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Original Research Article

Evaluation of CNS depressant, antidiarrheal and antipyretic activities of ethanolic leaf extract of *Calophyllum inophyllum* L. on swiss albino mice.

Abstract

Objective

The main objective of the present the study was to evaluate the CNS depressant, antidiarrheal and antipyretic activities of ethanolic leaf extract of *Calophyllum inophyllum* L.

Method

In the present study CNS depressant activity of *C. inophyllum* was carried out using hole-cross, hole-board and open-field models in swiss albino mice whereas castor-oil induced diarrhea was used to evaluate antidiarrheal activity. The antipyretic activity was conducted by Brewer's yeast-induced hyperthermia in mice.

Result

The experimental result indicates that the ethanolic extract of *C. inophyllum* reduced CNS depression significantly (P = 0.05) in tested animals and the observed effect was dose dependent as compared to standard drug diazepam. The plant extract at the dose of 250 and 500 mg/kg body weight showed significant inhibition 41.66% and 62.19% in castor oil induced diarrheal method. In yeast induced pyrexia, the plant extract displayed dose dependent inhibition as compared to paracetamol.

Conclusion

The investigated study concludes that *C. inophyllum* leaf has significant CNS depressant, antidiarrheal and antipyretic activities which could support to develop it's medicinal applications.

Keywords: CNS depressant; antidiarrheal; antipyretic; Calophyllum inophyllum.

Introduction

Calophyllum inophyllum (clusiaceae), commonly known as "Indian laurel" or Alexandrian laurel is a large evergreen tree with a broad spreading crown of irregular branches and thick trunk covered with a rough, black and cracked bark [1,2,3]. The tree is usually 2 to 3m in height with a dense canopy of glossy, shiny, tough, elliptical leaves that are rounded at the bases [4, 5].

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It has white flowers arranged in axillary cymes with a sweat lime-like fragrance [6]. Fruits are spherical drupe arranged in cluster having a single large seed. Once ripe, their smooth, yellow epidermis discloses a thin layer of pulp, which tastes somewhat of apple. The grey, ligneous and rather soft nut contains a pale yellow kernel, which is odorless when fresh. Once chewed, it coats the mouth and emulsifies saliva, and its insipid taste becomes bitter. It is widespread tree that is indigenous to tropical Asia and geographically distributed in Melanesia and Polynesia [3]. It grows widely in the coral sands and on the sea shore. The species was brought north to Hawai'i from the South Pacific islands in early migrations of Polynesian settlers. Several parts of *Calophyllum inophyllum* is traditionally used in folk medicine for treating various health problems. In Java, the tree is said to have diuretic properties [7, 8]. In China, this plant is used in the treatment of eye diseases, wounds, rheumatisms, and inflammations in traditional Chinese folk medicine [9, 10]. This plant is used in Asia and India for orchitis and in the treatment for gonorrhea. The fresh leaves infusion of *Calophyllum inophyllum* are frequently used for treating bacterial infection, fungal infection and dried leaf for different kinds of skin problems, sores, cuts, and wounds [11,12,13,14]. In Cambodia, the leaves are inhaled as a treatment for migraines and vertigo. The bark is used as an antiseptic and disinfectant. In Cameroon the aqueous extracts of the root bark and leaves are used as a cicatrisant and are also used in the treatment of wounds and herpes [15]. The root bark and nut of *Calophyllum inophyllum* possessed the most significant cytoxicity against the KB cell line and antimicrobial activities [15]. Seed oil from *Calophyllum inophyllum* has been used in folk medicine in the treatment of aching joints and rheumatism [16]. *Calophyllum inophyllum* possessed strong activity against human immunodeficiency virus type 1 [17, 18]. Some of these species are frequently employed in folk medicine to treat several injuries [2]. This plant has very antihyperlipidemic and antioxidant activity [1].

Preliminary phytochemical analysis of *Calophyllum inophyllum* resulted a wide variety of chemical constituents including Coumarinic derivatives: calophyllolide [20] , inophyllolids including inphyllum B ,P , C and E [15, 20, 21] , calophyllic acid containing calophynic acid [22] , brasiliensic acid and inophylloidic acid [23] . Xanthone derivative such as inoxanthone [15], caloxanthone A and B, macluraxanthine, 1,5-dihydroxyxanthone [24], caloxanthone D ,caloxanthone E , caloxanthone N [9], gerontoxanthone C [25] and 2-hydroxyxanthone [26] ,tannins ,alkaloids [27] , chalcones, benzofurans and triterpenes [4, 5, 28, 29, 30] flavonoid compound Amentoflavone [31,32].

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2. Material and methods

2.1. Drugs and chemicals

Ethanol was bought from SIGMA chemical Co. St Louis, USA). Castor oil was purchased from WELL's Heath Care, Spain. Normal saline solution (0.9% NaC1) (Beximeo Infusion Ltd.), Diazepam (Incepta Pharmaceuticals Ltd.), loperamide manufactured by Square Pharmaceuticals Ltd., Bangladesh, was bought from local pharmacy. All the chemicals and reagents were analytical grade.

2.2. Plant materials

Fresh leaves of *C. inophyllum* leaves for experiment was collected by author from Botanical garden, Mirpur, Dhaka, Bangladesh.The plant was identified by the taxonomist of Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh (accession number DACB:39674) where voucher specimen was deposited as documentation.

2.3. Preparation of extract

250mg of plant extract was soaked in 1000 ml of 99% ethanol in clean, sterilized, and flat-bottomed glass container and shaken two times a day for 15days at room temperature. Afterwards the plant extract was subjected to filtration with sterilized cotton material followed by Whatman No.1 filter paper. After filtration the extract was evaporated using rotary evaporator (Bibby RE-200, Sterilin Ltd., UK) at 4 rpm and 65 ºC temperature and the remaining gummy concentrate was taken as crude extract or ethanolic extract. The extract was stored in refrigerator at 4 °C until used for treatment [33].

2.4. Experimental animals

Weighted between 25-30g of young Swiss albino mice of either sex were collected from the animal Resource Branch of the International center For Diarrheal Disease and Research, Bangladesh (ICDDR, B). Animals placed under favorable environmental conditions maintained optimum temperature (25±2°C), humidity (45-55 %) and rodent food and water were provided formulated by the ICDDR, B. During experiment all animals received human care according to the guidelines of 'Guide for the Care and Use of Laboratory Animals', 8th edition, prepared by the National Academy of Sciences and published by the National Institute of Health (US).

2.5. CNS depressant activity test

2.5.1. Hole-cross test

The method was adopted as described by Hussain et al (34). A steel partition was fixed in the middle position of a cage having a size of 30 cm×20 cm×14 cm. A hole of 3 cm diameter was made in the partition for easy passage of mice in the cage. The mice were divided into control,

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standard control and test group. The test groups received ethanolic extract of *C. inophyllum* at the dose of 500 and 250 mg/kg body weight orally whereas control group received vehicle (1% Tween 80 in water) at 10 mL/kg body weight orally and standard group received diazepam at a dose of 1 mg/kg body weight orally. The number of movement from one compartment to the other through the hole was counted at 0, 30, 60, 90 and 120 min after oral administration of test drugs for a period of 3 min.

2.5.2. Hole board test

The experiment was performed as described by Emran and Rahman (2014) [35]. A 30 × 20 × 14cm3 size hole cross box was used to carry out the experiment. A steel partition was fixed in the middle of a cage. There was a 3 cm hole in the partition of the cage. The animals were divided into control, standard , and test groups containing five mice each. The test groups received the ethanolic extract of *C. inophyllum* orally at a dose of 250 and 500 mg/kg body weight whereas the normal control group received vehicle (1% Tween-80 in water orally). The number of movement through the hole from one compartment to the other was counted at 0, 30, 60, 90 and 120 min for a period of 3 min. Diazepam (1 mg/kg body weight i.p.) was used as positive control in the hole board test.

2.5.3. Open field test

The experiment was followed by the method described by Gupta et al. (1971) [36]. The animals were divided into control, standard and test groups containing five mice each The test groups received the ethanolic extract of *C. inophyllum* at a dose of 250 and 500 mg/kg body weight orally whereas the normal control group received vehicle (1% Tween-80 in water orally). The floor of open field was divided into a series of squares and each square alternately colored black and white. The apparatus of open field had a 40 cm height wall. Each mouse was placed at the center of the field and the number of squares traveled by the animals was counted at 0, 30, 60, 90 and 120 min for 3 min.

2.6. Antidiarrheal activity test

2.6.1. Castor oil-induced diarrhea

The method described by Shoba and Thomas (2001) [37] was followed for this study. Animals were divided into four groups of four mices each. Two test groups administered orally with ethanolic extract of *C. inophyllum* orally at a dose of 250 and 500 mg/kg body weight, respectively. Positive control group was administered loperamide 5 mg/kg as standard , while the control group was administered with only distilled water. One hour after administration of extract and drug each mice was administered of standard dose of 2 ml of castor oil and housed in a separate cage where stools were collected on blotting paper sheet of uniform weight placed for collecting of fecal material. The number of fecal material in terms of both hard and soft pellet was counted up to 5 h at each hour interval. Percent inhibition (PI) was calculated as follows:

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$$PI = \frac{\text{Mean defecation (control group-treated group)} \times 100}{\text{Mean defecation of control group}}$$

2.7. Antipyretic activity test

Brewer's yeast induced pyrexia

The antipyretic activity was evaluated by Brewer's yeast induced pyrexia in experimental animal.The animals were injected with 10 ml/kg body weight 20% aqueous suspension of brewer's yeast sub-cutaneously and rectal temperature of animals was recorded using an Ellab thermometer (33.19 ± 0.40 °C). The animals that after 18 h of subcutaneous administration showed an increase of 0.3–0.5 °C in rectal temperature were selected for the antipyretic activity. Treated groups received ethanolic extract of *C. inophyllum* (500 mg/kg) orally ,paracetamol (150 mg/kg) as reference drug whereas control group received distilled water (10 ml/kg) only. The rectal temperature was recorded at 1 h intervals up to 3 h after administration of drug [39].

2.8. Statistical analysis

Data obtained was analyzed by one-way ANOVA test followed by Dunnett's multiple comparisons post-hoc test using SPSS version 16.0 for Windows® and the results obtained were compared with the control group. $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. CNS depressant activity

3.1.1. Hole-cross test

The table-1 demonstrates the result of hole-cross test of *C. inophyllum* leaves. 2nd observation period (30 min) manifests the locomotors lowering activity which was maintained up to the 5th observation period (120 min) for extracts. The extract showed the dose dependent lowering of locomotion activity which was comparable to standard drug diazepam. The extract showed dose dependent activity of 5.00 ± 0.41and 3.50 ± 0.96 respectively at the dose of 250 and 500 mg/kg body weight whereas the standard drug diazepam showed 4.75 ± 1.38 at the dose of 1 mg/kg.

Table 1: Effect of ethanolic extract of the *C. inophyllum* leaves on hole cross test in mice

Group	**Number of Movements (Mean±SEM)**				
	0 min	**30 min**	**60 min**	**90 min**	**120 min**
Control	5.00 ± 1.08	5.25 ± 2.02	3.25 ± 0.63	2.25 ± 0.48	2.00 ± 0.71
Standard	6.00 ± 1.92	9.00 ± 3.42	8.00 ± 2.04	5.50 ± 1.85	4.75 ± 1.38
C. inophyllum (	0.50 ± 0.29*	1.75 ± 1.11	4.00 ± 0.71	2.50 ± 1.04	5.00 ± 0.41*

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250 mg/kg)					
C. inophyllum (500 mg/kg)	5.00 ± 1.47	3.50 ± 0.65	4.00 ± 0.91	2.25 ± 0.63	3.50 ± 0.96

Values are reported as mean ± S.E.M. for group of five animals (n = 5). Values are analyzed as compared to control using one way ANOVA followed by Dunnett's post Hoc test. Asterisks indicated statistically significant values from control, * indicates P= 0.05, ** indicates P < 0.01 and *** indicates P < 0.001.

3.1.2. Hole-board test

The table-2 demonstrates the result of hole-board test of *C. inophyllum* leaves. After 90 and 120 minutes in hole board test, the extract at the dose 500 mg/kg body weight displayed a significant (p<0.05) number of head dipping behavior and the results were 13.50 ± 2.22 and 15.00 ± 3.29 respectively compared to control group. The Standard drug diazepam at 120 min showed significant (p<0.05) number of head dipping (21.50 ± 3.30) behavior.

Table 2: Effect of ethanolic extract of the *C. inophyllum* leaves on hole board test in mice

Group	**Number of Movements (Mean±SEM)**				
	0 min	**30 min**	**60 min**	**90 min**	**120 min**
Control	39.75 ± 6.71	23.75 ± 1.89	17.00 ± 2.08	20.75 ± 3.92	20.00 ± 1.87
Standard	47.50 ± 4.79	23.25 ± 2.84	27.25 ± 3.79	20.00 ± 1.35	21.50 ± 3.30
C. inophyllum (250 mg/kg)	28.75 ± 0.48	14.25 ± 2.29**	15.00 ± 4.02	17.50 ± 4.33	25.50 ± 2.10
C. inophyllum (500 mg/kg)	42.50 ± 1.04	13.00 ± 1.96*	8.00 ± 2.48*	13.50 ± 2.22	15.00 ± 3.29

Values are reported as mean ± S.E.M. for group of five animals (n = 5). Values are analyzed as compared to control using one way ANOVA followed by Dunnett's post Hoc test. Asterisks indicated statistically significant values from control, * indicates P= 0.05, ** indicates P < 0.01 and *** indicates P < 0.001.

3.1.3. Open Field Test

In open field test, the extract displayed a significant suppression of the number of squires traveled by the mice and the data has been represented into table-3. At 120 min the test sample showed remarkable dose dependant CNS depression activity. The extract at the dose of 500 mg/kg (28.25 ± 5.76) body weight showed significant (p<0.001) reduction in the number of movement as compared to control group (39.75 ± 14.76) and standard group (144.3 ± 24.35).

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Table 3: Effect of ethanolic extract of the *C. inophyllum* leaves on open field test in mice

Group	Number of Movements (Mean±SEM)				
	0 min	**30 min**	**60 min**	**90 min**	**120 min**
Control	122.3 ± 12.15	111.3 ± 10.44	97.00 ± 15.15	35.50 ± 9.579	39.75 ± 14.76
Standard	175.8 ± 10.27	169.3 ± 23.23	134.3 ± 18.96	96.25 ± 35.97	144.3 ± 24.35**
C. inophyllum (250 mg/kg)	161.8 ± 6.29	107.3 ± 7.857	82.50 ± 5.95	59.50 ± 5.74	45.00 ± 13.03
C. inophyllum (500 mg/kg)	135.5 ± 25.59	97.25 ± 12.46	73.75 ± 3.15	57.00 ± 8.89	28.25 ± 5.76

Values are reported as mean ± S.E.M. for group of five animals (n = 5). Values are analyzed as compared to control using one way ANOVA followed by Dunnett's post Hoc test. Asterisks indicated statistically significant values from control, * indicates P= 0.05, ** indicates P < 0.01 and *** indicates P < 0.001.

3.2. Antidiarrheal activity

3.2.1. Castor oil-induced diarrhea

The results at table-4 showed that *C. inophyllum* leaves extract significantly (p<0.001) reduced the defecation number as compared to control group of diarrhea which was induced by castor oil. The extract treated group significantly (p<0.01) decreased the number of stools at 1 to 5 hours when compared to control group. *C. inophyllum*, showed 64.29% inhibition of diarrheal stools at the dose of 500 mg/kg body weight whereas the standard drug loperamide at the dose of 5mg/kg showed 71.43% diarrheal inhibitory activity.

Table 4: Effect of ethanolic extract of *C. inophyllum* leaves on the castor oil-induced diarrhea in mice

Group	Total number of feces	Number of diarrhea feces	Inhibition of diarrhea (%)
Control	11.50 ± 1.29	3.50 ± 0.58	-
Standard (Loperamide 5 mg/kg)	6.50 ± 1.26 ***	1.00 ± 0.82 **	71.43
C. inophyllum (250 mg/kg)	4.50 ± 2.44 **	2.00 ± 1.10 *	41.66

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C. inophyllum (500 mg/kg)	3.15 ± 2.12 ***	1.12 ± 0.5 **	62.19

Values are reported as mean ± S.E.M. for group of five animals (n = 5). Values are analyzed as compared to control using one way ANOVA followed by Dunnett's post Hoc test. Asterisks indicated statistically significant values from control, * indicates P= 0.05, ** indicates P < 0.01 and *** indicates P < 0.001.

3.3. Antipyratic activity

Brewer's yeast-induced pyrexia

The results at table-4 showed that after 18th of Brewer's Yeast injection experimental mice displayed a increase in rectal temperature. The extract at the dose of 250 and 500 mg/kg body weight showed marked reduction of rectal temperature in 1h, 2h and 3h after administration of the extract and displayed peak anti-pyretic effect at the dose of 500 mg/kg in 3rd h (96.23 ± 0.83˚C, p<0.001) as compared to control (96.35 ± 0.44˚C). Standard drug paracetamol showed significant anti-pyretic effect and reached the peak in 3rd h (96.35 ± 0.44˚C, p<0.001) as compared to control group.

Table 6: Effect of ethanolic extract of *C. inophyllum* leaves on Brewer's yeast-induced pyrexia in mice.

Treatment	**Dose (mg/kg)**	**Temperature in ˚C**				
		Initial	**Pyretic**	**1 h**	**2 h**	**3 h**
Control	10	96.23 ± 0.49	99.00 ± 0.58	98.68 ± 0.85	96.05 ± 0.20	96.35 ± 0.44
Standard	150	97.35± 0.39*	97.30± 0.34*	96.58 ± 0.81**	95.33 ± 0.20	95.35 ± 0.31
Calophyllum inophyllum (250 mg/kg)	250	96.80 ± 0.26	98.55 ± 0.40	95.28 ± 0.81	96.63 ± 0.21	96.43 ± 0.31
Calophyllum inophyllum (500 mg/kg)	500	96.58 ± 0.55	99.70 ± 0.47	94.83 ± 0.79	95.45 ± 0.42	96.23 ± 0.83

Values are reported as mean ± S.E.M. for group of five animals (n = 5). Values are analyzed as compared to control using one way ANOVA followed by Dunnett's post t-test. Asterisks indicated statistically significant values from control, * indicates P= 0.05, ** indicates P < 0.01 and *** indicates P < 0.001.

4. Discussion

Diarrhoea is gastrointestinal disorder, characterized by an excess fluid loss in the faeces results from an imbalance between the absorptive and secretory mechanisms in the intestinal tract (40). The liberation of ricinoleic acid from castor oil results in diarrheal effect by inducing permeability changes in mucosal fluid and electrolyte transport (41). The results of the present

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study show that the extract of *C. inophyllum* exerted a significant reduction in the frequency of diarrhoea produced by castor oil. Since ricinoleic acid from castor oil stimulates motility and secretion by the release of prostaglandins (42). It can be assumed that the anti-diarrhoeal effect of ethanol and aqueous extracts may be due to the inhibition of prostaglandin biosynthesis. To obtain the effect of *C. inophyllum* on the central nervous system (CNS) activity, a number of methods namely open field, hole cross, hole-board were used (43).

In all the three experimental antipyretic test calo ino gradually decreased locomotor activity and produced a significant increase in sedative effect during the period of observation. The locomotor activity is a useful measure of the CNS awareness and any decrease of this activity resulting from depression of the central nervous system indicates a sedative effect(44). Sedation are principally mediated in the CNS by the GABAA receptor complex which are the most abundant inhibitory neurotransmitter in the central nervous system (CNS) (45) and it is commonly thought that central nervous system depressant drugs exert their actions through gamma-aminobutyrate (GABA)-mediated mechanisms (46).

All the above experimental analgesic methods reveal the potential central and peripheral antinociceptive activity of C. inophyllum and it is supposed to assume that the central activity of the plant via potentiating GABA inhibition. *C. inophyllum* having many bioactive compounds like flavonoids alkaloids, saponins and steroids are found to be ligands for the gamma amin-obutyric acid type A (GABAA) receptors in the central nervous system (CNS) that provides further evidence(47).

Brewer's yeast-induced pyrexia was employed in evaluating antipyretic activities of ethanolic extracts of *C. inophyllum*. The present study showed that the ethanolic extract of *C. inophyllum* leaves caused a better antipyretic action in yeast induced elevation of body temperature. It is well known that the non-steroidal anti-inflammatory drugs control fever by the inhibition of prostaglandin synthesis within the hypothalamus that regulates body temperature (48). It may be assumed that the extract revealed their antipyretic action through inhibition of prostaglandin synthesis within the hypothalamus .

Conclusion

From the above study we can make a conclusion that *C. inophyllum* possess significant CNS depressant, anti-diarrheal and antipyretic activities and various phytochemicals present in the extract may attribute these activities. However, to be clear about the above pharmacological activities further research is needed.

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