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EVALUATION OF CNS DEPRESSANT AND ANALGESIC ACTIVITIES OF THE METHANOL EXTRACT OF *PIPER LONGUM* LINN. LEAVES

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ABSTRACT

Present study reports CNS depressant and analgesic activities of methanol extract obtained from the leaves of *Piper longum* L (MEPL). CNS depressant activity was evaluated by using open field and hole cross tests at doses of 250 and 500 mg/kg body weight while peripheral analgesic activity was evaluated by using acetic acid induced writhing method and formalin test respectively in rat model at 100 and 200 mg/kg body weight. The results of the statistical analysis showed that the plant extract had significant ($p<0.01$) dose dependent CNS depressant and analgesic activities. Locomotor activity and exploratory behavior of rats in hole cross and open field test were decreased in the test group comparing the control group indicating CNS depressant effect of the extract which was comparable with the standard drug diazepam. The extract also showed better analgesic effects at both doses characterized by reduction in the number of writhes in the acetic acid-induced writhing model and reduction of licking time in the formalin test when compared to the control group. The extract, at the dose of 200 mg/kg, exerted a maximum of 57.58% inhibition of writhing response and 58.8% inhibition was observed for reference drug Indomethacin. So, the present results suggest that the methanol extract of *P. longum* leaves possesses remarkable CNS depressant and analgesic activities.

INTRODUCTION: *Piper longum* L. is very important for its medicinal values from ancient times to present day. *Piper longum* L. (Piperaceae), commonly known as "long pepper", is a slender aromatic climber with perennial woody roots and numerous simple, ovate, cordate leaves. Flowers are yellow, in elongate spikes and the fruits are small, ovoid berries, shiny blackish green, embedded in fleshy spikes [1]. It is widely distributed in the tropical and subtropical regions of the world, throughout the Bangladesh, China, Indian, Sri Lanka, Middle Eastern countries and the America. The fruits are used as spice and as a preservative in pickle. They have a pungent taste and cause salivation and numbness of the mouth [2]. In the traditional medicine, mature spikes of female plants, thick stems, roots and leaves are extensively used in the treatment of bronchial diseases, dyspepsia, worms, amoebiasis and aphrodisiac agent. Pepper is an important drug capable of improving intellect and memory power and also to regain health dispelling diseases. It is reportedly acrid, hot, light, digestive, appetizer and tonic.

It cures cough, dyspnoea, ascites, leprosy, diabetes, piles, colic indigestion, anemia, thirst, and dispels cardiac and spleen disorders, chronic fever and loss of appetite. Dried ripe fruits and roots are the officinal parts.[3] It is carminative sedative, emollient, demulcent, general tonic and hematinic. It enhances thermogenic response or release of metabolic heat energy. The fruits and roots of long pepper are used as snuff in comma and drowsiness, as sedative in insomnia and epilepsy, as cholagogue in obstruction of bile duct and gall-bladder, as emmenagogue, abortification and as antihelmintic [2].

Long pepper is very effective in the treatment of bronchial asthma in children [4, 5]. Long pepper is used in Ayurvedic treatment for abdominal tumors and distention, to improve the digestive fire, flatulence, gout, laryngitis, paralysis, rheumatic pain, sciatica, worms, and for the immune system. It is used in manufacturing cold relief balm, pain balm, joint care balm and in heart and stress care and coughs syrups.

Due to its great importance in traditional medicine, many research works have already been carried out and it is reported that the fruits extract of the plant had antidepressant, antinociceptive, anti-inflammatory, antioxidant, anticancer, antidiabetic, antibacterial, antifungal, antitumor, antiallergic, antiasthmatic, antitubercu-lar, antifertility, antiulcer, antihypertensive, antiplatelet, antithyroid, imminomodulatory, antiamoebic, hepato- protective, vasodilating, insecticidal and mosquito larvicidal activit-ies [6-8].

Piperine is an alkaloid present in the fruits extract mainly responsible for the pharmacological activities of the fruits and its modes of action for versatile pharmaco-logical functions have already been established. But there is no scientific report on CNS depressant and analgesic activities of the leaves extract of the plant. Hence attempt has been taken to study the CNS depressant and analgesic effects of the *Piper longum*.

MATERIALS AND METHODS:

Plant material: For this present investigation, leaves of *Piper longum* were collected from Mirpur, Dhaka, Bangladesh in October, 2010 and were identified by the experts of Bangladesh National Herbarium, Dhaka, where a voucher specimen has been retained. The collected plant parts were dried for one week and pulverized into a coarse powder with the help of a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced.

Preparation of the extract: About 150 gm of powdered material was taken in a clean, flat bottomed glass container and soaked in 200 ml of 85% methanol. The container with its contents was sealed and kept for a period of 7 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then it was filtered through Whatman filter paper (Bibby RE200, Sterilin Ltd., UK). The filtrate (methanol extract) obtained was evaporated using rotary evaporator. It rendered a gummy concentrate of reddish black color. The gummy concentrate was designated as crude extract of methanol. The extract was transferred to a closed container for further use and protection.

Animals: Young Long-Evans rats of either sex weighing about 80-120gm were used for the experiment. The rats were purchased from the animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDRB). They were kept in standard environmental condition (at 24.0±0°C temperature & 55-65% relative humidity and 12 hours light/dark cycle) for one week for acclimation after their purchase and fed ICDDRB formulated rodent food and water ad libitum. The set of rules followed for animal experiment were approved by the institutional animal ethical committee.[9]

Drugs and chemicals: Acetic acid was obtained from Merck, Germany. Tween-80 was obtained from BDH Chemicals, UK. Formalin was purchased from CDH, India. Normal saline solution was purchased from Beximco Infusion Ltd., and indomethacin and diazepam were obtained from Square Pharma- ceuticals Ltd., Bangladesh. All chemicals used were of analytical reagent grade.

Acute toxicity: The 50% lethal dose (LD50) of the extract of *P. longum* in rats was estimated by the up and down method.[10] Doses were adjusted up or down by a constant multiplicative factor (1.5) depending on the previous outcome.

CNS Depressant Activity:

Hole cross test: The method was carried out as described by Takagi et al., (1971).[11] A steel partition was fixed in the middle of a cage having a size of 30×20×14 cm. A hole of 3 cm diameter was made at a height of 7.5 cm in the center of the cage. Twenty animals were divided into four groups with five rats in each group. Group I animals received vehicle (1% Tween 80 in water, 10 ml kg^{-1} p.o.), animals of Group II received diazepam at 1 mg kg^{-1} body weight (p.o.) while animals of Group III and Group IV were treated with 250 and 500 mg kg^{-1} body weight (p.o.) of the extract. The number of passages of a rat through the hole from one chamber to other was counted for a period of 3 min on 0, 30, 60, 90 and 120 min after oral administration of test drugs

Open field test: The animals were treated as discussed above. The experiment was carried out according to the methods described by Gupta et al., (1971).[12] The floor of an open field of half square meter was divided into a series of squares each alternatively colored black and white. The apparatus had 40 cm height a wall. The number of squares visited by the animals was counted for 3 min, on 0, 30, 60, 90 and 120 min after oral administration of test drugs.

Analgesic Activity:

Acetic acid induced writhing method: The analgesic activity of the samples was also studied using acetic acid-induced writhing model in rats.[13] Test samples (100 and 200 mg/kg body weight), vehicle (1% tween 80 in water) and indomethacin (10mg/kg) were administered orally 30 min before intraperitoneal administration of 0.7%, 0.1 ml/10gm acetic acid. Then the rats were observed for specific contraction of body referred to as 'writhing' for the next 20 min.[13]

Full writhing was not always accomplished by the animal, because sometimes the animals started to give writhing but they did not complete it. This incomplete writhing was considered as half-writhing. Accordingly, two half-writhing were taken as one full writhing. The number of writhes in each treated group was compared to that of a control group while indomethacin (10mg/kg) was used as a reference substance (positive control). The percent inhibition (% analgesic activity) was calculated by;

$$\% \text{ inhibition} = \{(A-B)/A\}X\ 100$$

Where, A= Average number of writhing of the control group; B= Average number of writhing of the test group.

Formalin test: The antinociceptive activity of the drugs was determined using the formalin test described by Sharma *et al*. (2010).[13] Control group received 5% formalin. 20 µl of 5% formalin was injected into the dorsal surface of the right hind paw 60 min after administration of extract (100 and 200 mg/kg, p.o.) and Indomethacin (10 mg/kg, p.o.). The rats were observed 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 period between 15 and 30 min as the late phase. The total time spent licking or biting the injured paw (pain behavior) was measured with a stop watch.

Statistical Analysis

Data are expressed as mean ± STD and were analyzed statistically by one-way ANOVA procedures, followed by using Dunnett's test. A difference was considered significant at $p<0.01$.

RESULTS:

Acute toxicity: In the acute toxicity test, no any toxicity was observed within 7 days after oral administration at the high dose of 15 g/kg methanol extract of *P. longum leaves* in rats.

CNS Depressant Activity:

Hole cross test: In the animal treated with methanol extract at two doses (250 mg/kg & 500 mg/kg) showed dose dependent reduction in the locomotor activity and at higher dose, it was comparable with that of standard drug diazepam. Diazepam was used as the standard drug in the experimental animals to evaluate the CNS depressant effect of the plant extract. The extract produced reduction in spontaneous motor activity, and this effect may be attributed to CNS depression, as depression of locomotor activity is common to most neuroleptics. The CNS was depressed till observation and the results were statistically significant (**Table 1**).

TABLE 1: CNS DEPRESSANT ACTIVITY OF METHANOLIC EXTRACT OF LEAVES OF *P. LONGUM* ON HOLE CROSS TEST IN RATS

Group	Treatment	Dose, Route	Number of Movements				
			0 min	30 min	60 min	90 min	120 min
Group-I (Control)	1% tween 80 in water	10ml/kg, p.o	12.8±2.59	13±3.16	13.6±2.07	14.2±1.92	14±1.22
Group-II (Standard)	Diazepam	1mg/kg, p.o	11.2±1.30	6±1.58*	4±1.87*	2.4±1.82*	1.8±0.84*
Group-III (Extract)	MEPL	250 mg/kg, p.o	12.6±3.43	6.8±2.39*	5.8±1.1*	4.4±1.14*	3.6±2.07*
Group-IV (Extract)	MEPL	500 mg/kg, p.o	12.2±1.48	5.8±1.48*	4.2±1.09*	3±1.41*	1.8±1.64*

All values are expressed as mean ± STD (n=5); One way Analysis of Variance (ANOVA) followed by Dunnet's test. *P<0.01, significant compared to control

Open field test: Open field test was carried out to determine the depressive action of the test drugs on CNS in rats [12]. In the test, the extract showed a noticeable decrease in locomotion in the test animals from the second observation period to last study period at both dose levels (250 and 500 mg/kg body weight). The effect observed was increasing with time and a noticeable result was found at 120 min of test sample administration. Test animals showed significant decrease in number of movement in the dosages of 250 and 500mg/kg (24.8±3.11, 15.8±3.77, respectively, as compared to 118.0±1.58 in the control group and 9.6±1.14 in the standard group) at 120 min of administration of the extract. So, the extract showed dose dependent CNS depressant activities which were statistically significant (**Table 2**).

TABLE 2: CNS DEPRESSANT ACTIVITY OF METHANOLIC EXTRACT OF LEAVES OF *PIPER LONGUM* ON OPEN FIELD TEST IN RATS

Group	Treatment	Dose, Route	Number of Movements				
			0 min	30 min	60 min	90 min	120 min
Group-I	1% tween 80 in water	10ml/kg, p.o	118.4±2.7	118.0±2.92	115.4±1.14	117.4±2.61	118.0±1.58
Group-II	Diazepam	1mg/kg, p.o	117.2±2.59	64.6±3.21*	40.8±1.30*	18.8±1.92*	9.6±1.14*
Group-III	MEPL	250 mg/kg, p.o	119.4±1.14	73.2±2.17*	57.2±4.60*	35.8±3.77*	24.8±3.11*
Group-IV	MEPL	500 mg/kg, p.o	120.2±3.19	67±4.69*	42±1.87*	25.4±3.85*	15.8±3.77*

All values are expressed as mean ± STD (n=5); One way Analysis of Variance (ANOVA) followed by Dunnet's test. *P<0.01, significant compared to control

Analgesic Activity:

Acetic acid- induced Writhing Test: In acetic acid induced writhing test, the methanol extract of leaves of *P. longum* significantly and dose dependently suppressed the frequency of acetic acid-induced writhing in rats after oral administration. At 100 mg/kg body weight, the extract showed 48.73% writhing inhibition, at 200 mg/kg body weight, the extract showed 57.58% writhing inhibition (**Table 3**). So, the plant extract showed analgesic activity at the dose of 200 mg/kg body weight was comparable to the standard drug indomethacin that inhibited 58.87% writhing at the dose of 10 mg/kg body weight.

TABLE 3: ANALGESIC ACTIVITY OF METHANOLIC EXTRACT OF LEAVES OF *PIPER LONGUM* BY ACETIC ACID INDUCED WRITHING METHOD IN RATS

Groups	Treatment	Dose, route	No. of writhing	Percent inhibition
Group-I (Control)	1% Tween 80 in water	0.1 ml/10gm body weight	26.33±1.37	-
Group-II (Standard)	Indomethacin	10mg/kg	10.83±2.99*	58.87
Group-III (Extract)	MEPL	100mg/kg	13.5±3.87*	48.73
Group-IV (Extract)	MEPL	200mg/kg	11.17±1.83*	57.58

All values are expressed as mean ± STD (n=5); One way Analysis of Variance (ANOVA) followed by Dunnet's test. *P<0.01, significant compared to control

Formalin test: The methanol extract of leaves of *P. longum* (100 and 200 mg/kg, p.o.) significantly suppressed the licking activity in either phase of the formalin-induced pain in rats (**Table 4**) in a dose dependant manner. The reference analgesic drug Indomethacin (10 mg/kg) also significantly inhibited the licking activity against both phases of formalin-induced pain.

TABLE 4: EFFECT OF THE METHANOLIC EXTRACT OF BARKS OF PIPER LONGUM ON HINDPAW LICKING IN THE FORMALIN TEST IN RATS

Groups	Treatment	Dose, route	Early phase (Sec)	Late phase (Sec)
Group-I (Control)	Distilled water	10 ml/kg	35.67±3.39	46±2.53
Group-II (Standard)	Indomethacin	10 mg/kg	16.83±2.23*	21.83±1.72*
Group-III (Extract)	MEHS	100 mg/kg	23.33±5.04*	25.17±2.79*
Group-IV (Extract)	MEHS	200mg/kg	17±8.48*	22.67±2.50*

All values are expressed as mean ± STD (n=5); One way Analysis of Variance (ANOVA) followed by Dunnet's test. *P<0.01, significant compared to control

DISCUSSIONS: Our present study revealed that the leaves extract of *P. longum* showed dose dependent CNS depressant and analgesic activities. Locomotor activity is often used to assess the depressant effects of the crude extract through Open field and Hole cross methods. Locomotor activity considered as an increase in alertness and decrease in locomotor activity indicated sedative effect [14]. Extracts of *P. longum* decreased locomotor activity indicates its CNS depressant activity. Gamma-amino-butyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system.

Different anxiolytic, muscle relaxant, sedative-hypnotic drugs are elucidation their action through $GABA_A$, therefore it is possible that extract of *P. longum* may acts by potentiating GABAergic inhibition in the CNS via membrane hyperpolarization which leads to a decrease in the firing rate of critical neurons in the brain or may be due to direct activation of GABA receptor by the extract [15]. The fruits of *Piper longum* L. contains amide alkaloids, flavonoids, essential oils, lignans and the root contains amide alkaloids but the phytochemical compositions of the leaves is still unknown. So, further investigations of the mechanism(s) of action of the leaves extract, and the active substance(s) responsible for its CNS depressant action, are necessary.

Acetic acid induced writhing in rats attributed visceral pain finds much attention of screening analgesic drugs.[16] The two different doses (100 & 200 mg/kg b. wt.) of crude extract of the plant showed significant analgesic action compared to the reference drug Indomethacin but higher dose (200 mg/kg) was found to exhibit higher analgesic activity against acetic acid induced pain in rats. Pain sensation in acetic acid induced writhing method is elicited by triggering localized inflammatory response resulting release of free arachidonic acid from tissue phospholipid via cyclooxygenase (COX), and prostaglandin biosynthesis [17, 18].

In other words, the acetic acid induced writhing has been associated with increased level of PGE2 and PGF2α in peritoneal fluids as well as lipoxygenase product [19]. The increase in prostaglandin levels within the peritoneal cavity then enhances inflammatory pain by increasing capillary permeability [20].

The acetic acid induced writhing method was found effective to evaluate peripherally active analgesics. The agent reducing the number of writhing will render analgesic effect preferably by inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition [21].

The significant pain reduction of the plant extract might be due to the presence of analgesic principles acting with the prostaglandin pathways. The abdominal writhing induced by acetic acid was also reported to be less selective and proposed to act indirectly by releasing endogenous mediators stimulating neurons that are sensitive to other drugs such as narcotics and centrally acting agents [22, 23].

The pain in the early phase of formalin test was due to the direct stimulation of the sensory nerve fibers by formalin while the pain in the late phase was due to inflammatory mediators, like histamine, prosta-glandins, serotonin and bradykinins [24]. This test is believed to be a more valid analgesic model which is better correlated with clinical pain [25, 26].

In this study, the extract caused a dose-dependent decrease in licking time (Table 4) by the rats injected with formalin signifying the analgesic effect of the extract.

There is no scientific report on phytochemical composition of the leaves of the *P. longum*. The leaves of the *Piper nigrum* belongs to the same family of *Piper longum* contain lignan compounds that show analgesic activity like NSAIDs which may also be responsible for analgesic activity of our test sample. But to ascertain the mode of analgesic action, phytochemical analysis of the methanolic leaves extract and their biological activities are essential to study.

CONCLUSION: On the basis of results obtained from the present study, it can be concluded that the plant extract possesses remarkable CNS depressant and analgesic activities, thereby lends support to the traditional use of the plant in painful and inflammatory disorders. Present work was a preliminary effort which will require further detailed investigation including characterization of active compounds and requires preformulation studies for development of a potential dosage form.

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