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Analgesic, Anti-diarrheal, CNS-depressant, Membrane Stabilizing and Cytotoxic Activities of *Canavalia virosa* (Roxb.) W&A

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Abstract

The methanol extract of the leaves of *Canavalia virosa* (Roxb.) W&A was investigated for the evaluation of analgesic, anti-diarrheal and CNS-depressant activities in Swiss albino mice. The analgesic activity was assessed by formalin-induced paw licking method, where the crude extract of *C. virosa* (400 mg/kg, b.w.) exhibited 41.46% reduction of licking response in mice as compared to 73.17% reduction exhibited by standard acetylsalicylic acid. In the castor oil-induced diarrhea in mice, the plant extract at the dose of 200 mg/kg, b.w., demonstrated 56% reduction of diarrheal feces in mice, while the standard loperamide revealed 76% reduction of diarrheal feces. The CNS-depressant activity of the plant extract was assessed through phenobarbitone-Na induced sleeping time test. The methanol extract of *C. virosa* and its different Kupchan fractions were also subjected to screen membrane stabilizing and cytotoxic activities using acetylsalicylic acid and vincristine sulphate as standard, respectively.

Key words: *Canavalia virosa*, analgesic, anti-diarrheal, CNS-depressant, membrane stabilizing, cytotoxic.

Introduction

Plants have always been a vital source of medicine for mankind. Lead structures elucidated from the medicinal plants are targeted for the development of new therapeutic agent with enhanced activity and reduced toxicity. Approximately 119 pure chemical substances extracted from higher plants are used as medicines throughout the world (Farnsworth *et al.*, 1985). According to World Health Organization (WHO), 80% of the population in Africa and the majority of the populations in Asia and Latin America use traditional medicines for their primary healthcare needs.(Ghani *et al.*, 2003; Ezuruike *et al.*, 2014; Samsam and Moatar *et al.*, 1991). *Canavalia virosa* (Roxb.) (Local name: Kathshim; Family: Fabaceace) is a climbing perennial, common throughout India and found wild in scrub jungles. *C. virosa* extends southward as from Arabia, Socotra and India, through tropical Africa into north-east South Africa (Westphal *et al.*, 1974). Seeds are used in scorpion sting (Raju *et al.*, 1996). Root is used in malaria (Karuppusamy *et al.*, 1998). Where as root and bark are used to eradicate intestinal worms (Sudhakar *et al.*, 1985). Chemical analysis of seeds showed the presence of crude protein (26%), pentosan (11.6%), water-soluble mucilage (4.1%), protein and pentosan were absent in the mucilage (Kapoor *et al.*, 1978). The aqueous extract can potentiate pentobarbitone hypnosis in mice and morphine catalepsy in albino rats (Mukhopadhyay *et al.*, 1986). The application of seeds, after removal of seed coat, was effective in giving complete relief of the symptoms of scorpion and centipede poisoning within 5-7 hrs

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(Jayavardhanan *et al.,* 1986). Leaves are reported to possess antiulcer activity (Lavanya *et al.,* 2012). As the leaves are predominately employed as medicine, the present study was designed to evoluete the analyses, antidiarrhed, CNS depressant, mambrane stabilizing and cytotoxic activities in Swiss albino mice.

Materials and Methods

Plant material: Leaves of *C. virosa* (Roxb.) W&A were collected from Savar, Dhaka, Bangladesh, and identified in Bangladesh National Herbarium, Mirpur, Dhaka where a voucher specimen has been deposited.

The plant sample was air-dried and ground to a coarse powder using a grinding machine. The powdered material (425 g) of *C. virosa* was macerated in 2.5 L of methanol for 15 days and finally, filtered through Whatman filter paper number 1. The filtrate was concentrated using rotary evaporator at 40°C under reduced pressure. About 5 g of the concentrated extract of *C. virosa* was subjected to solvent-solvent partitioning following the modified Kupchan method (Van Wagenen *et al.*, 1993) to yield petroleum-ether, dichloromethane, chloroform and aqueous soluble fractions. Then the crude extract and its Kupchan fractions were separately evaluated for biological activities.

Drugs and reagents: Methanol, formalin, Tween-80, loperamide (Square Pharmaceuticals Ltd.), normal saline (Opsonin Pharmaceuticals Ltd.), morphine and phenobarbitone-Na (Gonoshasthaya Pharmaceuticals Ltd.), acetyl salicylic acid (Essential Drugs Company Ltd.) and castor oil were collected from local market. All other chemicals and solvents were of analytical grade.

Experimental animal: Swiss Albino mice (28-30 of either sex aged 4-5 weeks were collected from the Animal Resource Branch of the International Centre for Diarrheal Diseases and Research, Bangladesh (icddr,b). The mice were housed in standard polypropylene cages and kept at room temperature (24 ± 2ºC) and relative humidity (60-70%) in a 12 hrs light-dark cycle. The animals were fed with icddr,b formulated diet and water *ad libitum.*

Analgesic activity: The analgesic activity of *C. virosa* extract was determined by formalin-induced paw licking method in mice (Barua *et al.*, 2011). In this method, the test groups received the plant extract at 200 and 400 mg/kg b.w., while the positive control- and negative control-mice were treated orally with standard drug acetylsalicylic acid (50 mg/kg**,** p.o.) and 1% Tween-80 in normal saline, respectively. About 30 min after treatment, 1% formalin (0.1 ml/10 g, b.w.) was injected subcutaneously under dorsal surface of the hind paw and the time spent for licking the injected paw with formalin was counted for 5 min post formalin injection and considered as indications of the pain stimuli. The percent (%) inhibition of licking response in comparison to control group was taken as an index of analgesia and was calculated using the following formula:

$$\% \text{ Inhibition} = (L_C - L_T)/L_C \times 100\%$$

where, L_C = licking response by control and L_T = licking response by test groups.

Anti-diarrheal activity: Anti-diarrheal activity of the crude methanolic extract of *C. virosa* was carried out following the published method (Shoba and Thomas, 2001) with slight modification. The negative control group received vehicle (1% Tween-80 in normal saline) at 10 ml/kg b.w. orally, while the positive control group received loperamide (50 mg/kg b.w.) orally. The test group received the extract of *C. virosa* at 200 and 400 mg/kg b.w. orally. After 30 min intervals to ensure proper absorption of the administered substance, 1.0 ml of castor oil was fed to each mouse to induce diarrhea. Each animal was placed in an individual case, the floor of which was lined with blotting paper. Each of the mice was observed for 4 h to record the number of stool giving instances. The average of total number of stool given by the test group, and the control group was compared and the percent inhibition of defecation in mice was calculated by using the following equation:

$$\% \text{ Inhibition} = (M_C - M_T)/M_C \times 100$$

where, M_C = mean defecation of control and M_T = mean defecation of test groups.

CNS-depressant activity: For CNS-depressant assay the crude methanolic extract of *C. virosa* was subjected to phenobarbitone-Na induced sleeping time test (Rahman *et al.,* 2015). Here, the test groups were orally administered with test samples prepared with normal saline water and Tween-80 at doses of 200 and 400 mg/kg b.w., while the positive control group was treated with diazepam (1 mg/kg b.w.) and the negative control group with normal saline water containing 1% Tween-80. After 30 min, phenobarbitone-Na (40 mg/kg b.w.) was administered intraperitoneally to each mouse to induce sleep. The animals were observed for the latent period for time of onset of sleep (*i.e.,* time between phenobarbitone-Na injection and loss of righting reflex) and duration of sleep (*i.e.,* time between the loss and recovery of righting reflex).

Membrane stabilizing activity: The *C. virosa* extracts and its Kupchan partitionates were subjected to assay for membrane stabilizing activity following the method (Shinde *et al.,* 1999) of hypotonic-and heat-induced hemolysis of human erythrocyte using acetylsalicylic acid as standard.

Cytotoxic activity: To determine the cytotoxic activity, the plant extractives dissolved in dimethyl sulfoxide (DMSO) were applied against *Artemia salina* in a one-day assay using vincristine sulphate and DMSO as the positive and negative control, respectively (Rashid *et al.,* 2016).

Results and Discussion

The analgesic activity of *C. virosa* extract (Table 1) was assessed by formalin-induced paw licking method in mice. The formalin test has been narrated as a recognized method for producing and quantifying pain in rats (Dubuisson *et al.*, 1977). The test exerts a painful stimulus to which the animals show a spontaneous response and it is sensitive to commonly used analgesics. Subcutaneous injection of 1% formalin evoked a characteristic licking response in the Albino mice.

Table 1. Analgesic activity of methanol extract of *C. virosa* in Swiss Albino mice.

Test groups	Average time (s) of licking response	% Inhibition of licking response
Control	13.67	-
ASA (50 mg/kg b.w.)	3.67	73.17
MECV (200 mg/kg b.w.)	10.33	24.39
MECV (400 mg/kg b.w.)	8.00	41.46

Here, MECV = Methanol extract of *C. virosa*, ASA = Acetylsalicylic acid.

In this study, the plant extract showed activity in a dose-dependent manner where decrease in licking time and licking frequency were observed in the mice injected with formalin. There signify the analgesic effect of the extract. Although, the active doses of the plant extract were higher than those of the reference drug, it should be mentioned that the crude extract is made up of different compositions of many substances.

In the assay of anti-diarrheal activity induced by castor oil, the crude methanol extract of *C. virosa* showed marked anti-diarrheal effect in the mice, as shown in table 2. Here, the plant extract at the dose of 200- and 400-mg/kg b.w. demonstrated reduction of diarrheal feces by 46.15% and 57.67%, respectively when compared to loperamide which reduced the same by 61.59%. In the evaluation of anti-diarrheal activity, the crude extract revealed dose-dependant maaner which is also statistically significant.

The methanol extract of leaves of *C. virosa* slightly increased the sodium thiosulphate-induced sleeping time in a dose dependent manner (Table 3). The time of onset of sleep was 151.67 min in control group whereas in experimental group it was 162.00 min and 203.00 min at 400 and 200 mg/kg body weight, respectively.

In the present study, dichloromethane, chloroform and aqueous soluble fractions of the crude methanol extract of leaf of *C. virosa* showed

cytotoxicity in brine shrimp lethality bioassay which suggested that the test samples are biologically active. Each of the test samples showed different mortality rates at different concentrations. Plotting of log of concentration versus percent mortality for all test samples showed an approximate linear correlation. From the graphs, the median lethal concentration (LC_{50} = the concentration at which 50% mortality of brine shrimp nauplii occurred) was determined for the samples (Table 4).

Table 2. Antidiarrheal activity of methanol extract of *C. virosa* in Swiss Albino mice.

Test groups	No. of diarrheal feces	% Reduction of diarrheal feces
Control	8.33	-
Loperamide (50 mg/kg b.w.)	2.00	76.00
MECV (200 mg/kg b.w.)	3.67	56.00
MECV (400 mg/kg b.w.)	5.33	36.00

Table 3. CNS-depressant activity of crude extract of *C. virosa* in Swiss Albino mice.

Test groups	Time of onset of sleep (min.)	Total sleeping time (min)
Control	151.67	88.33
Diazepam (1 mg/kg b.w.)	117.33	122.67
MECV (200 mg/kg b.w.)	203.00	37.00
MECV (400 mg/kg b.w.)	162.00	78.00

Table 4. Membrane stabilizing and cytotoxic activities of different extracts of *C. virosa.*

Sample	% Inhibition of hemolysis		Cytotoxic activity (LC_{50}, µg/ml)
	Hypotonic solution-induced	Heat-induced	
MECV	75.19	67.27	133.18
PESF	58.31	57.93	207.57
DCMSF	27.82	3.96	6.50
CSF	33.33	4.65	14.19
AQSF	38.39	32.09	47.95
VS	--	--	0.451
ASA	61.90	42.00	--

Here, PESF = Petroleum-ether soluble fraction, DCMSF = Dichloromethane soluble fraction, CSF = Chloroform soluble fraction, AQSF = Aqueous soluble fraction of methanolic extract of *C. virosa*, VS= Vincristine sulphate.

The methanolic extract and its different fractions of leaves of *C. Virosa* were effective in the membrane stabilizing activity as the extractives prevented the lysis of erythrocytes. The highest level of membrane stabilizing activity was noticed by methanol extract in both solution and treat-induced hemolysis (Table 4).

Conclusion

The results of our study, being reported for the first time, provide reasonable indication that the methanol extract and its organic soluble partionates of leaves of *C. virosa* possess significant analgesic, anti-diarrheal, membrane stabilizing, ,cytotoxic and CNS-depressant properties. However, additional studies are necessary to isolate and characterize the active compounds responsible for those activities.

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