Journal of Medicinal Plants Studies
www.PlantsJournal.com

ISSN 2320-3862
JMPS 2016; 4(3): 140-143

Received: 20-03-2016
Accepted: 22-04-2016

Anjan Adhikari
Department of Pharmacology, R. G. Kar Medical College, 1 Kshudiram Bose Sarani, Kolkata-700004, West Bengal, India

Moumita Ray
Department of Pharmacology, R. G. Kar Medical College, 1 Kshudiram Bose Sarani, Kolkata-700004, West Bengal, India

Tapas Kumar Sur
Department of Pharmacology, Institute of Post Graduate Medical Education & Research, 244, A.J.C. Bose Road, Kolkata-700020, West Bengal, India

Satyajyoti Kanjilal
Research & Development center, Healthcare Division, Emami Limited, 13, BT Road, Kolkata-700056, West Bengal, India

Ranendra Kumar Roy
Department of Pharmacology, College of Medicine & Sagore Dutta Hospital, 578, B.T. Road, Kamarhati, Kolkata-700058, West Bengal, India

Anup Kumar Das
Department of Pharmacology, R. G. Kar Medical College, 1 Kshudiram Bose Sarani, Kolkata-700004, West Bengal, India

Correspondence
Anjan Adhikari
Department of Pharmacology, R. G. Kar Medical College, 1 Kshudiram Bose Sarani, Kolkata-700004, West Bengal, India.

Analgesic actions of Sunderban mangrove, *Rhizophora mucronata* L. leaves

Anjan Adhikari, Moumita Ray, Tapas Kumar Sur, Satyajyoti Kanjilal, Ranendra Kumar Roy, Anup Kumar Das

Abstract
The widely used analgesic drugs are associated with several harmful effects, which initiate search for new drug development from medicinal plants with better therapeutic efficacy and minimum toxicity. *Rhizophora mucronata* is a very popular mangrove plant which has been used as ethno-medicine from ancient time. In the present study the ethanolic extract of *Rhizophora mucronata* L. leaves was found to be safe upto 2gm/kg body weight (orally) in mice in acute toxicity study. The extract in 200 mg/kg body weight dose (orally) showed significant inhibition in acetic acid induced writhing response in mice. In the hot plate method, it increased the latency period than control to some extent, but in tail immersion test, did not showed any significant result. Present study revealed that the ethanolic extract of *Rhizophora mucronata* L. (Sunderban mangrove) leaves is rich in tannin, flavonoids, polyphenols and possess potential peripheral analgesic and mild central analgesic action.

Keywords: Analgesic, Mangrove, Ethno-medicine, *Rhizophora mucronata*, Mice

Introduction
Pain is a common noxious phenomenon, causing one of the most common healthcare problems and leads to different complicated physical and psychological problems [1]. Pain management with several pain controlling drugs cause only symptomatic relief. Analgesics are such type of drugs; which can be classified generally into two types- centrally acting analgesics and peripheral analgesics. Presently the widely prescribed and most commonly used over the counter analgesic drugs include Non-steroidal anti-inflammatory drugs (NSAIDs), steroids and opiates, but they are associated with several adverse effects like for central analgesics respiratory depression, addiction, abuse potentiality and for NSAIDs gastric ulceration, gastrointestinal bleeding etc [2]. Therefore research is focused on natural products and phytotherapy may contribute better therapeutic performance than conventional drugs. *Papaver somniferum*, *Cannabis sativa*, *Capsicum* and *Salix* species are some plants which have a great contribution in relevant drug development for pain management [3].
Besides the commonly used medicinally important terrestrial plants, several mangrove plants also have ethnopharmacological relevance. Mangroves synthesize some novel natural compounds or secondary metabolites, which have significant pharmacological properties and used as ethnomedicine for treating number of ailments [4, 5]. *Rhizophora mucronata* is one of the medicinally important mangrove plant (family Rhizophoraceae), abundantly found in a number of South Asian countries including India and different parts of this plant are being used traditionally in the treatment of diabetes, diarrhoea, wounds, ulcers and liver disorders [6, 7, 8].
The present study was designed for preliminary phytochemical screening and to evaluate the anti-nociceptive activity of the ethanolic extract of *Rhizophora mucronata* L. leaves (Sunderban mangrove) in different *in-vivo* analgesic models.

Materials and Methods
Materials
Quercetin, Diclofenac sodium, Fehling's solution, Molisch's reagent, Mayer's reagent, Dragendorff's reagent, sulfuric acid (Merck), hydrochloric acid (Merck), picric acid, lead acetate, ethanol, chloroform (Merck), ferric chloride (Merck), acetic acid (Merck), all the other chemicals and reagents are of standard analytical grade.

Collection of Plant material and Identification
Rhizophora mucronata leaves were collected from Sunderban, West Bengal and were

authenticated from Botanical Survey of India, Howrah, West Bengal (CNH/55/2013/Tech. II/19 dated 02.12.2013) as *Rhizophora mucronata* L. (Family- Rhizophoraceae).

Extraction of leaves of Plant material

Fresh *Rhizophora mucronata* L. leaves were cleaned, shade-dried and crushed to powder by grinder. They were extracted with absolute ethanol in Soxhlet apparatus. Thereafter, the solvent was removed under reduced pressure to dry it (RME) and kept in desiccators for further experiments.

Phytochemical screening

Qualitative standard chemical methods were used to identify different phytoconstituents [9], like reducing sugar (Fehling's test), non-reducing sugar (Molisch's test), alkaloids (Wagner, Hager, Mayer, Dragendorff's test), glycosides (Killer Killani test), tannins and phenols (ferric chloride test), terpinoids (Salkowski test) present in the ethanolic extract of *R. mucronata* L. leaves. Further High performance thin layer chromatography (HPTLC) analysis of RME was done. The extract in 1mg/ml concentration was dissolved in methanol and filtered by Millipore sample filtration unit. It was applied on a precoated silica gel plates (Merck, 60F254, 20 x 20 cm) in band form and the plates were developed for 30mins in a solvent system (toluene:ethyl acetate:formic acid= 4.5:3:0.2) [10]. Quercetin (3,4,5-5,7,3',4'-tetrahydroxy flavonol) was used to compare as a bio-marker. Camag TLC Scanner 3 was used for scanning at absorbance 280 nm (D2 lamp) and operated by multi-level winCATS planar chromatography manager.

Animals

Swiss albino mice of both sexes weighing 20-25gm were used for these studies. The animals were kept in the departmental animal house, maintaining standard condition and fed with proper diet and water *ad libitum*. Ethical clearance for animal studies was obtained from Institutional Animal Ethics Committee (RKC/IAEC/13/17 dated May 10, 2013).

Acute toxicity study

Acute oral toxicity study of RME was performed according to Organization for Economic Co-operation and Development (OECD) guidelines for the testing of chemicals [11]. RME was administered orally in different doses i.e. 50mg/kg, 100mg/kg, 500mg/kg, 1000mg/kg and 2000mg/kg in mice following the OECD guideline no. 423- acute oral toxicity study. The animals were observed for any sign of toxicity, morbidity or mortality for initially 24hrs and followed by next 72hrs.

In-vivo analgesic study

Treatment groups

The mice were randomly divided into five groups of six mice per group (n=6). The group division for the analgesic studies with their respective treatments is given below:

Groups	Group Types	Treatment
Group I	**Control**	0.5ml distilled water/kg body weight (b.w.) orally
Group II	**Standard**	Diclofenac Sodium 10mg/kg body weight orally
Group III	**Test group 1**	RME 50mg/kg body weight orally
Group IV	**Test group 2**	RME 100mg/kg body weight orally
Group V	**Test group 3**	RME 200mg/kg body weight orally

Acetic acid induced writhing

Animals were fasted overnight with water *ad libitum* and then drug treatment was done. After 30mins of the treatment, 0.1ml of 0.6% glacial acetic acid was injected intraperitoneally (i.p) in each mice of the above five groups and the pain sensation, characterized by abdominal constrictions or writhes was counted for 10 minutes. The percentage inhibitions of abdominal writhing were calculated and compared with the untreated control group [12, 13].

Thermal nociception by Eddy's hot plate method

The all animals were individually kept on Eddy's hot plate having a constant temperature of 55±0.1 ºC. The initiation of response to the pain sensation due to the heat, like either paw licking or jumping for each mice were recorded. Mice showing response within 15secs were included in the study. The initial response time before starting the experiment (0hr) was recorded and then the treatment was done. Then again after 30mins, 60mins and 90mins of drug administration the response times were recorded [12, 13].

Tail immersion method

In this study the tail tips of the mice were dipped into hot water maintaining a constant temperature of 55±0.1 ºC and the time for withdrawing their tail from hot water were recorded. Mice showing response within 15secs were included in the study. The initial response time before starting the experiment (0hr) was recorded and then the treatment was done. Then 30mins, 60mins and 90mins after drug administration, the response times were again recorded [12, 13].

Result

Phytochemical screening

The phytochemical screening of the ethanolic extract of the leaves of *Rhizophora mucronata* L. revealed the presence of non-reducing sugars, flavonoids, polyphenols, glycosides, terpinoids and good amount of tannins in it [Table 1]. The HPTLC study revealed the presence of quercetin in the ethanolic extract of *R. mucronata* L. leaf. The concentration of quercetin was 1.65% µg per gm of leaf extract.

Table 1: The phytochemical screening of the ethanolic extract of *Rhizophora mucronata* L. leaves

Phytochemical parameters in RME	Test results
Reducing sugars	-
Non-reducing sugars	+
Alkaloids	-
Tannins	++
Glycosides	+
Flavonoids	+
Phenolics	+
Terpinoids	+

Positive +, Highly positive ++, Negative –

Acute toxicity study

The ethanolic extract of *Rhizophora mucronata* L. leaves (RME) was found to be safe up to 2gm/kg body weight dose orally in mice.

***In-vivo* analgesic study**

Acetic acid induced writhing

RME showed significant analgesic activity in the acetic acid induced writhing in mice. The study result showed that the extract significantly reduced the writhing response in mice in a dose dependant manner compared to the untreated control group [Figure 1]. The activity was comparable with the standard drug Diclofenac sodium in the dose 10 mg/kg b.w. orally. Diclofenac sodium reduced the writhing response 58.78% in mice compared to the control whereas the test

sample in 100mg/kg dose orally reduced the same by 30.08% and the most effective dose of RME in a dose of 200mg/kg orally reduced the same by 44.05% [Table 2].

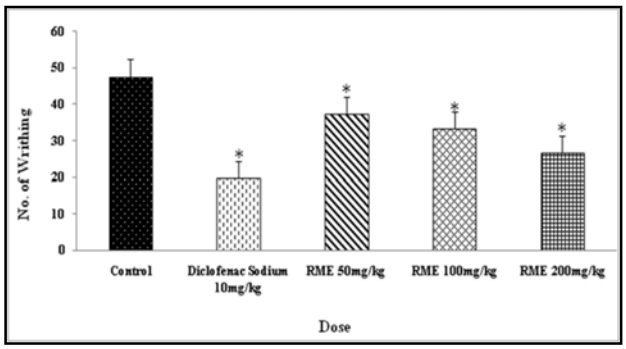

Fig 1: Distribution status of writhing response in acetic acid induced model in mice

Table 2: Inhibition of writhing responses (percentage) in the acetic acid induced writhing model in mice

Sl. No.	Groups (n=6)	No. of writhes	Percentage of inhibition
1.	Control	47.67 ± 2.33	----
2.	Diclofenac Sodium 10mg/kg	19.65 ± 3.21 *	58.78 *
3.	RME 50mg/kg	37.33 ± 1.45 *	21.69 *
4.	RME 100mg/kg	33.33 ± 1.20 *	30.08 *
5.	RME 200mg/kg	26.67 ± 4.63 *	44.05 *

Data are mean ± S.E.M (n=6).

The data were analyzed by one way ANOVA followed by Dunnett test, * denotes level of significance $p < 0.05$
Standard drug Diclofenac sodium (10mg/kg).
RME- *Rhizophora mucronata* L. leaf ethanolic extract

Thermal nociception by Eddy's hot plate method

In the thermal nociception model, RME at a dose of 200mg/kg body weight (orally) possesses analgesic activity to some extent but the other doses failed to show significant analgesic effect. Standard analgesic drug Diclofenac sodium 10mg/kg orally was used for comparison. RME in a dose of 200mg/kg orally reduced the pain response and increased the latency period in hot plate by 37.31% in mice after end of the study compared to the control whereas the standard drug Diclofenac sodium reduced the pain sensation by 49.01% [Table 3].

Table 3: Inhibition of pain responses (percentage) in the Eddy's hot plate method in mice

Sl. No.	Groups (n=6)	Percentage of inhibition (%)		
		30mins	60mins	90mins
1.	Control	----	----	----
2.	Diclofenac Sodium 10mg/kg	45.02749 *	47.91667 *	49.01 *
3.	RME 50mg/kg	2.48227	21.76871	13.75
4.	RME 100mg/kg	18.87906	18.38183	21.838
5.	RME 200mg/kg	0.900901	23.10264	37.31

Data are mean ± S.E.M (n=6).
The data were analyzed by one way ANOVA followed by Dunnett test, * denotes level of significance $p < 0.05$
Standard drug Diclofenac sodium (10mg/kg).
RME- *Rhizophora mucronata* L. leaf ethanolic extract

Tail immersion method

RME at a dose of 200mg/kg body weight (orally) slightly increased the reaction time of the treated mice compared to control. Standard analgesic drug Diclofenac sodium (10mg/kg orally) was used for comparison. The test sample did not revealed significant analgesic activity in this model [Figure 2].

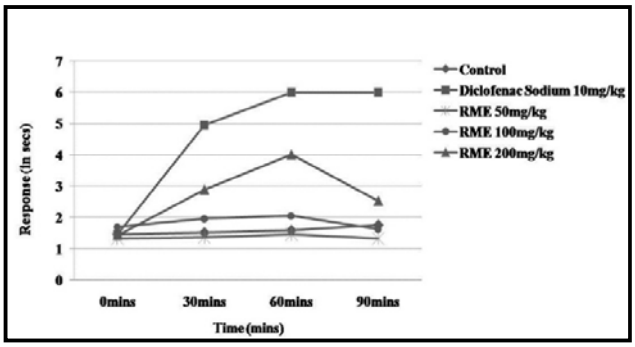

Fig 2: Distribution status of reaction response of mice (in secs) in tail immersion method

Data are mean ± S.E.M (n=6).
The data were analyzed by one way ANOVA followed by Dunnett test $p < 0.05$
Standard drug Diclofenac sodium (10mg/kg).
RME- *Rhizophora mucronata* L. leaf ethanolic extract

Discussion

A survey based study from the Bhitarkanika wildlife sanctuary, Orissa reported that *Rhizophora mucronata* Lamk is one of the widely distributed and beneficial mangrove plants which is rich in tannin and has been used to treat diseases like hepatitis, diabetes [14]. *R. mucronata* fresh leaves showed the presence of a good amount of polyphenols, like flavonoid, tannin, glycoside, phenolic compounds and the fresh juice of *R. mucronata* leaves showed significant anti-hyperglycemic effect in alloxan induced rats [15]. A study from Mangalore reported that, the ethanol extract of *R. mucronata* leaves showed the presence of saponins, tannins, flavonoids, phenols and volatile oils [16]. In the present study, the ethanolic extract of *Rhizophora mucronata* L. leaves (RME) from Sunderban mangrove also revealed the presence of non-reducing sugars, flavonoids, polyphenols, glycosides, terpinoids and good amount of tannins. Presence of Quercetin in the extract is also a significant finding.

The extract revealed significant analgesic activity in the acetic acid induced writhing response in mice in a dose dependant manner. Intraperitoneally injected acetic acid elicited the visceral pain sensation in abdomen triggering the localized inflammatory response which increases the pain mediators like prostaglandins specially PGE2, PGF2α and the lipoxygenase mediated eicosanoids present in the peritoneal fluid, and result in abdominal constriction or writhing in mice [17, 18]. This model is well-established for evaluating peripheral analgesic activity, compounds possessing analgesic activity act on visceral receptors, inhibit the pain mediators and as a result reduce the number of writhes. RME reduced the writhing response in a dose dependant manner; therefore it may be suggested that the leaf extract must have peripheral analgesic activity.

The Eddy's hot plate and mice tail immersion in hot water are the two validated methods for evaluating the efficacy of centrally acting analgesics, where non-inflammatory, central nociceptive reaction is induced by thermal stimuli [19, 20]. Hot-plate test is a widely used model for neurologic pain, any agent acting centrally may result in prolongation of the hot plate latency in this model [21, 22]. In the present study, the RME

extract slightly increased the reaction time in the hot plate model [Table 3]. In the tail immersion method also the test sample only in the 200mg/kg body weight dose (orally) exhibited very less response. Therefore, the ethanolic extracts of the *Rhizophora mucronata* L. leaves have central analgesic activity to a lesser extent.

There are very few studies till now revealing the analgesic effect of *Rhizophora mucronata* L. (Sunderban mangrove) leaves. A study from Dhaka, Bangladesh reported that the ethanolic extract of the *Rhizophora mucronata* Lam. bark from the Sunderban of Bangladesh region in doses of 250mg/kg & 500mg/kg body weight showed marked analgesic activity [23].

It is well known that the opioid or narcotic analgesics inhibit both the peripheral and central action of pain, whereas the peripheral pain is inhibited only by the non-steroidal anti-inflammatory drugs (NSAIDs) [24]. In the present study, the ethanolic extract of *Rhizophora mucronata* L. leaves potentially inhibited the acetic acid induced writhing response in mice in a dose dependant manner but did not reveal significant efficacy in the other two analgesic models. Therefore the extract exhibited similar properties like NSAIDs.

Conclusion

It can be stated that the ethanolic extract of *Rhizophora mucronata* L. (Sunderban mangrove) leaves possess potential peripheral analgesic action, may be mediated through peripheral mechanism. It may be due to the phyto-constituents like flavonoids, polyphenols, tannins etc. present in it. Further study is in research stage to confirm its analgesic effect and to validate its medicinal use.

Acknowledgement

The authors would like to express gratitude to the Principal, R. G. Kar Medical College, Kolkata for providing support in every aspects for conducting the research. Authors also acknowledge support from West Bengal University of Health Sciences, Kolkata in this research.

References

1. Zareba G. Phytotherapy for pain relief. Drugs Today (Barc). 2009; 45(6):445-67.
2. Barua CC, Buragohain B, Roy JD, Talukdar A, Barua AG, Borah P. Evaluation of analgesic activity of hydroethanol extract of Plantago erosa Ex Roxb. Pharmacologyonline. 2011; 2:86-95.
3. Calixto JB, Scheidt C, Otuki M, Santos AR. Biological activity of plant extracts: novel analgesic drugs. Expert Opin Emerg Drugs. 2001; 6(2):261-79.
4. Salini G. Pharmacological Profile of Mangrove Endophytes - A Review. Int J Pharm Pharm Sci. 2015; 7(1):6-15.
5. Bandaranayake WM. Bioactivities, bioactive compounds and chemical constituents of mangrove plants. Wetlands Ecol Mangt. 2002; 10:421-452.
6. Hoppe-Speer SCL, Adams JB, Rajkaran A, Bailey D. The response of the red mangrove Rhizophora mucronata Lam. to salinity and inundation in South Africa. Aquatic Botany. 2011; 95(2):71-76.
7. Manilal A. An *in vitro* antagonistic efficacy validation of Rhizophora mucronata. Asian Pacific Journal of Tropical Disease. 2015; 5(1):28-32.
8. Arumugam S, Palanisamy D, Sambandam RT. Identification of Bioactive Compounds of Rhizophora Mucronata Poir. Leaves Using Supercritical Fluid Extraction and GC-MS. World Journal of Pharmacy and Pharmaceutical Sciences. 2014; 3(10):1621-1631.
9. Kokate CK. Practical Pharmacognosy. 4th ed. Vallabh Prakashan, New Delhi. 2008; 107-111.
10. Rohini RM, Das AK. Determination of Lupiol, beta-sitosterol and quercetin from ethyl acetate extract of Rhizophora mucronata bark by hptlc technique. Asian J Pharmaceut Clin Res. 2011; 4:103-105.
11. OECD guidelines 423. Guideline for the testing of chemicals. Acute oral toxicity in animals. Adopted 21st september, 1998. Available from: http://www.oecd.org.
12. Vogel HG. Analgesic, anti-inflammatory, and anti-pyretic activity. Drug Discovery and Evaluation Pharmacological Assays. 2nd ed. Springer. Chapter H. 2002, 670.
13. Hossain H, Ismet JA, Howlader SI, Dey SK, Hira A, Ahmed A. Phytochemical Screening and Anti-nociceptive Properties of the Ethanolic Leaf Extract of *Trema Cannabina* Lour. Advanced Pharmaceutical Bulletin. 2013; 3(1):103-108.
14. Pattanaik C, Reddy CS, Dhal NK, Das R. Utilisation of mangrove forests in Bhitarkanika wildlife sanctuary, Orissa. Indian Journal of Traditional Knowledge. 2008; 7(4):598-603.
15. Ray M, Adhikari A, Sur TK, Mondal C, Pathak A, Das AK. Pharmacognostic and Anti-Hyperglycemic Evaluation of the Leaves of Sunderban Mangrove, Rhizophora Mucronata L. Pharmanest. 2014; 5(5):2289-2294.
16. Sahoo G, Mulla NSS, Ansari ZA, Mohandass C. Antibacterial Activity of Mangrove Leaf Extracts against Human Pathogens. Indian J Pharm Sci. 2012; 74(4):348-351.
17. Derardt R, Jongney S, Delvalcee F, Falhout M. Release of prostaglandins E and F in an algogenic reaction and its inhibition. Eur J Pharmacol. 1980; 51:17-24.
18. Dhara AK, Suba V, Sen T, Pal S, Chaudhuri AK. Preliminary studies on the anti-inflammatory and analgesic activity of methanolic fraction of the root of Tragia involucrate. J Ethnopharmacol. 2000; 72:265-268.
19. Sewell RDE, Spencer PSJ. Anti-nociceptive activity of narcotic agonist and partial agonist analgesics and other agents in the tail-immersion test in mice and rats. Neuropharmacol. 1976; 15(11):683-8.
20. D'Amour FE, Smith DL. A method for determining loss of pain sensation. J Pharmacol Exper Therap. 1941; 72:74-9.
21. Sabina EP, Chandel S, Rasool MK. Evaluation of analgesic, antipyretic and ulcerogenic effect of Withaferin A. International Journal of Integrative Biology. 2009; 6(2):52-56.
22. Ibironke GF, Ajiboye KI. Studies on the anti-inflammatory and analgesic properties of Chenopodium ambrosioides leaf extract in rats. International Journal of Pharmacology. 2007; 3:111-115.
23. Howlader SI, Ahmed J, Kabir ANMH, Uddin G, Hossain K. Antibacterial, cytotoxic, analgesic and diuretic activities of Rhizophora mucronata Lam. bark. Indian Journal of Natural Products and Resources. 2013; 4(3):229-232.
24. Pal S, Sen T, Chaudhuri AK. Neuropsychopharmacological profile of the methanolic fraction of Bryophyllum pinnatum leaf extract. Journal of Pharmacy and Pharmacology. 1999; 51:313-318.