperature for 48 hours. The methanolic extract was filtered and concentrated to a dark viscous mass (Yield 15.6 % w/w) under reduced pressure at 50-55°C. The methanolic extract was examined chemically to screen the presence of different phytoconstituents. The extract was stored in a refrigerator and a weighed amount of the extract was suspended in 5% aqueous Tween 80 solution and used for the present study.

Preliminary phytochemical screening

The methanolic extract was subjected to phytochemical screening tests for the detection of various phytoconstituents using conventional methods[11]

Animals

Albino (Wister) rats 180-200 g of either sex were used. The animals were kept in the standard polypropylene cages and provided with food and water *ad libitum.* The animals were acclimatized for a period of 14 days prior to performing the experiments.

Acute toxicity

The acute toxicity study was carried out for methanolic extract of *Avicennia alba* to evaluate any possible toxicity. Albino mice (n = 6) of either sex were treated with different doses (500, 1000 and 2000mg/kg, p.o.), while the control group received saline (10ml/kg). All the groups were observed for any gross effect for first 4h and then mortality was observed after 24h[12].

Analgesic activity:

Radiant heat method

First the 24 rats are taken and weighed properly. Then they are divided into 4 groups i.e. control, standard, test-I and test -II with 6 rats in each group. Their tails are exposed to the radiant heat apparatus and the basal reaction time of each rat is recorded before drug is administered to each group of animals. The 'control' group is administered with normal saline. The 'standard' group is administered with acetyl salicylic acid 150mg/kg body wt. The 'test' groups are administered with 100mg/kg body wt and 200mg/kg wt of methanolic extract of crude drug. The Reaction times are measured in30 minute intervals for 180 minutes.

The latency time for all groups was recorded at 0, 30, 60, 90, 120, 150 and 180 min[13].

Tail immersion test

Albino rats of either sex were divided into four groups each of six animals (120-150 g). They are marked in groups of three as head, body and colorless and divided into four groups, control, standard, test (methanolic extract 100mg/kg body wt and methanolic extract 200mg/kg body wt).

Water is heated in a beaker by a hot plate up to 55-58°C. Tails of all rats are immersed in the water and the time of the tail removing reflex is noted before the administration of the drug. This is the basal reaction time of the animal.

The animals in the 'control' group are administered normal saline, the 'standard' group animals are administered with standard analgesic drug, acetyl salicylic acid 150mg/kg body wt. Test animals are administered with methanolic extract of 100 mg/kg body wt. and 200 mg/kg body wt. respectively. Their reaction times are noted every 30 minute interval for 180 minutes[14].

The cut-off time, i.e. time of no response was put at 30s, while Tb was consider the reaction time for control group.

Anti-pyretic Activity

The albino rats of either sex (120-160 g) are divided into four groups each of six animals, namely control, standard, test-I (methanolic extract 100mg /kg body wt) and test-II (200mg/kg body wt). The body temperatures of the rats are measured before they are administered subcutaneously with 10ml/kg of 20% aqueous suspension of brewer's yeast. Acetyl salicylic acid (150mg/kg orally) was used as reference drug. Control group received only distilled water (10ml/kg). The rectal temperature of each group is taken by digital Telethermometer at 0, 30, 60, 90 and 120 min after treatment[15]

Results:

Preliminary phytochemical screening

Preliminary phytochemical analysis of methanolic extract of aerial part of *Avicennia alba* indicated the presence of carbohydrates, alkaloids, tannins, Glycosides, steroids and flavonoids.

Radiant heat method

In the radiant heat method, results presented in Table 1,showed that the methanolic extract of *Avicennia alba* at doses of 100mg/kg and 200mg/kg significantly ($p<0.05$ and $p<0.01$) increase pain threshold of rats in dose dependant manner. The methanolic extract at a dose of 100 and 200mg/kg showed significant increase in reaction time i.e. 9.74 s and 11.71 s, respectively after 90 min. when compared to control (6.25) in Table 1. The standard drug acetyl salicylic acid at dose level of 150mg/kg showed significant increase in reaction time i.e. 13.24 s after 90 min. when compared to control. However the analgesic effect of methanolic extract of *Avicennia alba* was less when compared to the standard drug, acetyl salicylic acid. The present investigations suggested that the methanolic extract showed a significant analgesic effect in mechanical induced pain models.

Tail immersion test

In the tail immersion test, the mean reaction time shown by the rats before treatment with methanolic extract of *Avicennia alba* at the dose level of 100 mg/kg body weight was 2.16 s while after 90 min interval of treatment it became 5.71s. Similarly, methanolic extract at the dose level of 200 mg/kg body weight was 2.24s while after 90 min interval of treatment it became 6.55s.which is significantly ($P< 0.01$) higher than result of treatment with extract at 100 mg/kg (Table 2). In the dose level 100 mg/kg body wt. there is a significant effect ($P< 0.05$) of the drug but it much lower than the therapeutic effect of standard drug ($P< 0.01$).

Antipyretic test

The results of the antipyretic effect of the control, standard drug (acetyl salicylic acid) and the methanolic extract of *Avicennia alba* at doses of 100mg/kg and 200mg/kg are depicted in Table 3. The asprin as well as methanolic extract of *Avicennia alba* at doses of 100mg/kg and 200mg/kg started showing significant antipyretic activity after 30 min of post dosing when compared with the control group. Antipyretic activity was observed up to 1 hr (60 min.) after aspirin and test extracts administration.

The methanolic extract of *Avicennia alba* (200mg/kg) is found to have very significant anti-pyretic effect ($P< 0.01$) almost comparable to standard drug.After 60 min,Acetyl salicylic acid (150 mg/kg, body wt) was observed to decrease the rectal temperature from 37.70°C to 37.50°C while 200mg/kg body wt. of methanolic extract of crude drug decreases the temperature from 38.20 °C to 37.84°C and 100mg/kg body wt. decrease the temperature from 38.17 to 38°C. Thus both dose levels seem to have similar anti-pyretic effect compared to standard.

Table-1: Effect of methanolic extract of *Avicennia albav* on radiant heat method in rats

Reaction time in sec								
Groups	**Dose (mg/kg)**	**0min**	**30min**	**60min**	**90min**	**120min**	**150min**	**180min**
Control (normal saline)	10 (ml/kg)	6.13± 1.31	6.61± 1.52	6.46± 1.26	6.25± 1.28	6.23± 1.52	6.63± 1.76	6.16± 1.44
Standard (Acetyl salicylic acid)	150	6.21± 2.22	9.05± 2.33*	11.24± 2.45**	13.24± 3.62**	11.95± 3.22*	9.62± 2.31	8.17± 3.82
Methanolic extract	100	6.34± 1.21	7.72± 1.42	8.85±* 1.33	9.74±** 0.93	7.93± 0.98	6.55.± 0.47	6.13± 1.13
Methanolic extract	200	6.94± 0.86	8.37± 0.98	10.46±* 0.68	11.71±** 0.16	8.12± 0.77	7.85± 0.64	6.88± 0.46

Values are mean ± SEM from 6 rats in each group.
*Statistically significant at p<0.05; **statistically significant at p<0.01.

Table-2: Effect of methanolic extract of *Avicennia alba* on tail immersion method in rats

Reaction time in sec								
Groups	**Dose (mg/kg)**	**0min**	**30min**	**60min**	**90min**	**120min**	**150min**	**180min**
Control (normal saline)	10 (ml/kg)	3.03± 0.41	3.12± 0.35	2.84± 0.27	3.53± 0.77	3.10± 0.39	2.87± 0.34	2.66± 0.23
Standard (Acetyl salicylic acid)	150	2.65± 0.53	5.86± 0.38	6.33± 0.14	8.47±** 0.73	7.96± 0.14	5.30± 0.38	4.31± 0.41
Methanolic extract	100	2.16± 0.45	2.94± 0.32	4.38± 0.43	5.71±0.59 **	4.07± 0.38	3.11± 0.42	2.24± 0.08
Methanolic extract	200	2.24± 0.79	4.68± 0.43	5.12± 0.14	6.55±** 0.62	5.63±* 0.56	3.94± 0.49	2.89± 0.27

Values are mean ± SEM from 6 rats in each group.
*Statistically significant at p<0.05; **statistically significant at p<0.01.

Table-3: Effect of methanolic extract of *Avicennia alba* on brewer's yeast induced method in rats

Groups	**Dose (mg/kg)**	**Rectal temp. (°C) before yeast injection**	**Rectal temperature °C after Yeast injection**				
			0 min	**30 min**	**60 min**	**90min**	**120min**
Control	10 (ml/kg)	38.37± 0.32	39.20± 0.11	39.55± 0.27	39.56± 0.08	39.60± 0.15	39.65± 0.48
Standard (Acetyl salicylic acid)	150	37.55± 0.07	38.90± 0.06 **	38.10± 0.17**	37.70± 0.36*	37.60± 0.41	36.50± 0.14
Methanolic extract	100	37.40± 0.29	38.35± 0.14	38.25± 0.06	38.17± 0.26	38.06± 0.23	38.00± 0.12
Methanolic extract	200	37.30± 0. 80	38.55± 0.18**	38.30± 0.15**	38.20± 0.17**	37.90± 0.05*	37.840± 0.12

Values are mean ± SEM from 6 rats in each group.
*Statistically significant at p<0.05; **statistically significant at p<0.01.

Discussion

The radiant heat and tail immersion are basically used to study the peripheral and central effects. So the results obtained may be supported by the ability of the methanolic extract of the plant to have peripheral and central pain inhibition mechanisms.

In skin both delta fibres and C fibers sensory neurons are present, which are associated to the thermal pain generation. Besides, there are many ion channels in the skin that respond to temperature. These ion channels are transmembrane proteins in the plasma membrane which let in both calcium ions and sodium ions. The generated action potential by the ions sends message through the nerve fibres to the spinal cord and finally to brain to understand the pain. The tested effective analgesic plants in the tail immersion assay may have the ability to modulate the action potential and signal transmission for pain relieving originated by heat[17].

The inhibition of prostaglandin may be responsible for antipyretic effect of methanolic extract of extract *Avicennia alba*. The inhibition of prostaglandin synthesis could be the possible mechanism of antipyretic action as that of acetyl salicylic acid and the inhibition of prostaglandin can be achieved by blocking the cyclo-oxygenase enzyme activity. Several chemical mediatorsmay cause pyrexia and the inhibition of these mediators are responsible for the antipyretic effect[18]. The oral administration of methanolic extract of extract of *Avicennia alba* significantly alleviated rectal temperature of yeast induced rats. The flavonoid present in methanolic extract of *Avicennia alba* may also be responsible for its antipyretic activity by inhibiting prostaglandin synthesis in hypothalamus[19]. Hence the presence of flavanoids in the methanol extract of *Avicennia alba* may be contributory to its antipyretic activity.

Conclusion:

In conclusion, the methanolic extract of *Avicennia alba* was proved a natural safe remedy for the treatment of pyrexia and analgesia. Our current findings demonstrated scientific rationale for the folk use of the plant as antipyretic and analgesic. Interestingly the methanolic extract of *Avicennia alba* exhibited both peripheral as well as central analgesic effect which might have been attributed to the presence of such active principles, due to which it has proven folk use in pain and fever.

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