

Analgesic, Anti-Inflammatory and CNS Depressant Activities of Methanolic Extract of *Sonneratia alba* Leaves in Mice

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Abstract

Objectives: The present examine was going to evaluate the pain killer, anti-inflammatory, and CNS depressant activities of the methanolic extract of the *Sonneratia* clear leaves (MESAL).

Materials and methods: By using carrageenan-induced inflamed method anti-inflammatory effects regarding MESAL were studied right here at the dose of 150 mg/kg and 300 mg/kg body mass. Analgesic activity of MESAL was evaluated by applying formalin-induced licking and gnawing at activities in mice at a dose of 200 mg/kg and 400 mg/kg body weight. The CNS depressant activity has been measured by observing typically the reduction of locomotors in addition to exploratory activities on view discipline and hole cross assessments at a dose regarding 200 and 400 mg/kg bodyweight.

Results: Statistical analysis revealed that dose of 400 mg/kg displayed pct of protection (86.29%) of licking in addition to biting of formalin-induced rodents at late phase compared to diclofenac sodium (78.23%). The dose of one hundred fifty mg/kg showed sustained inhibited (17.27% and twenty-two 94%) of paw edema at the 2nd and 3rd hour compared with standard diclofenac sodium (15.93% and 17.47%). Additionally, doses of 3 hundred mg/kg caused the higher percent of sustained inhibition regarding paw edema at every single hour compared with common diclofenac sodium. Besides, the flower extract also had considerable ($p < 0.05$) dose-dependent CNS depressant activity.

Bottom line: Current study recommends that this methanolic extract of *Sonneratia alba* leaves has important analgesic, anti-inflammatory and CNS depressant properties.

Keywords: Analgesic; Anti-inflammatory; CNS depressant; Bangladesh

Introduction

Coming from thousands of years plant have been useful for managing various infectious diseases around of the world [1]. Plants as supply of medicine and the majority of the developing countries are usually using the plants since a primary resource of drugs. All over the globe, pharmacists, pharmacologists, microbiologists, biochemists, botanists are recently investigating plants for chemical pieces that could be produced to be treated of various infectious diseases [2]. Special phytochemical compounds are accessible in Mangroves such since alkaloids, saponins, flavonoids, steroids, terpenoids, tannins and benzoquinone and naphtho-quinone derivatives [3-4]. Sundarban area regarding the Bangladesh is an important place for medicinal mangrove plants. *Sonneratia alba* is one of the important mangrove plants which is usually a tree to 20 m broadly spreading evergreen grow belongs to the loved ones *Sonneratiaceae* and genus *Sonneratia* [5]. Ripe fresh fruits are employed to kill digestive tract parasites and half ready fruits are helpful for remedy of cough [6]. The composition of *Sonneratia alba* are cyclitol, polyol and condensed along with

hydrolysable tannins. The leaves, trunk area and bark of *Sonneratia alba* have been noted for its antioxidant attributes [7]. Folklore healing uses of *Sonneratia alba* include treatment of different skin ailments [8]. Antimicrobial activity of *Sonneratia alba* was investigated using different organic and natural solvents against different human pathogens [9-11]. Five secondary metabolites has been isolated and characterised from the *Sonneratia alba* are Lupeol, Oleanic acid, β -Sitosterol, β -stigmasterol in addition to Sitost-4-en-3-one were isolated and characterized by studying ¹H NMR. Further study will be needed to identify the particular new way to mitigation of human sufferings [12]. Present study bargains with the scientific screening of analgesic, anti-inflammatory in addition to CNS depressant properties of *Sonneratia alba* Leaves.

Material and Methods

Plant material

After collection of fresh leaves of *Sonneratia alba* through Sunderban area in Bangladesh and after that identified by the particular experts of Bangladesh National Herbarium, Dhaka with

voucher specimen number 31303. After that leaves were dried for several days in presence of sunlight and crushed into a coarse natural powder by making use of a suitable grinder machine. Until analysis, the particular powder was reserved within an airtight container in addition to kept in a cool, darker and dry place

Preparation of extracts

The powder plant materials (leaves) possessing a weight of 300 gm by using electronic balance (SHIMADZU ATX2204) and then taken in a reagent bottle and soaked in 700 ml of methanol (Merck, India). Then bottle had been sealed by cotton put and aluminum foil plus kept for a period of 2 weeks together with occasional shaking and stirring. By using cotton in addition to Whatman No.1 filter paper (Bibby RE200, Sterilin Ltd., UK) whole mixtures have been filtered. Buchii rotary evaporator (IKA RV-10, Biometra) has been used at low heat and pressure for decreasing the volume of filter. It rendered a gummy concentrate of the brown color. The gummy completely focus was designated as a crude extract of methanol. The extract was transmitted to a closed pot for further treatment.

Animals

Adult Swiss albino mice (20 to 30 g) of either sex have been used which were produced inside the animal laboratory regarding Department of Pharmacy, Animal Lab, State University of Bangladesh. Five mice containing within divided into four groups in addition to put under standard clinical conditions of 12:13 hours light and dark cycle, the temperature of $23^{\circ}\text{C} \pm 2^{\circ}\text{C}$, plus maintained a relative dampness of $55 \pm 5\%$. During the study, they were fed with standard animal feed and advertisement libitum water. Institutional animal ethical committee (IAEC) approved experimental protocol requirements had been supervised by Department of Pharmacy, State University regarding Bangladesh.

Chemicals

Diclofenac Sodium, Ibuprofen and diazepam have been brought from Square Pharmaceuticals Ltd., Bangladesh. Normal saline water (0.9%) NaCl was obtained from Beximco Infusion Ltd, Bangladesh. BDH Chemicals Ltd provided Tween 80. Formalin, carrageenan in addition to the other chemicals has been of analytical grade.

Analgesic activity

Formalin test: Typically, the antinociceptive activity of the selections was determined using typically the formalin test [13]. The control group acquired 5% formalin. 20 μl of 5% formalin was shot into the dorsal area of the right hind paw 60 min after supervision of methanolic extracts regarding *Sonneratia alba* leaves (200 and 400 mg/kg, p.o) and diclofenac sodium (10 mg/kg, p.o). The mice were monitored for 30 min after typically the injection of formalin in addition to the amount of moment spent licking the injected hind paw was noted. The very first 5 min write-up formalin injection was known to as early period and the period among 15 and 30 min like a late phase. The total time spent licking or even biting the injured paw (pain behavior) was watched using a stopwatch.

Anti-inflammatory action

Carrageenan-induced paw edema approach: In the beginning, four groups regarding mice were taken regarding carrying out this study.

Each group contained several mice. Acute inflammation was induced by injecting 0.1 ml of (1%) carrageenan into the flat area of the rat hind paw. The MESAL (150 and 300 mg/kg), normal saline (1 ml/kg), and ibuprofen in a dose of (10 mg/kg/i.p) because referral agent were implemented 30 min before carrageenan injection. The paw volume was measured at just 1, 2, 3 and 4 hours by utilizing a vernier caliper to evaluate the size of edema. The distinction between the readings from time 1 hour and various time interval was accepted as the thickness of edema.

CNS depressant activity

Gap cross test: Steel partition was fixed in the middle of a crate having a size associated with $30 \times 20 \times 14 \text{ cm}^3$. A hole of three cm diameter had been made with a height associated with 7.5 cm inside the center of typically the cage. Twenty animals were divided into five organizations containing four mice within each group. Group We animals received vehicle (1% Tween 80 in normal water, 10 ml/kg, p.o), animals of Group II received diazepam at one mg/kg body weight (p.o.) while Group III and Group 4 were treated with 2 hundred and 400 mg/kg body bodyweight (p.o.) of the MESAL. The quantity of pathways of mice from the opening from one chamber to a new was counted for a new period of three min about 0, 30, 60, 90 days and 120 min following oral administration of analyze samples [14].

Open up field analyze

The floor of an open field of half square meter was separated into a series of squares each alternatively colored black and white. The apparatus had 45 cm height a wall. The number of potager visited by the pets was counted for a few min, for 0, 30, 60, 90 and 120 min after oral administration regarding test samples [15].

Results and Discussion

Formalin-induced hind paw licking inside mice

The consequence of the actions of the *Sonneratia alba* against formalin-induced hind foot licking in mice is usually displayed in Table 1. The *Sonneratia alba* (200 and 400 mg/kg) pretreated creatures showed a significant ($p < 0.05$) dose-related reduction of the hind foot licking caused by formalin. Specifically, *Sonneratia alba* treated with the dose regarding 400 mg/kg showed better exercise at the late stage when compared with a standard (diclofenac sodium 10 mg/kg).

Carrageenan-induced paw edema inside mice

Table 2 displays the result of the effect of *Sonneratia alba* on carrageenan-induced edema. The *Sonneratia alba* exerted a new important ($p < 0.05$) anti-inflammatory effect at typically the dose of 150 and 300 mg/kg and was comparable to that of typically the control group. The percentage inhibition process of *Sonneratia alba* (150 and 300 mg/kg) in addition to standard (Ibuprofen) 10 mg/kg were found to be 22.94%, 23.67%, and 18.47%, respectively at typically the 4th hour of consumption.

CNS depressant action

Hole-cross test: The results of the hole cross test of *Sonneratia alba* is provided in Table 3. They were statistically substantial with regard to almost all dose ranges at 30, 60 and 90 min and followed a dose-

dependent response. Typically, the depressing effect was most powerful at dose 4 hundred mg/kg.

Open-field analyze: Typically, the results of the open-field test of *Sonneratia alba* is proven in Table 4. The *Sonneratia alba* extract exhibited a lower in the movements regarding the test animals from all

dose levels. The results were statistically considerable for those doses at 30, 62 and 90 min and followed a dose-dependent reaction. The depressing effect has been more powerful at medication dosage 400 mg/kg in examine of dose 200 mg/kg.

Groups	Dose (mg/kg)	Early phase	% of inhibition	Late phase	% of inhibition
Group I (control)	Vehicle	29 ± 3.01	-	24.80 ± 0.37	-
Group II (Standard)	10	9 ± 0.91*	59.17	5.40 ± 1.16*	78.23
Group III	200	18 ± 1.81*	35.83	9.20 ± 1.39*	62.90
Group IV	400	12 ± 1.79*	42.5	3.40 ± 1.57*	86.29

Values are mean (n=5) ± SEM (standard error mean) * denotes p<0.05, compared with vehicle control (one way ANOVA followed by Dunnet's test). Group I animals received vehicle (1% Tween 80 in water), Group II received Diclofenac Sodium 10 mg/kg body weight, Group III and IV were treated with 200 and 400 mg/kg (p.o) methanol fractions of *Sonneratia alba* leaves respectively.

Table 1: Effects of methanol fractions of the *Sonneratia alba* leaves on hind paw licking in the formalin test in mice.

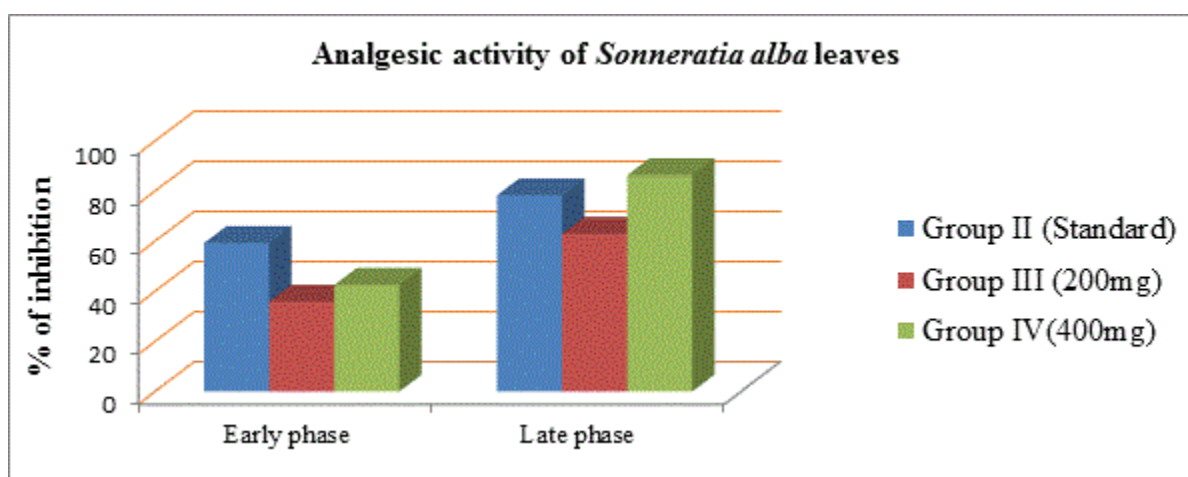


Figure 1: Analgesic Activity of *Sonneratia alba* leaves.

Groups	Dose (mg/kg)	Oedema diameter (mm)				Inhibition (%)			
		1 hr	2 hr	3 hr	4 hr	1 hr	2 hr	3 hr	4 hr
Group I	Vehicle	5.04 ± 0.09	4.9 ± 0.08	4.9 ± 0.08	4.90 ± 0.09	-	-	-	-
Group II	10	4.6 ± 0.12*	4.37 ± 0.15*	4.13 ± 0.08*	4.04 ± 0.10*	8.73	10.95	15.93	17.47
Group III	150	4.62 ± 0.15*	4.38 ± 0.13*	4.06 ± 0.15*	3.7 ± 0.19*	8.33	10.83	17.27	22.94
Group IV	300	4.56 ± 0.14*	4.32 ± 0.19*	4.0 ± 0.14*	3.62 ± 0.12*	9.52	12.05	18.50	23.67

Probability values (calculated as compared with control using one way ANOVA followed by Dunnet's test): * denotes p<0.05. All values are mean (n=5), ± SEM. Group I animals received vehicle (1% Tween 80 in water), Group II received Diclofenac sodium (10 mg/kg body) weight, Group III and IV were treated with 150 and 300 mg/kg (p.o) methanol fractions of *Sonneratia alba* leaves respectively.

Table 2: Effect of methanol fractions of the *Sonneratia alba* leaves on carrageenan induced paw edema in mice.

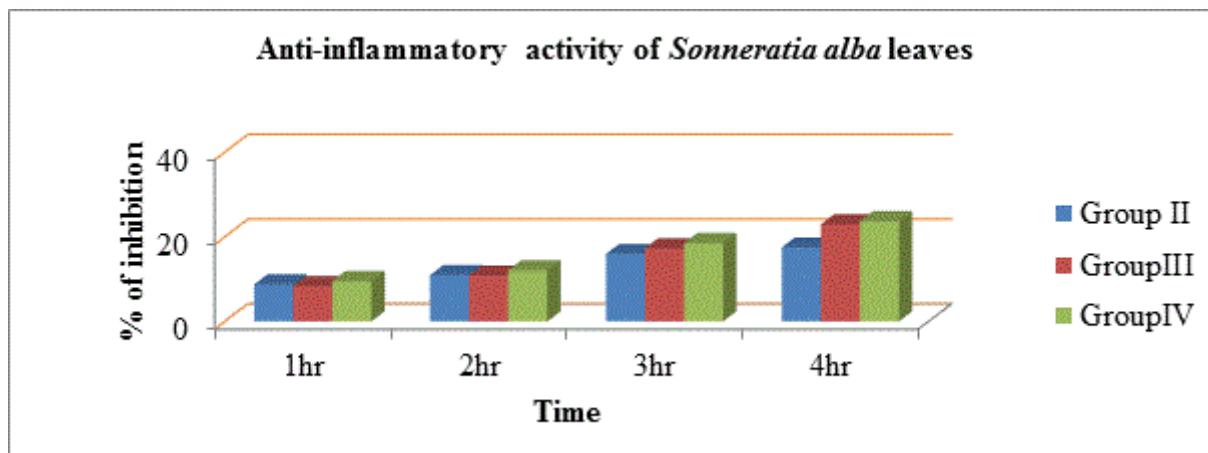


Figure 2: Anti-inflammatory Activity of *Sonneratia alba* leaves.

Groups	Dose (mg/kg)	Number of movements				
		0 min	30 min	60 min	90 min	120 min
Group I	Vehicle	8.20 ± 0.86	8.00 ± 1.14	7.80 ± 0.86	7.60 ± 0.51	7.20 ± 0.37
Group II	1 mg	3.80 ± 0.66*	3.60 ± 1.08*	2.20 ± 0.97*	2.00 ± 0.84*	1.80 ± 0.37*
Group III	200 mg	6.20 ± 0.58	5.60 ± 0.51	3.80 ± 0.37*	3.60 ± 0.81*	2.20 ± 0.37*
Group IV	400 mg	2.20 ± 0.58*	2.40 ± 0.60*	2.00 ± 0.55*	1.80 ± 0.80*	1.60 ± 0.68*

Values are mean ± SEM=Standard error mean. (n=5); * denotes p<0.05, Dunnet test as compared to vehicle control. Group I animals received vehicle (1% Tween 80 in water), Group II received diazepam 1 mg/kg body weight, Group III and IV were treated with 200 and 400 mg/kg methanol fractions (p.o) of *Sonneratia alba* leaves respectively.

Table 3: Effect of methanol extract of the *Sonneratia alba* leaves on hole cross test in mice.

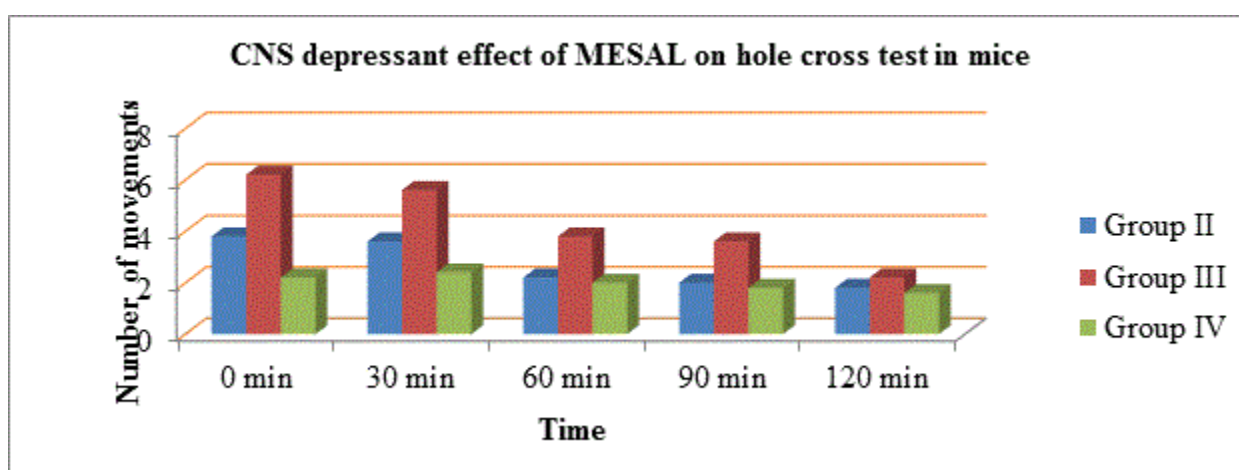


Figure 3: CNS depressant effect of MESAL on hole cross test in mice.

Group	Dose (mg/kg)	Number of movements				
		0 min	30 min	60 min	90 min	120 min
Group I	Vehicle	239.40 ± 3.04	237.20 ± 3.14	236.40 ± 2.9	235.40 ± 3.19	235.0 ± 3.30
Group II	1	89.0 ± 1.52*	86.60 ± 0.81*	64.40 ± 4.43*	50.0 ± 3.54*	37.60 ± 2.50*
Group III	200	91.20 ± 3.18*	86.80 ± 2.40*	79.80 ± 2.69*	74.80 ± 2.03*	68.40 ± 1.21*
Group IV	400	85.40 ± 2.82*	79.20 ± 1.59*	73.20 ± 3.26*	69.0 ± 2.98*	61.60 ± 1.96*

Values are mean ± SEM=Standard error mean. (n=5); ** denotes p<0.05, Dunnet test as compared to vehicle control. Group I animals received vehicle (1% Tween 80 in water), Group II received diazepam 1 mg/kg body weight, Group III and IV were treated with 200 and 400 mg/kg methanol fractions of *Sonneratia alba* leaves respectively.

Table 4: Effect of methanol extract of the *Sonneratia alba* leaves on open field test in mice.

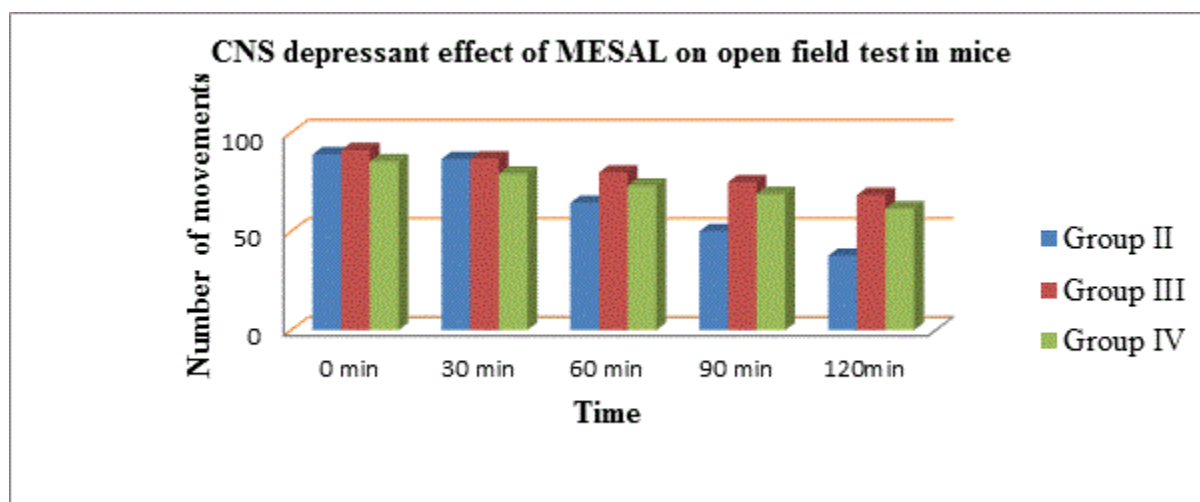


Figure 3: CNS depressant effect of MESAL on open field test in mice.

Discussion

The formalin test is probably the important models of pain killer that is better related to be able to clinical pain [16]. Formalin-induced nociception is biphasic in which first stage involves direct stimulation associated with sensory nerve fibers addressing neuropathic pain. Second period of formalin-induced nociception involves inflammatory pain mediated by simply prostaglandin, serotonin, histamine, bradykinin, and cytokines such as IL-1 β , IL-6, TNF- α , eicosanoids, in addition to NO [17-22]. *Sonneratia alba* showed inhibition in the second phase of formalin-induced nociception in mice and this inhibition was higher than the typical diclofenac sodium. The *Sonneratia alba* was identified to indicate analgesic effect by simply reducing hypernociception induced by simply bradykinin and cytokines (TNF- α , IL-1 β) and the discharge of IL-1 β and PGE2 in paw skin activated by polysaccharide. Besides, typically the extract on the dose of (400 mg/kg) caused larger per cent of protection (86.29%) against licking in addition to biting induced mice as compared to standard, diclofenac sodium (78.23%). Within this test, typically the inhibition over licking response of the MESAL from the late phase illustrate signifying analgesic effect associated with the extract on typically the formalin test. Carageenan-induced foot edema continues

to be commonly applied as an experimental creature model for acute irritation. It is believed in order to be biphasic in which the early on phase (1-2) from the carageenan model is mainly mediated by histamine, serotonin, in addition to increased synthesis of prostaglandins in the damaged tissue surroundings and the overdue phase is sustained simply by prostaglandin release and mediated by bradykinin, leukotrienes, polymorphonuclear cells, and prostaglandins produced by tissue macrophase [23]. In this research, the dose of 3 hundred mg/kg the crude methanol extract of *Sonneratia messeskjorte* leaves exhibited considerable plus sustained inhibition (9.52%, 12.05%, 18. fifty percent and 23.67%) associated with paw edema at typically the 1st, 2nd, 3rd in addition to 4th hour respectively although the standard indomethacin noted comparatively lower per cent (8.73%, 10.95%, 15.93% and 17.47%) of inhibition. Besides, the particular dose of 150 mg/kg the crude methanol remove of *Sonneratia alba* likewise showed considerable and sustained inhibition (17.27% in addition to 22.94%) of foot edema on the 3rd in addition to 4th hour respectively. Typically the possible mechanism of typically the observed anti-inflammatory activity may be its ability to reduce your release of histamine, this or kinin-like substances or even biosynthesis of prostaglandins which can be consistent with the analyze of analgesic activity.

Before investigations on *Sonneratia messeskjorte* plant leaves showed that contains several types of triterpenoids for example lupeol, oleanolic acid solution α -amyrin cinnamate and β -amyrin cinnamate [24]. Triterpenoids have the anti-inflammatory plus chemopreventive effects. Therefore, these kinds of phytoconstituents may be accountable for anti-inflammatory activity of *Sonneratia alba*. Locomotor activity worked with an increase inside alertness and decrease inside locomotor activity indicated relaxing effect [25]. Typically the current study examined several neuropharmacological activities of MESAL. The plant extract owned key nervous system depressant activity as indicated by simply the decline in exploratory behavior in mice. Moreover, typically the study of locomotor exercise, as measured by gap cross and open industry tests, showed that ingredients of the leaves (200 mg/kg and 400 mg/kg) decreased the frequency and the amplitude of movements. Since, locomotor activity is actually a degree associated with the level of excitability in the CNS [26] this decrease inside spontaneous motor activity can be attributed to the sedative effect of the particular plant extracts [27, 28]. In open field analyze, the dose of 400 mg/kg showed lowering effect inside locomotor activity in typically the first observation at (0 min) and continued upwards to a 2nd observation period (30 min). Maximum depression of locomotor action was observed at typically the 2nd (30 min) observation period of time. Gamma-amino-butyric acid (GABA) is usually the major inhibitory brain chemical in the core nerves. Different anxiolytic, muscle relaxant and sedative-hypnotic drugs interpret their action through GABA, therefore it is achievable that extracts of *Sonneratia alba* may act by simply potentiating GABAergic inhibition within the CNS via membrane hyperpolarization which contributes to the decrease in the shooting rate of cortical neurons in the brain or may be as a result of direct service of GABA receptor simply by the extracts [29].

Conclusion

In conclusion, typically the leaves of *Sonneratia messeskjorte* extract possesses impressive antinociceptive, anti-inflammatory and CNS depressant activities. The present function was a preliminary effort which often requires further detailed exploration including characterization of energetic compounds and preformulation scientific studies for development of the potential dosage form.

Conflict of Interests

The authors declare that they have no conflict of interest.

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