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Original Research Article

Central Nervous System Depressant, Analgesic and Antidiarrheal Effects of the Seed Extracts of *Dimocarpus longan* Lour in Rats

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Abstract

Purpose: *To assess the central nervous system (CNS) depressant, analgesic and antidiarrheal activities of the dried seed crude extracts of Dimocarpus longan Lour in rodents.*
Methods: *Selected pharmacological effects of the ethanol (ENLS), petroleum ether (PELS), chloroform (CHLS) and ethyl acetate (EALS) extracts of D. longan fruit seeds were investigated. CNS depressant activity was evaluated by open field and hole cross tests; analgesic activity by acetic acid-induced writhing test and formalin-induced licking test; and anti-diarrheal activity was assessed in castor oil and magnesium-induced diarrhea rat model. The extracts were given orally in a rat model at doses of 200 and 300 mg/kg body weight. Normal saline served as control in all experiment. In CNS depressant test, diazepam (1 mg/kg) was used as reference drug while indomethacin (10 mg/kg) and loperamide(2 mg/kg) were used as standard drugs in analgesic and antidiarrheal tests, respectively.*
Results: *In hole cross method, EALS showed the most effective depressant effect, viz, 1.17±0.17 for 200 mg/kg dose and 0.83±0.31 number of movements for 300 mg/kg dose after 120 min ($p < 0.01$), whereas in the open field test, all the extracts exhibited significant ($p < 0.01$) depressant effect in relation to positive control, diazepam. In acetic acid-induced pain test, PELS gave the lowest number of writhing (2.83±0.307) and the highest inhibition (88.45 %, 300 mg/kg dose) which was statistically significant. All the extracts also significantly ($p < 0.01$) suppressed licking activity in both phases of the formalin-induced licking test, in contrast to indomethacin. In the antidiarrheal tests, diarrheal suppression was highest at 300 mg/kg dose for all the extracts, compared with loperamide in both castor oil and magnesium sulphate induced diarrhea model.*
Conclusion: *The extracts of Dimocarpus longan tested demonstrated significant CNS depressant, analgesic and antidiarrheal activities in a rodent model.*

Keywords: *Dimocarpus longan Lour, CNS depressant, Analgesic, Anti-diarrheal.*

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INTRODUCTION

The use of medicinal plants to treat diseases is as old as human civilization. Synthetic drugs are very expensive to develop. On the contrary, many medicines of plant origin have been used foe centuries without any undesirable effects. It is therefore essential that efforts should be made to introduce new medicinal plants to develop new drugs [1]. Since medicinal plants are believed to be an important source of novel chemical substances with potential remedial effects, they

are used in Bangladesh traditional medical practice for the treatment of various diseases [2]. *Dimocarpus longan* Lour. syn. *Euphoria longana* Lam. (locally known as Longan fruit) is a subtropical evergreen tree of Sapindaceae family, extensively grown in southern China, India and South East Asia [3]. The appearances of the fruits are similar to those of lychee, but are smaller, smoother and yellow-tan in colour. The color of fleshy aril is white to off-white or pinkish which surrounds a red brown, brown to black seed, which separates easily from the flesh. Its fruit is accepted by consumers over the world due to its sweet and juicy sensation in the mouth and its health benefits. Longan fruit is rich in carbohydrates, protein, fiber, fat, vitamin C, amino acids, and minerals.4 The fruit has been used traditionally in Chinese medicinal formulations, serving as a agent for the relief of neural pain and swelling5. In recent years, the extracts from longan fruit including aril, pericarp and seed, have exhibited excellent antioxidant ability, anti-tyrosinase, anticancer, antifatigue and hypoglycemic activities [4-14].

Longan seeds are used to prevent pain, hemorrhage, hernia, and skin diseases in Chinese folk medicine [14]. The seeds act as antioxidant [], prevent human colorectal carcinoma cells [4], and they contain gallic acid, ellagic acid,and corilagin [9]. The current study was undertaken to investigate the analgesic, CNS depressant and anti-diarrheal effects of some solvent extracts of the seeds of *D. longan*.

EXPERIMENTAL

Collection and identification of plant

Fresh fruits of *D. longan* were collected from Dhaka, Bangladesh in August, 2012. The fresh fruits of *D. longan* were identified by Dr Mahbubur Rahaman, Associate Professor, Department of Botany, Rajshahi University, Rajshahi and National Herbarium of Bangladesh, with voucher specimen no. 36664. The seeds were separated from the fruits, dried for one week and pulverized into a coarse powder with a suitable grinder (Palm Leaf Grinder PK 300). The powder was stored in an airtight container, and kept in a cool, and dry place pending analysis.

Drugs

Diazepam, indomethacin and loperamide were obtained from Square Pharmaceuticals Ltd., Bangladesh

Preparation of extract

The dried seed powder (500 g) was soaked in 500 ml of 95 % ethanol for 7 days in cold condition with occasional shaking. The whole mixture was successively filtered through a piece of clean, white cotton material and filter paper. The filtrate was divided into two equal portions; one portion was further partitioned by solvent-solvent extraction with petroleum ether, chloroform and ethyl acetate and other portion was dried. After multiple extractions all filtrates were further evaporated to dryness to obtain the dried crude extracts. The ethanol, petroleum ether, chloroform and ethyl acetate extracts were tagged ENLS, PELS, CHLS and EALS, respectively.

Phytochemical analysis

The crude extracts were subjected to qualitative phytochemical screening using standard procedures [15].

Animals

Long-Evans rats (8 to 14 weeks old) of either sex weighing about 80 – 120 g and were used to conduct the research. The rats were procured from the animal research branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDRB). They were kept under standard environmental conditions (24.0 ± 0 °C and 55 - 65 % relative humidity with a 12 h light/dark cycle) for two weeks for acclimatization. They were fed ICDDRB formulated rodent food and had access to tap water *ad libitum*. All the animals were fasted over night prior to each test but had free access to water.

Ethical approval

The guidelines followed for animal experiment were accepted by the Southeast University institutional animal ethical committee [16].

CNS depressant activity

CNS depressant activity was assessed usng both the hole cross and open field tests.

Hole cross test

The aim of this study was to examine the effect of the extract on the emotional behavior of the rodents using the hole-board test. The method of Takagi *et al.* [17] was followed. A steel partition with a hole of 3 cm diameter fixed in the middle of a cage having a size of 30 × 20 × 14 cm was

used. The number of times a rat passed through the hole from one chamber to other was counted for a period of 3 min at 0, 30, 60, 90 and 120 min after oral administration of normal saline (10 ml/kg), experimental crude extracts (at dose of 200 mg/kg and 300 mg/kg) and diazepam as standard drug (1 mg/kg)

Open field test

This experiment evaluates a range of anxiety-induced locomotor activity and exploratory actions. The animals were treated at the as stated above and at the same doses. The test was performed according to Gupta et al [18]. The floor of an open field of half square meter was divided into a series of squares, each colored alternatively black and white. The apparatus had a 40 cm wall. The number of squares visited by the animals was calculated for 3 min, at 0, 30, 60, 90 and 120 min subsequent to oral administration of the normal saline (10 ml/kg), experimental crude extracts (doses) (200 mg/kg and 300 mg/kg) and diazepam as standard drug (1 mg/kg.)

Evaluation of analgesic activity

To assess the analgesic activity of the extracts, acetic acid-induced writhing and formalin-induced pain tests were used.

Acetic acid-induced writhing test

In this test, acetic acid was given intra-peritoneally to generate pain sensation [19]. Indomethacin (10 mg/kg) was used as standard. The plant extracts were administered orally at 200 and 300 mg/kg doses to test rats after an overnight fast. The extracts, normal saline and standard drug were administered orally 30 min prior to intraperitoneal administration of 0.7 %v/v acetic acid solution (0.1 ml/10 g). [19]The rodents were placed individually on an observation table to count the writhings made in 15 min commencing just 5 min after intra-peritoneal administration of acetic acid. The animals did not always give full writhing, sometimes they started to give but they did not complete it. This unfinished writhing was counted as half-writhing. Hence, two half-writhings were taken as one complete writhing. The number of writhes in each treated group was compared to those of the control and standard groups.

Formalin test

The antinociceptive activity of the extracts was determined using formalin test [20]. Control group received normal saline and 20 µl of 5 % formalin while the test groups and positive control groups received tested extracts (200 and 300 mg/kg, p.o.) and reference drug, indomethacin (10 mg/kg) 60 minutes before injection of 20 µl of 5 % formalin into the dorsal surface of the right hind paw. The rats were monitored for 30 min after the injection of formalin, and the amount of time spent licking the injected hind paw was recorded. The first 5 min post-formalin injection is referred to as the early phase and the tenure between 15 and 30 min as the late phase. The total time spent licking or biting the wounded paw (pain response) was determined with the aid of a stop watch.

Anti diarrheal tests

Anti-diarrheal effect was evaluated usng castor oil- and magnesium sulphate-induced diarrheal methods.

Castor oil-induced diarrhea test

Awouter et al [21] method was used for this test. Fifty rats were fasted for 18 h with free access to water and randomly separated into ten groups of five rodents each. Each extract was administered orally at 200 and 300 mg/kg doses. Group-I (control) received only normal saline (5 ml/kg), while group 2 received loperamide (3 mg/kg) as standard. One hour later, each animal received 1 ml of castor oil orally by gavage. Feces were collected with an absorbent sheet of paper placed below the transparent cages, the total number of diarrheal feces expelled in 4 h were compared with both the standard and control groups. The total score of diarrheal faeces for the control group was considered as 100 %. The results were expressed as % inhibition of diarrhea.

Magnesium sulphate-induced diarrhea test

This test was performed in a similar way to the above method. Here, diarrhea was induced by oral administration of magnesium sulphate [22](2 mg/kg) to the animals 30 min after oral pre-treatment with vehicle (1 % Tween 80 in water, 10 ml/kg, p.o.) to the control group, loperamide (3 mg/kg) to the positive control group; the plant extracts were given at 200 and 300 mg/kg doses to the test groups.

Statistical analysis

Data are expressed as mean ± SEM and evaluated by analysis of variance (ANOVA) followed by Dunnet's multiple comparisons test, using SPSS 15.00 (USA). Differences between means for various groups were considered significant *at* $p < 0.01$.

RESULTS

Phytochemical profile of extracts

The extracts gave positiveresults for tannins, alkaloids, and flavonoids.

CNS depressant activity

Rodents treated with different solvent extracts at two doses (200 and 300 mg/kg) showed dose dependent reduction in the locomotor activity comparable with the standard drug, diazepam (p < 0.01, Table 1). The control group showed insignificant change in locomotor activity (p < 0.01).

Analgesic activity of the extracts

Table 3 shows the effects of the extracts on acetic acid-induced writhing in rats. Both doses of the extracts showed significant reduction (p < 0.01) in writhing after oral administration. The extracts seemed to be more potent than the reference drug.

In the antinociceptive test based on the formalin model, the extracts significantly (p<0.01) suppressedlicking activity in both phases of pain (Table 4) in a dose-dependant manner. Standard.

Antidiarrhoeal activity

In the castor oil-induced diarrhea model, all the extracts at both doses significantly diminished the total number of diarrhoeial feces in a dose-dependent manner (p < 0.01, Table 5).

In the magnesium sulphate-induced diarrhea, pretreatment of rats with the different doses of the extracts caused significant dose-dependent decrease in the frequency of purging (p < 0.01, Table 6).

Table 1: CNS depressant activity of extracts in rats (hole cross method)

Treatment	Dose (mg/kg)	No. of movements				
		0 min	*30 min*	*60 min*	*90 min*	*120 min*
1% Tween 80 in water	10ml/kg,	17±0.57	16±0.51	14.33±0.55	13.5±0.84	12.17±0.30
Diazepam	1	15.5±0.71	6.67±0.49*	4.17±0.40*	2.33±0.21*	1.17±0.30*
ENLS	200	13.6±1.28	6.5±0.56*	4.2±0.30*	2.33±0.21*	1.83±0.30*
ENLS	300	12.2±0.79*	5.2±0.47*	4±0.36*	2.66±0.33*	1.67±0.21*
PELS	200	15.3±0.88	3.67±0.33*	2.83±0.30*	1.33±0.21*	1.16±0.16*
PELS	300	14.5 ± 0.92	5.83±0.47*	4.33±0.33*	2.5±0.34*	1.33±0.21*
CHLS	200	13.3±0.33*	7.33±0.33*	5.33±0.33*	3.2±0.30*	1.67±0.21*
CHLS	300	10.5±0.22*	6.8±0.40*	5.5±0.22*	2.67±0.33*	1.33±0.21*
EALS	200	15.3±0.40	6.5±0.22*	3.5±0.22*	2.33±0.21*	1.17±0.16*
EALS	300	11.83±0.65*	5.66±0.33*	3.33±0.21*	2.17±0.30*	0.83±0.30*

All values are expressed as mean ± SD (n = 6); *p < 0.01, significant compared to control. In this test, all extracts exhibited a perceptible decline in locomotion of test animals at 2nd observation (30 min) which continued up to 4th observation period (120 min) at both dose levels (200 and 300 mg/kg body weight). The results were dose dependent and statistically significant (P<0.01) (Table-2).

Table 2: CNS depressant activities of plant extracts in rats (open field method)

Treatment	Dose (mg/kg)	No. of movements				
		0 min	*30 min*	*60 min*	*90 min*	*120 min*
1% Tween 80 in H_2O	10ml/kg,	151.8±1.99	150.5±1.58	149.8±2.41	147.7±1.28	145.6±1.45
Diazepam	1	161.2±3.32*	86.2±1.10*	64.3±1.33*	43.8±1.13*	12.3±0.84*
ENLS	200	167.5±1.33*	122.8±1.2*	89.7±1.20*	61.8±1.51*	11.6±0.61*
ENLS	300	161.3±1.76*	105.8±2.15*	77.8±0.70*	54.2±1.19*	10.8±0.30*
PELS	200	158.5±2.06	82.7±1.33*	65.2±1.01*	44.5±0.92*	15.3±2.10*
PELS	300	153.7±0.55	80.5±0.76*	43.2±1.01*	38.3±1.11*	11.7±0.55*
CHLS	200	163.7±0.49*	84.8±0.60*	47.2±0.60*	37.5±0.92*	13.3±1.05*
CHLS	300	155.3±0.80	80.5±1.56*	41.3±1.05*	33.2±0.70*	10.8±0.40*
EALS	200	162.3±0.98*	84.3±0.33*	43.8±0.70*	36.8±1.95*	13.5±0.84*
EALS	300	154.3±0.55	79.8±1.07*	38.8±0.47*	32.8±0.60*	11.8±0.30*

*All values are expressed as mean ± STD (n=6); One way Analysis of Variance followed by Dunnet's test. *P<0.01, significant compared to control.*

Table 3: Analgesic activity of extracts by acetic acid-induced writhing method

Treatment	Dose (mg/kg)	No. of writhings	% Inhibition
1% Tween 80 in water	0.1 ml/10g	24.50 ± 1.43	-
Indomethacin	10	10.17 ± 0.79*	58.50
ENLS	200	7.67 ± 1.15*	68.69
ENLS	300	5.50 ± 0.84*	77.55
PELS	200	6.83 ± 1.08*	72.12
PELS	300	2.83 ± 0.30*	88.45
CHLS	200	9.17 ± 0.47*	62.57
CHLS	300	4.50 ± 0.22*	81.63
EALS	200	9.33 ± 2.02*	61.92
EALS	300	4.33 ± 0.21*	82.33

*All values are expressed as mean ± SD (n = 6); *p < 0.01 significant compared to control*

Table 4: Analgesic activity of extracts based on formalin-induced writhing method

Treatment	Dose (mg/kg)	Early phase (s)	Late phase (s)
Water	10 ml/kg	25.17 ± 0.86	42.50 ± 0.71
Indomethacin	10	12.83 ± 0.30*	24.00 ± 0.516*
ENLS	200	13.67 ± 0.49*	20.83 ± 0.30*
ENLS	300	11.83 ± 0.30*	17.83 ± 0.40*
PELS	200	14.50 ± 0.22*	20.50 ± 0.34*
PELS	300	12.67 ± 0.21*	18.00 ± 0.36*
CHLS	200	14.17 ± 0.40*	20.50 ± 0.22*
CHLS	300	12.67 ± 0.49*	16.17 ± 0.40*
EALS	200	15.33 ± 0.21*	21.17 ± 0.47*
EALS	300	13.50 ± 0.22*	17.50 ± 0.34*

*All values are expressed as mean ± STD (n=6); ANOVA followed by Dunnet's test. *P<0.01 significant compared to control.*

Table 5: Effect of the extracts on castor oil-induced diarrhoea

Treatment	Dose (mg/kg)	Total no of diarrhoeial faeces in 4 h	Inhibition (%)
Normal saline	5	20.5 ± 1.08	-
Reference	2	9.33 ± 0.80*	54.48
ENLS	200	12.83 ± 0.60*	37.41
ENLS	300	8.33 ± 0.66*	59.37
PES	200	13.8 ± 0.47*	32.68
PES	300	8.16 ± 0.30*	60.20
CHLS	200	15.16 ± 0.65*	26.05
CHLS	300	7.50 ± 0.65*	63.41
EAS	200	13.33 ± 0.42*	34.98
EAS	300	8.16 ± 0.40*	60.20

All values are expressed as mean ± SD (n = 6); (ANOVA) followed by Dunnet's test;
**p < 0.01 significant compared to control*

DISCUSSION

In the current study, we have evaluated the CNS depressant, analgesic and antidiarrheal effects of various solvent extracts of the seeds of *D. longan* in rodents. *In vivo* methods using intact animal models are thought to be good method for examining the action of drugs on the central nervous system. The most significant step in assessing drug action on the CNS is to monitor locomotor activity of the test models. To obtain evocative results regarding the activity of *D. longan* seeds extracts on the CNS of rats, two tests, namely hole cross and open field, were performed. The movement is a measure of the level of excitability of the CNS [23] and its decrease may be intimately related to sedation resulting from depression of the CNS [24].

All the extracts significantly decreased this activity as revealed by the results of the two aforementioned tests. Sedation may be due to interaction with benzodiazepines-like compounds. The seed extracts might have acted by potentiating GABAergic inhibition in the CNS by membrane hyperpolarization which diminish the firing rate of critical neurons in the brain or may be due to direct activation of GABA receptor by the extracts [24]. Previous investigation on phytoconstituents and plants indicate that many flavonoids and neuroactive steroids are ligands for $GABA_A$ receptors in the central nervous system, which led to the postulation that they can act as benzodiazepine [25]. Therefore, the phytoconstituents (tannin, alkaloids, flavonoids) may be responsible for their CNS depressant activity.

Table 6: Effect of the extracts on magnesium sulphate-induced diarrhea

Treatment	Dose (mg/kg)	Total no. of diarrhoeial faeces in 4 h	Inhibition (%)
(Control)	Water	20.5 ± 1.08781	-
(Standard)	2	9.33 ± 0.73*	54.48
(ENLS)	200	16.16 ± 0.60*	21.17
(ENLS)	300	7.83 ± 0.60*	61.80
(PES)	200	14.16 ± 0.47*	30.92
(PES)	300	7.16 ± 0.30*	65.07
(CHLS)	200	16.33 ± 0.30*	20.34
(CHLS)	300	8.33 ± 0.33*	59.37
(EAS)	200	13.8 ± 0.33*	32.68
(EAS)	300	8.16 ± 0.30*	60.20

All values are expressed as mean ± SD (n = 6); (ANOVA) followed by Dunnet's test;
**p < 0.01 significant compared to control*

To ascertain analgesic activity, we performed both acetic acid-induced writhing and formalin-induced pain tests. The first method is extensively used for the assessment of peripheral antinociceptive activity [26]. It is very sensitive and able to detect anti-nociceptive effect of compounds at dose levels that may appear inactive in other methods such as the tail-flick test [27]. The method has been associated with prostanoids in general, e.g., increased levels of PGE2 , PGF2α and lipoxygenase products in peritoneal fluids [28,29]. Therefore, the results of the acetic acid induced writhing; strongly suggest that the mechanism of action of these extracts may be linked partly to lipoxygenase or cyclooxygenase pathways.

Again, the second test is useful for elucidating mechanism of pain and analgesia. Drugs that principally act centrally such as narcotics hinder both phases of formalin-induced pain while peripherally-acting drugs such as indomethacin inhibit only the second phase [29]. Since the extracts exerted significant dose-related inhibition of both phases in the formalin-induced pain model, this probably indicates that the overall antinociceptive effect of the crude extracts involves central and peripheral mechanism. Indomethacin produced significant decline in the pain response of the rodents but the effect was chiefly noticed in the second phase of pain which is consistent with earlier reports that NSAIDs are efficient only in the second phase of the formalin test.[30]

Our investigation also points to an antidiarrheal activity of the extracts. The effect was close to that of the higher dose of the standard drug, loperamide. Castor oil produces diarrhea via ricinoleic acid [31] which elevates prostaglandin

biosynthesis. Prostaglandin participates in the patho-physiological functions in the GIT [32]. The extracts reduced this diarrhea probably through the inhibition of prostaglandin biosynthesis. On the other hand, magnesium sulphate accelerates the liberation of cholecystokinin from duodenal mucosa, which extends the secretion and motility of small intestine [33]. The extracts reduced the diarrheia, perhaps by increasing the absorption of water and electrolyte from GIT.

CONCLUSION

All the seed extracts of *D. longan* fruits investigated have potent CNS depressant, analgesic (both central and peripheral) and antidiarrheal activities. However, further research is needed to determine the precise mechanisms involved as well as the chemical constituents responsible for the pharmacological activities.

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