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CNS Depressant, Antidiarrheal and Antipyretic Activities of Ethanolic Leaf Extract of *Phyllanthus acidus* L. on Swiss Albino Mice

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Authors' contributions

This work was carried out in collaboration between all authors. Author MSS designed and supervised the study. Author AD wrote the first draft of the manuscript. Author MSH managed the literature searches, managed the experimental process and performed statistical analysis. Author SA collected the plant materials and participated in laboratory work. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The study was to evaluate the CNS depressant, antidiarrheal and antipyretic activities of ethanolic leaf extract of *Phyllanthus acidus* L.
Methodology: The ethanolic extract of *P. acidus* leaves was divided into two concentrations 250 mg/kg body weight and 500 mg/kg body weight. CNS depressant activity of *P. acidus* was investigated in swiss albino mice using hole-cross, hole-board and open-field models. Castor-oil induced diarrhea and gastrointestinal motility test with barium sulfate milk was used to assess antidiarrheal activity whereas Brewer's yeast-induced hyperthermia in mice was used to investigate the antipyretic activity respectively.
Results: *P. acidus* significantly (P = 0.05) reduce the CNS depression of the studied animals in different tested models and the effects were found to be dose-dependent which were comparable to the standard drug diazepam. In castor oil induced diarrheal model the plant extract reduced the

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time of onset and severity of diarrhea with significant (P = 0.05) inhibition of 42.86% and 64.29% for 250 and 500 mg/kg b.w extracts respectively whereas the standard drug loperamide (5 mg/kg) showed 71.43% diarrheal inhibitory activity. Distance of gastrointestinal motility was significantly (P = 0.05) reduced to 62.05±1.74% from control distance 79.06±2.93% in barium sulfate milk model by the highest dose 500 mg/kg. In yeast induced pyrexia, the plant extract demonstrated dose dependent protection at 250 and 500 mg/kg, similar to standard drug, paracetamol at 500 mg/kg.

Conclusion: In this study, we found that *P. acidus* possess significant CNS depressant, antidiarrheal and antipyretic activities and therefore it could be an excellent source for natural CNS depressant, antidiarrheal and antipyretic agents for medical applications.

Keywords: CNS depressant; antidiarrheal; antipyretic; Phyllanthus acidus.

1. INTRODUCTION

CNS depression may be taken into account as a major affective brain disorder which is mainly characterized by modification in mood, lack of interest about the surroundings, apathy and loss of energy, mental object retardation, and melancholia further as profound feelings of cheerlessness, despair and self-destructive cerebration. This disease is prevalent among about 5% of the general population [1]. Although there are several antipsychotics available in the market but these drugs are associated with a variety of autonomic, endocrine, allergic, hematopoietic and neurological side effects [2]. Therefore, a large number of complementary and alternative medicines are being used for the treatment of psychiatric disorders [3].

Diarrheal disease is a major health care problem leading to mortality and morbidity, especially among children in developing countries [4]. The major causative agents of diarrhea in humans include some microbes such as *Shigella flexneri, Staphylococcus aureus, Escherichia coli, Salmonella typhi and Candida albicans* [5,6]. Although a wide spectrum of approaches are available for the management of diarrhea but still a vast majority of people in developing countries rely on herbal drugs for the management of diarrhea. Therefore, WHO has encouraged studies for treatment and prevention of diarrheal diseases depending on traditional medical practices [7].

Pyrexia or fever occurs as a result of secondary implication of inflammation [8] while enhanced production of prostaglandins is the key factor for the induction of pain, inflammation and fever [9]. Thus, most anti-inflammatory agents are also expected to possess analgesic and antipyretic activities as they inhibit or prevent excess production of prostaglandins [10]. Due to the adverse effects of non-steroidal anti-inflammatory drugs (NSAIDs) and opioids, there is a high demand for these arches of new drugs with lesser or no side effects. In the context, current trend of research has shifted towards medicinal plants because of their affordability and accessibility with lesser side effects [11].

All plant species possess certain phytochemicals which can be poisonous, medicinal and nutritional [12]. For the treatment of various human diseases a wide spectrum of central nervous system (CNS) depressants, antipyretics and antidiarrheal drugs from both natural and synthetic origin have been proposed in several times [12,13]. But due to severe toxic effects (e.g. liver damage, mutagenesis) and emerging incidences of drug resistance, the search of newer drugs from natural origin has become essential for modern age [13]. *P. acidus* is a medicinal plant, belonging to Phyllanthaceae, having a wide spectrum of folkloric or traditional use [14]. The plant is well distributed throughout the sub-continental region [15] and is used in the treatment of various ailments like emetic and purgative [16], hypertension and respiratory disorder [17], nociceptive disorders [18] and as laxative [19]. In this context, the aim of the present study was to investigate the CNS depressant, antidiarrheal and antipyretic activities of *P. acidus* leaf extract with the intension of searching for newer drugs.

2. MATERIALS AND METHODS

2.1 Drugs and Chemicals

All the chemicals used in this study were of analytical grade, and purchased from Sigma Chemical Co. (St. Louis, MO, USA), and Merck (Darmstadt, Germany). Normal saline solution (0.9% NaC1) (Beximeo Infusion Ltd.), Diazepam

(Incepta Pharmaceuticals Ltd.), Loperamide (Square Pharmaceuticals Ltd.), etc. were used for conducting the tests.

2.2 Collection of Plants

The fresh leaves of the plant were collected from Ashuganj, Brahmanbaria, Bangladesh. The plant was identified by the taxonomist of Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh and a voucher specimen was deposited in the herbarium unit (accession number DACB: 39673).

2.3 Preparation of Extract

Weighed (500 g of the dried and powdered) sample was soaked in 2200 ml of 99% ethanol in clean, sterilized, and flat-bottomed glass container. Afterwards, it was sealed and maintained for 15 days accompanying occasional stirring and agitation. The complete mixture was then subjected to coarse filtration on a piece of clean, white sterilized cotton material and Whatman® filter paper. The extract was obtained by evaporation using rotary evaporator (Bibby RE-200, Sterilin Ltd., UK) at 4 rpm and 65ºC temperature. It rendered a gummy concentrate of greenish color. The gummy concentrate was designated as crude extract or ethanolic extract. Then the crude ethanolic extract was dried by freeze drier and preserved at +4ºC for further analysis.

2.4 Experimental Animals

Healthy albino mice of Swiss strain of either sex were used. They were collected from the animal Resource Branch of the "International center For Diarrheal Disease and Research, Bangladesh (ICDDR, B)" and were housed in standard conditions of temperature (25±2℃), 12 hours light per day cycle, relative humidity of 45-55 %. They were fed the ICDDR, B formulated rodent food and water. Animals were kept and all operation on animals was done in aseptic condition.

2.5 CNS Depressant Activity Test

2.5.1 Hole-cross test

The method was followed as described by Hussain et al. [20]. A cage with a fixed steel partition in the middle position having a size of 30 cm×20 cm×14 cm was taken. A hole of 3 cm diameter was made at a height of 7.5 cm in the midpoint of the cage. The mice were divided into group A (control), group B (standard) group C (250 mg/ kg body weight) and group D (500 mg/kg body weight). 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 passages of a mouse through the hole from one chamber to the other was counted for a period of 3 min at 0, 30, 60, 90 and 120 min after oral administration of test drugs.

2.5.2 Hole board test

The experiment was carried out by established method as described by Emran and Rahman [21]. A steel partition was fixed in the middle of a cage of 30 × 20 × 14 cm3. A hole of 3 cm diameter was made at a height of 7.5 cm in the center of the cage. The animals were divided into group A (control), group B (standard), group C (250 mg/ kg body weight) and group D (500 mg/kg body weight) containing five mice each. The number of passages of a mouse through the hole from one chamber to the other was counted for a period of 3 min at 0, 30, 60, 90 and 120 min after oral administration of the extract. 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 carried out according to the methods described by Gupta et al. [22]. In the open field test, the animals were divided into group A (control), group B (standard), group C (250 mg/ kg body weight) and group D (500 mg/kg body weight) containing five mice each. Similar doses mentioned in previous section were used for both test and control groups. The floor of a half square meter open field was divided into a series of squares each alternately colored black and white. The apparatus had a 40 cm height wall. The number of squares visited by the animals was counted for 3 min at 0, 30, 60, 90 and 120 min after oral administration of the test drug.

2.6 Antidiarrheal Activity Test

2.6.1 Castor oil-induced diarrhea

The method described by Shoba and Thomas [23] was followed for this study. Twenty swiss albino mice were randomly divided into four equal groups (n = 5) as group A (control group), group B (standard group) and two individual treated groups. Control group received only

distilled water, standard group received loperamide 5 mg/kg body weight and two treated groups as group C and group D received 250 mg/kg body weight and 500 mg/kg body weight of ethanolic extract of *P. acidus* leaf orally. Mice were housed in separate cages having blotting paper placed below for collection of fecal matters. Diarrhea was induced by oral administration of castor oil (2 ml/mouse). Extract and drugs were given orally 1 h before the administration of standard dose of 2 ml of castor oil. Diarrhea was defined as the presence of fluid material in the stool, which was stained by the absorbent paper placed beneath the cage. The number of diarrheal episodes in terms of both hard and soft pellet was counted at every hour over 5 h periods for each mouse. Percent inhibition (PI) was calculated as follows:

PI = Mean defecation (control group-treated group) × 100 / Mean defecation of control group

2.6.2 Gastrointestinal motility test with barium sulfate milk

This experiment was carried out by the method described by Chatterjee [24]. Overnight fasted twenty swiss albino mice were randomly divided in to four equal groups (n=5). Group A(Control) received only distilled water 2 mL/mouse orally whereas group B (standard) received commercially available anti-diarrheal drug loperamide 5 mg/kg orally. Two treated groups as group C and group D received 250 mg/kg body weight and 500 mg/kg body weight of ethanolic extract of *P. acidus*. Thirty minutes later 2 mL of 10% barium sulfate solution were administered in all groups of mice. After 30 min mice were sacrificed. And the distance traveled by $BaSO_4$ milk was measured and expressed as a percentage of the total length of small intestine (from pylorus to the ileo-cecal junction).

2.7 Antipyretic Activity Test

2.7.1 Brewer's yeast induced pyrexia

The antipyretic activity was evaluated by Brewer's yeast induced pyrexia in experimental animal. Hyperpyrexia was induced by subcutaneous administration of 10 ml/kg body weight 20% aqueous suspension of brewer's yeast. The selected animals were fasted overnight with water ad libitum before the experiments. Initial rectal temperature of animals was recorded using an Ellab thermometer (33.19±0.40℃). After 18 h of subcutaneous administration, the animals that showed an increase of 0.3–0.5℃ in rectal temperature were selected for the antipyretic activity. Crude ethanolic extract of plant was given at the dose of 250 mg/kg body weight and 500 mg/kg body weight to two treated groups. Paracetamol as standard drug was given at the dose of 150 mg/kg whereas distilled water (10 ml/kg body weight) was given to control group. The rectal temperature was recorded at 1 h intervals for 3 h after treatment [25].

2.8 Statistical Analysis

One way ANOVA with Dunnett's post Hoc test for this experiment was carried out with SPSS 16.0 for Windows® software 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

Results of the hole-cross test of *P. acidus* leaves are shown in Table 1. The locomotors activity lowering effect was manifested at the 2nd observation (30 min) period and was sustained up to the 5th observation period (120 min) for extracts. The extracts diminished the locomotion activity in a dose dependent manner which was comparable with standard diazepam. At 250 and 500 mg/kg, the extract showed depressant activity of 5.25±0.96 and 4.00±3.65 respectively whereas the standard drug diazepam showed 2.75±0.75 at the dose of 1 mg/kg.

3.1.2 Hole-board test

In the hole-board test, the extract at dose 500 mg/kg body weight showed a significant (P= 0.05) number of head dipping behavior after 90 and 120 minutes (6.25±4.35 and 7.00±3.37 respectively) when compared to the control group. However, at 120 min the standard drug, diazepam at dose 1 mg/kg, showed significant (P= 0.05) head dipping (12.50±1.61) behavior compared with the control group (Table 2).

3.1.3 Open field test

After statistical analysis of the experimental data, it was observed that in open field test, the number of squares traveled by the mice was suppressed significantly in the test group throughout the study period (Table 3). The CNS

depressant activity observed for the extract was dose dependent and a noticeable result was found at 120 min of test sample administration. Test animals were showing significant (P < 0.001) decrease in number of movement at the dosages of 500 mg/kg (14.75±0.94), as compared to 39.75±2.51 in the control group and 12.25±0.70 in the standard group) after 120 min of administration of the extract.

3.2 Antidiarrheal Activity

3.2.1 Castor oil-induced diarrhea

The study results showed that *P. acidus* plant extract significantly (P < 0.001) inhibited the mean number of defecation when compared to control group of diarrhea induced by castor oil (Table 4). The number of stools at 1 to 5 hours for methanol extract treated group was significantly (P < 0.01) decreased as compared to control group. *P. acidus*, at the dose of 500 mg/kg b.w. showed 64.29% inhibition of diarrheal stools. While at the dose of 250 mg/kg b.w. it showed 42.86% inhibition. The standard drug loperamide at the dose of 5mg/kg showed 71.43% diarrheal inhibitory potency.

3.2.2 Gastrointestinal motility test with barium sulfate milk

The methanol extract of *P. acidus* showed dose-dependent inhibition of barium sulfate induced gastrointestinal motility in albino rat. This effect was significant even at lower dose of 250 mg/kg over 30 min as compared to normal control, however, this activity was less as compared to loperamide as shown in Table 5. *P. acidus* leaf extract decreased the distance of gastrointestinal motility of rats from 79.06±2.93% (control group) to 62.05±1.74% (treated group). However, loperamide (1 mg/kg) exhibited much more marked reduction of 60.38±1.53% with barium sulfate milk at 30 min study.

3.3 Antipyratic Activity

3.3.1 Brewer's yeast-induced pyrexia

From the results (Table 6), it was observed that, experimental mice showed a marked increase in rectal temperature, 18th h after Brewer's Yeast injection. In the first 30 minute (0.5 h) the extract did not show anti-pyretic activity. The extract treatment, with 250 and 500 mg/kg, significantly reduced the rectal temperature of the animals in the first, second and third hour after administration, reaching the peak of antipyretic effect with the highest dose (500 mg/kg) in the 3rd h (35.26±0.52℃, P < 0.001), in relation to control (35.75±0.49℃). The paracetamol treatment (150 mg/kg) caused significant antipyretic effect at all time periods, reaching the peak in the 3rd h (35.75±0.49℃, P < 0.001), in comparison to control.

Table 1. Effect of ethanolic extract of the *P. acidus* leaves on hole cross test in mice

Group	Number of movements (Mean±SEM)				
	0 min	30 min	60 min	90 min	120 min
Group A	5.00±2.16	5.25±4.03	3.25±1.26	2.25±0.96	2.00±1.41
Group B	6.00±3.83	9.00±2.83	8.00±4.08	5.50±1.70	2.75±0.75
Group C	4.25±2.87	5.75±2.87	4.75±0.96	3.75±1.06	5.25±0.96
Group D	9.50±1.73*	5.00±1.82	4.50±1.91	3.75±1.30	4.00±0.65

*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*

Table 2. Effect of ethanolic extract of the *P. acidus* leaves on hole board test in mice

Group	Number of movements (Mean±SEM)				
	0 min	30 min	60 min	90 min	120 min
Group A	39.75±3.42	23.75±3.77	17.00±4.16	20.75±2.85	20.00±3.74
Group B	47.50±2.57	23.25±3.68	20.25±2.59*	16.00±2.71*	12.50±1.61*
Group C	24.00±0.82**	27.75±2.07	16.75±3.91	19.50±3.55	22.50±2.35
Group D	28.50±2.39	16.50±2.23	9.25±1.25	6.25±4.35*	7.00±3.37*

*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*

Table 3. Effect of ethanolic extract of the *P. acidus* leaves on open field test in mice

Group	Number of movements (Mean±SEM)				
	0 min	30 min	60 min	90 min	120 min
Group A	122.25±2.31	111.25±2.87	97.00±3.32	35.50±1. 16	39.75±2.51
Group B	175.75±2.53*	89.25±1.46**	48.25±1.92***	26 .25±71.94***	12.25±0.70***
Group C	123.75±2.53	91.75±1.51**	58.25±1.02**	33.50±1.59**	20.00±0.49**
Group D	143.25±1.17*	86.25±1.83**	46.75±1.56***	24. 25±1.07***	14.75±0.94***

*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*

Table 4. Effect of ethanolic extract of *P. acidus* leaves on the castor oil-induced diarrhea in mice

Group	Total number of feces	Number of diarrhea feces	Inhibition of diarrhea (%)
Group A	11.50±1.29	3.50±0.58	-
Group B	6.50±1.26 ***	1.00±0.82 **	71.43
Group C	5.50±2.38 **	2.00±1.15 *	42.86
Group D	3.25±2.22 ***	1.25±0.5 **	64.29

*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*

Table 5. Effect of ethanolic extract of *P. acidus* leaves on gastrointestinal motility with Barium sulfate milk on mice

Group	Gastro-intestinal motility			
	Total length of GI (cm)	Length of $BaSO_4$ travelled (cm)	% motility	Mean±SEM
Group A	42	30	71.43	79.06±2.93
	53	49	92.45	
	53	32	60.38	
	50	46	92.00	
Group B	46	25	54.35	60.38±1.53*
	51	32.5	63.73	
	52	28.5	54.81	
	51	35	68.63	
Group C	43	31	72.09	73.07±2.12
	56	39.5	70.54	
	51	34.5	67.65	
	50	41	82.00	
Group D	48	19	39.58	62.05±1.74*
	50	30	60.00	
	49	40	81.63	
	53	35.5	66.98	

*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*

Table 6. Effect of ethanolic extract of *P. acidus* leaves on Brewer's yeast-induced pyrexia in mice

Treatment	Dose (mg/kg)	Temperature in ℃				
		Initial	Pyretic	1 h	2 h	3 h
Group A	10	35.68±0.55	37.22±0.65	37.04±0.95	35.58±0 .22	35.75±0.49
Group B	150	36.31±0.44	36.28±0.38	35.88±0.90 ***	35.18±0.22 ***	35.19±0.35 ***
Group C	250	36.31±0.73	37.32±0.87	36.06±0.70 ***	35 .68±0.61 *	35.63±0.45 **
Group D	500	35.81±0.63	36.92±0.67	34.96±0.26 ***	35 .32±0.17 **	35.26±0.52 ***

*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 t-test. Asterisks indicated statistically significant values from control, * indicates P= 0.05, ** indicates P < 0.01 and *** indicates P < 0.001*

4. DISCUSSION

The ethanolic extract of *P. acidus* leaves were studied and showed significant CNS depressant, antidiarrheal and antipyretic effects in animal models. This plant has long been used for its different medicinal values but there is limited data regarding leaf extract. Hence it was considered that investigation for the medicinal properties of this plant might give scientific authentication to the traditional claims.

The plant extract showed marked CNS depressant activity in hole cross, hole broad and open field methods in comparison to the control drug and the effects were dose dependent. It is known that Gamma-amino-butyric acid (GABA) act as a major inhibitory neurotransmitter in the central nervous system [26] and many anxiolytic, muscle relaxants and sedative-hypnotic drugs exert their actions via GABA [27]. Previous researches showed that phytoconstituents like flavonoids and neuroactive steroids act as ligands for the $GABA_A$ receptors in the central nervous system which led to the postulation that these compounds may act as benzodiazepine like molecules [27]. Therefore it may be assumed that the plant extract may exert it action by potentiating GABAergic inhibition in the CNS by membrane hyperpolarization [28]. Phytochemical assessment showed the presence of alkaloids, flavonoids, saponins and steroids in the plant [29] which supports the result its CNS depressant activity of our present study.

Our research focused on the anti-diarrheal activity of the plant extract using two different methods namely castor oil induced diarrhea and gastrointestinal motility test with barium sulfate milk. There are several proposed mechanisms to describe the effect of castor oil in the induction of diarrhea which include inhibition of intestinal Na^+,K^+-ATPase activity, activation of adenylate cyclase or mucosal cAMP mediated active secretion, stimulation of prostaglandin formation, platelet activating factor. Most recently it was claimed that nitric oxide contributes to the diarrhoeal effect of castor oil [30]. Although there are numerous methods regarding the mood of action of castor oil induced diarrhea but still the exact mechanism of action is unknown [31]. Among these methods it is well documented that castor oil produces diarrhea via ricinoleic acid which elevates prostaglandin biosynthesis [32]. Since the methanol extract of *P. acidus* successfully inhibited the castor oil-induced diarrhoea, therefore the extracts might have exerted their antidiarrhoeal action through the inhibition of prostaglandin biosynthesis by antisecretory mechanism.

Furthermore, barium sulphate induced diarrhea is presumed to be by osmotic properties and chole-cystokinin production [33]. Barium sulphate increases the volume of the intestinal content by preventing the reabsorption of water. It also promotes the liberation of cholecystokinin from duodenal mucosa, which increases the secretion and motility of small intestine and also prevents the reabsorption of NaCl and water [34]. In our study we have found that the plant extract showed significant gut motility inhibitory activity which is one of the important factors contributing to antidiarrheal activity of extract.

Subcutaneous injection of Brewer's yeast induces pyrexia by increasing the synthesis of prostaglandin. It is considered as a useful test for the screening of plants materials as well as synthetic drugs for their antipyretic effect [35]. Yeast induced pyrexia is called pathogenic fever and its etiology could be the production of prostaglandins [36]. The inhibition of prostaglandin synthesis could be the possible mechanism of antipyretic action as that of paracetamol and the inhibition of prostaglandin can be achieved by blocking the cyclooxygenase enzyme activity. There are several mediators for pyrexia and the inhibition of these mediators is responsible for the antipyretic effect [37]. The intraperitoneal administration of *P. acidus* significantly attenuated rectal temperature of yeast induced febrile mice. Thus it can be postulated that *P. acidus* contained pharmacologically active principle(s) that interfere with the release of prostaglandins.

5. CONCLUSION

From the essence of the study, it can be postulated that the leaf extract of *P. acidus* has potent CNS depression, antidiarrheal and antipyretic activities and these activities may be attributed due to the presence of various phytochemicals in the extract. However, further research is needed in order to find out the precise mechanisms and responsible chemical constituents for the above mentioned pharmacological activities.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Hoskeri JH, Venkatarangaiah K, Hanumanthappa SK, Vootla SK, Gadwala M. CNS depressant activity of extracts from *Flaveria trinervia* Spring C. Mohr. Phytopharmacol. 2011;1:101-108.
2. Zhang ZJ. Therapeutic effect of herbal extract and constituents in animal models of psychiatric disorders. Life Sci. 2004;75: 1659-1699.
3. Selvi PT, Kumar MS, Yaswanth T, Adiyaman E, Anusha PT. Central nervous system depressant activity of aqueous extract of leaves of *Azadirachta indica* Linn in mice. Asian J Pharm Res. 2012;2:97-99.
4. Havagiray R, Ramesh C, Sadhna K. Study of antidiarrhoeal activity of *Calotropis gigantea* R.Br. in experimental animals. J Pharm Pharm Sci. 2004;7:70–75.
5. Toyin MY, Khadijat OF, Saoban SS, Olakunle AT, Abraham BF, Luqman QA. Antidiarrheal activity of aqueous leaf extract of *Ceratotheca sesamoides* in rats. Bangladesh J. Pharmacol. 2012;7:14-20.
6. Robert K, Egon S, Daniela B, Florian D, Christoph W, Gunter JK, Emil CR. Role of *Candida* in antibiotic-associated diarrhoea. J. Infect. Dis. 2001;184:1065–1069.
7. Umer S, Tekewe A, Kebede N. Antidiarrhoeal and antimicrobial activity of *Calpurnia aurea* leaf extract. BMC Complement. Alt. Med. 2013;13:21.
8. Khan A, Baki MA, Al-Bari MAA, Hasan S, Mosaddik MA, Rahman MM, Haque ME. Antipyretic activity of roots of *Laportea crenulata* gaud in rabbit. Research Journal of Medicine and Medical Sciences. 2007; 2:58–61.
9. Rang HP, Dale M, Ritter J. Pharmacology. 4th Ed. New York. Churchill Livingstone; 2001.
10. Dewanjee S, Maiti A, Sahu R, Dua TK, Mandal SC. Study of anti-inflammatory and antinociceptive activity of hydroalcoholic extract of *Schima wallichii* bark. Pharm Biol. 2009;47:402–407.
11. Ibrahim B, Sowemimo A, Rooyen AV, Venter MV. Antiinflammatory, analgesic and antioxidant activities of *Cyathula prostrata* (Linn.) Blume (Amaranthaceae). J Ethnopharmacol. 2012;141:282–289.
12. Khatoon MM, Khatun MH, Islam ME, Parvin MS. Analgesic, antibacterial and central nervous system depressant activities of *Albizia procera* leaves. Asian Pac J Trop Biomed. 2014;4(4):279-284.
13. Bhattacharjee I, Chatterjee SK, Ghosh A, Chandra G. Antibacterial activities of some plant extracts used in Indian traditional folk medicine. Asian Pac J Trop Biomed. 2011; 1(2):165-169.
14. Chakraborty R, Biplab D, Devanna N, Sen S. Antiinflammatory, antinociceptive and antioxidant activities of *Phyllanthus acidus* L. extracts. Asian Pac J Trop Biomed. 2012;2(2):953-961.
15. Devi SS, Paul SB. An overview on *Cicca acida* (*Phyllanthus acidus*). Assam University Journal of Science and Technology. 2011;7(1):156-160.
16. Lemmens RH, Bunyapraphatsara M1, Padua De LS. Plant resources of south-East Asia. Medicinal and poisonous plants I. Prosea Foundation, Bogor, Indonesia. 1999;12(1):386-387.
17. Sousa M, Ousingsawat J, Seitz R, Puntheeranurak S, Regalado A, Schmidt A, Grego T, Jansakul E, Amaral MD, Schreiber R, Karl KK. An extract from the medicinal plant *Phyllanthus acidus* and Its isolated compounds induce airway secretion: A potential treatment for cystric fibrosis. Mol. Pharmacol. 2007;7(1):336-337.
18. Catapan E, Otuki M, Viana AM, Yunes RA, Bresciani LF, Ferreira, Santos AR, Calixto JB, Cechinel-Filho V. Pharmacological activity and chemical composition of callus cultures extracts from selected species of *Phyllanthus*. Pharmazie. 2000;55(12):945-946.
19. Prasad D. Edible fruits and vegetables of the English speaking Caribbean. Caribbean Food and Nutrition Institute. Kingston, Jamaica; 1986.
20. Hussain J, Ur Rehman N, Hussain H, Al-Harrasi A, Ali L, Rizvi TS. Analgesic, anti-inflammatory, and CNS depressant

activities of new constituents of *Nepeta clarkei*. Fitoterapia. 2012;83(3):593-598.
21. Emran TB, Rahman MA. Sedative, anxiolytic and analgesic effects of *Urena sinuata* L. leaf extract in animal models. Int Food Res J. 2014;21(5):2069-2075.
22. Gupta BD, Dandiya PC, Gupta ML. A psychopharmacological analysis of behavior in rat. Jpn J Pharmacol. 1971;21: 293.
23. Shoba FG, Thomas M. Study of antidiarrheal activity of four medicinal plants in castor oil-induced diarrhea. J. Ethnopharmacol. 2001;76(1):73-76.
24. Chatterjee TK. Handbook on laboratory mice and rats. 1st ed. Kolkata, Department of Pharmaceutical Technology, Jadavpur University. 1993;157.
25. Bhowmick R, Sarwar MS, Dewan SMR, Das A, Das B, Uddin MMN, Islam MS. In vivo analgesic, antipyretic, and anti-inflammatory potential in Swiss albino mice and *in vitro* thrombolytic activity of hydroalcoholic extract from *Litsea glutinosa* leaves. Biol Res. 2014;47:1-8.
26. Ripa FA, Dash PR, Faruk MO. CNS depressant, analgesic and anti-inflammatory activities of methanolic seed extract of *Calamus rotang* Linn. fruits in rat. J Pharmacogn Phytochem. 2015;3(5):121-125.
27. Verma A, Jana GK, Sen S, Chakraborty R, Sachan S, Mishra A. Pharmacological evaluation of *Saraca indica* leaves for central nervous system depressant activity in mice. J Pharm Sci Res. 2010;2(6):338-343.
28. Kolawole OT, Makinde JM, Olajide OA. Central nervous system depressant activity of *Russelia equisetiformis*. Niger J Physiol Sci. 2007;22:59-63.
29. Kumari OS, Rao DNB, Chawhan LP, Rachel B. Phyto-chemical analysis of *Phyllanthus amarus* (Nela Usiri), *Phyllanthus emblica* and *Phyllanthus acidus*. World Journal of Pharmaceutical Research. 2014;4(1):1457-1462.
30. Rouf R, Uddin SJ, Shilpi JA, Alamgir M. Assessment of antidiarrhoeal activity of the methanol extract of *Xylocarpus granatum* bark in mice model. J. Ethnopharmacol. 2007;109(3):539-542.
31. Mascolo N, Izzo AA, Autore G, Barbato F, Capasso F. Nitric oxide and castor oil-induced diarrhea. J. Pharm. Exp. Ther. 1994;268:291–295.
32. Ripa FA, Morshed MTI, Sharmin AA, Papon SB, Islam R, Sheikh Z. Central nervous system depressant, analgesic and antidiarrheal effects of the seed extracts of *Dimocarpus longan* Lour in rats. Trop. J. Pharm. Res. 2014;13:235-242.
33. Galvez J, Zarzuelo A, Crespo ME, Lorente MD, Ocete MA, Jimenez J. Antidiarrhoeic activity of *Euphorbia hirta* extract and isolation of an active flavonoid constituent. Planta Med. 1993;59(4):333-336.
34. Rahman MA, Sultana R, Akter R, Islam MS. Antidiarrheal and antidiabetic effect of ethanol extract of whole *Ageratum conyzoides* L. in albino rat model. Afr. J. Pharm. Pharmacol. 2013;7(23):1537-1545.
35. Khan I, Nisar M, Ebad F, Nadeem S, Saeed M, Khan H. Anti-inflammatory activities of Sieboldogenin from *Smilax china* Linn.: Experimental and computational studies. J Ethnopharmacol. 2009;121(1):175–177.
36. Muhammad N, Saeed M, Khan H. Antipyretic, analgesic and anti-inflammatory activity of *Viola betonicifolia* whole plant. BMC Complement Altern Med. 2012;12(1):59.
37. Rawlins M. Mechanism of salicylate-induced antipyresis. S Karger AG. 1973;311.

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