

***In vivo* Analgesic, Anti-hyperglycaemic and CNS Depressant Studies of *Commelina paludosa* Blume**

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Abstract

The methanol extract of whole plant of *Commelina paludosa* Blume (family: Commelinaceae) has been screened to explore its *in vivo* central and peripheral analgesic, hypoglycaemic and central nervous system depressant activities in Swiss Albino mice. The crude extract significantly increased the latent response time in tail-flick method ($p < 0.001$) at the doses of 200 and 400mg/kg body weight for a 90 minute period when compared to control group. The peripheral analgesic activity was evaluated by acetic acid-induced writhing method and the extract showed significant results ($p < 0.001$) with 51.72 and 67.24% inhibition at 200- and 400-mg/kg body weight, respectively. In addition, a dose dependant blood glucose lowering and CNS depressant activities were also observed by the crude extract.

Key words: Central analgesic activity, peripheral analgesic activity, anti-hyperglycaemic activity, depressant activity

Introduction

From time immemorial, plants have been used as health care remedies and spread over all around the world populated by human beings. The rich tradition of nature-based interventions to counteract any difficulty or abnormality in daily life has made them a genuine source of reliable and ancient symptomatic remedies. Bangladesh has a profound tradition of medicinal practices. It has more than 5000 floral species, among them around 750 species are generally used in conventional medicines (Pasha *et al.*, 2013; Yousuf *et al.*, 1994; Ghani, 1998).

Commelinaceae family includes 41 genera and 731 species worldwide and is commonly known as the dayflower family or spiderwort family (Christenhusz and Byng, 2016). This ecologically diverse family is usually found in forests and grasslands (Kubitzia, 1998). *Commelina paludosa* Blume (Bengali name: Batboittashak/Kanshira/Kannyalata; English name: Dayflower) belonging to the Commelinaceae family,

is a perennial herb up to 45-60 cm in height with largest leaves and petals among taxa of *Commelina* (Gajurel and Shrestha, 2009). It is widely distributed in Bangladesh, India, Sri Lanka, Pakistan, Malaysia, Indonesia and the Philippines (Nordal, 1963). The boiled leaves mixed with crushed peppers are taken orally 2-3 times a day to treat dysentery by the Chakma tribes of Rangamati district in Bangladesh (Rahmatullah *et al.*, 2011; Rahman *et al.*, 2007). The people of Tripura rub small blue flowers on the body during itching (Majumdar and Datta, 2007). The root is used to treat vertigo, fevers, and bilious afflictions (Nordal, 1963). In 2018, Riaz *et al.* postulated that the whole plant possessed significant antioxidant, antibacterial, antidiarrheal as well as antipyretic activity in the *in vitro* and *in vivo* test systems.

A quick literature survey revealed little pharmacological studies with this plant, and we herein, report the results of our preliminary pharmacological investigation.

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Materials and Methods

Collection of plant materials and extraction: The whole plants of *C. paludosa* were collected from Chittagong. The plant was identified in Bangladesh National Herbarium, Dhaka, Bangladesh where a voucher specimen (Accession no. DACB-48274) has been perpetuated for future reference.

The collected plant materials were cleaned, sun dried and powdered using a grinding machine. The ground material (500 g) was soaked in 2.5 litre of methanol in an amber glass bottle at room temperature for almost 15 days. The mixture was filtered through a piece of clean cotton and then filtered with Whatman filter paper number 1. The filtrate was evaporated with a rotary evaporator and a dense gummy mass was obtained which was known as crude methanol extract. This extract was transferred to a clean beaker and preserved in refrigerator for further experiments.

Drugs and chemicals: Methanol, castor oil, glucose, Tween-80 (BDH Chemicals, UK), normal saline solution (Beximco Infusion Ltd. Bangladesh), Morphine (Gonoshashto Pharmaceuticals Ltd., Bangladesh), diclofenac sodium, glibenclamide and diazepam (Square Pharmaceuticals Ltd., Bangladesh) were used in the present study. All other reagents were of analytical grade.

Experimental animals: Swiss Albino mice of both sexes (weighing between 25-35 gm) and 4-5 weeks old were taken for the experiments. The mice were kept in the animal house of State University of Bangladesh under standard environmental condition (at $24.0 \pm 1^\circ\text{C}$ and 55-65% relative humidity and 12 hrs light/12 hrs dark cycle) and fed with rodent feed collected from the icddr, b and water *ad libitum*. The animals were acclimatized for a week in the laboratory environment prior to use for the experiments. The Federation of European Laboratory Animal Science Associations (FELASA) guidelines

and recommendations were followed to reduce the pain and stress of the experimental mice.

Grouping of mice: Twenty Swiss Albino mice were randomly divided into four groups having five animals in each group for each bioassay: positive control (STD), negative control (CTL) and two test groups receiving methanolic extract at doses of 200(CP 200) and 400(CP 400) mg/kg of body weight.

Central analgesic activity: The central analgesic activity was investigated by tail flicking method (Pizziketti *et al.*, 1985). Two doses were fed orally to the mice and the tips of their tail were immersed in hot water which acted as pain stimulus. The reaction time required by the mice to deflect their tails was termed as tail immersion time which was recorded at 30, 60 and 90minutes after the administration of the extract or drug. The positive control group received morphine (2-mg/kg b.w.) subcutaneously and the negative control group was given 1% Tween 80 in saline mixture (0.1mL/10mg) orally. The pain inhibition percentage (PIP) was calculated using the following formula:

$$\text{Pain inhibition percentage (PIP)} = \left[\frac{T_1 - T_0}{T_0} \right] \times 100$$

where, T_1 = Post-drug latency and T_0 = Pre-drug latency

Peripheral analgesic activity: The acetic acid-induced writhing method was followed to evaluate peripheral analgesic activity as described by Koster *et al.* (1959). In this method, pain sensation was induced by administering 0.1mL of acetic acid to the experimental animals intraperitoneally. Diclofenac sodium (5-mg/kg b.w.) was given intraperitoneally to the positive control group. In the experimental groups, the crude extract was used for the evaluation of analgesic activity at 200-and 400-mg/kg of b.w. and the percentage of writhing inhibition was calculated by using the following equation:

$$\text{Percentage inhibition} = \frac{\text{Control licking response} - \text{Test licking response}}{\text{Control licking response}} \times 100\%$$

Anti-hyperglycaemic activity: The anti-hyperglycaemic activity of the test samples was assessed by following the tail tipping method

described by Durschlag *et al.* (1996), where the lowering of blood glucose level of the experimental animals was measured. The control group received

1% Tween 80 in normal saline (10 ml/kg body weight), the standard group received glibenclamide (5-mg/kg b.w.) and the experimental groups were fed with crude extract of 200- and 400-mg/kg b.w. The blood glucose level (mmol/L) of the experimental animals was measured at zero minute using a glucometer (Bioland G-423 S). Then the control, standard and methanolic crude extract (200- and 400-mg/kg b.w.) were administered orally to the experimental animals with the help of feeding needle. After 30 minutes, all groups were treated with 10% glucose solution (2-g/kg b.w.). Then 60, 120 and 180min after glucose loading, blood samples were collected from tail vein. By using glucometer, the blood glucose level of the experimental animals was measured to observe the anti-hyperglycaemic effect of the crude methanol extract of *C. paludosa* with respect to control and standard groups.

Determination of CNS depressant activity by open field test: The experiment was carried out according to the method described by Zhao *et al.* (1992). The floor of an open field was divided into alternatively colored black and white squares and the wall height was 40 cm. The negative control, positive control and test group received 1% Tween 80 in water, diazepam (1-mg/kg b.w., p.o.) and methanol extract (200- and 400-mg/kg b.w.), respectively. After giving test drugs, the number of animal movements was counted up to 3 min at 0, 30, 60, 90 and 120 min.

Determination of CNS depressant activity by hole cross test: The hole cross test was conducted to determine the sedative activity of the plant following the method explained by Takagi *et al.* (1971). A specific type of cage is used in this method which consists of a steel partition fixed in the middle of the cage having a size of 30 × 20 × 14 cm. In the center of the cage, a hole of 3 cm diameter was made at a height of 7.5 cm. Control mice received vehicle (1% Tween 80 in water), positive control received diazepam (1-mg/kg b.w., p.o.) and the experimental animals received methanol extract (200- and 400-mg/kg b.w.) of *C. Paludosa*, respectively. After oral administration of test drugs, the number of passages of mice through the hole from one chamber to other

was calculated for a period of 3 min at 0, 30, 60, 90 and 120 min.

Statistical analysis: The values are presented as mean ± standard error of mean (M±SEM) and One way ANOVA followed by Dunnett's test were used to determine the significance difference between the control group and experimental groups, the p values < 0.05 were considered to be statistically significant.

Results and Discussion

The central analgesic effects of methanolic extract of the whole plant of *C. paludosa* in the tail flicking method are shown in table 1. The extract increased the response time at both the doses (200- and 400-mg/kg b.w.) by 63.37 and 77.17%, respectively in the first 30 mins as compared to the standard, morphine (86.75%). The percent of elongation of flicking response kept on increasing in a dose and time dependent manner. Even though in every case the values for the two doses of the sample were lower compared to morphine, the data were found to be statistically significant. In the present study, central analgesic activity was evaluated by using the tail flick method which is considered to be a spinal reflex induced by heat according to Pizziketti *et al.* (1985). But it could also involve higher neural structures (central analgesic activity). Pain is centrally regulated via a number of complex processes which include opiate, dopaminergic descending noradrenergic and serotonergic systems (Mishra *et al.*, 2011). *C. paludosa* produced significant increase in pain threshold at both the doses in tail flick model. The analgesic effect produced by the doses of test samples and standard may be acting through central mechanisms involving these receptor systems or via peripheral mechanisms involved in the inhibition of prostaglandins, leukotrienes and other endogenous substances that are key mediators in pain.

The peripheral analgesic activity exhibited by the plant extract in acetic acid-induced writhing method is presented in table 2. The number of abdominal muscle contraction induced by 0.1 ml of acetic acid was effectively reduced by the extract in a dose dependant manner. Statistical evaluation of the data

confirmed that the crude extract of whole plant of *C. paludosa* demonstrated significant peripheral analgesic activity having writhing inhibition of 50.00 and 62.22% at 200- and 400-mg/kg of b.w., respectively when compared to that of the standard diclofenac sodium (72.22% inhibition). Acetic acid-induced writhing test was done to evaluate the peripheral analgesic activity of *C. paludosa* in mice according to the method of Koster *et al.* (1959). In this test, pain is induced in peripheral origin by injection of irritant principles such as acetic acid in mice. The writhing response is considered as a reflexive test which is highly useful for screening analgesic drugs (Gawade, 2012). Acetic acid generates pain through the activation of chemosensitive nociceptors or irritation of the visceral

surface, which lead to the liberation of endogenous substances such as serotonin, histamine, prostaglandins, bradykinins (Schumacher *et al.*, 1940). Local peritoneal receptors are presumed to be involved in the abdominal constriction response (Bentley *et al.*, 1983). The reduction of the writhing frequency or abdominal constrictions deduces the analgesic activity of the test compound. Intraperitoneal injection of acetic acid produced abdominal writhing in this study. The extracts derived from *C. paludosa* whole plant exhibited significant analgesic activity in Swiss Albino mice by inhibiting acetic acid induced writhing. Therefore, the extract might be inhibiting synthesis or release of these endogenous substances.

Table 1. Central analgesic activity of crude methanol extract of *C. paludosa* whole plant.

Animal group	After 30 min		After 60 min		After 90 min	
	M±SEM	% Elongation	M±SEM	% Elongation	M±SEM	% Elongation
CTL	2.32±0.08*	-	2.35±0.19*	-	2.21±0.06*	-
STD	4.34±0.13*	86.75	8.35±0.04*	255.32	12.81±0.16*	478.86
CP 200	3.80±0.13*	63.37	5.53±0.08*	135.40	7.11±0.18*	221.36
CP 400	4.12±0.13*	77.17	6.22±0.25*	164.79	8.10±0.18*	265.93

Values are expressed as Mean ± SEM (n=5). *p < 0.001 compared to negative control. CTL = negative control (1% Tween 80 in water), STD = positive control (Morphine at 2-mg/kg b.w.), CP 200 = Methanolic extract of *C. paludosa* whole plant at 200-mg/kg b.w., CP 400 = Methanolic extract of *C. paludosa* whole plant at 400-mg/kg b.w.

Table 2. Peripheral analgesic activity of crude methanol extract of *C. paludosa* whole plant.

Animal group	Writhing count (sec)					Number of writhing (Mean ± SEM)	% Inhibition of writhing
	M-1	M-2	M-3	M-4	M-5		
CTL	19	18	16	17	20	18.00 ± 0.71*	-
STD	7	4	4	4	6	5.00 ± 0.63*	72.22
CP 200	9	11	8	7	10	9.00 ± 0.71*	50.00
CP 400	5	6	10	6	7	6.80 ± 0.86*	62.22

Values are expressed as Mean ± SEM (n=5). *p < 0.001 compared to negative control. CTL = negative control (1% Tween 80 in water), STD = positive control (Diclofenac sodium at 5mg/kg body weight). M1, M2, M3, M4, M5 = Mice 1, Mice 2, Mice 3, Mice 4, Mice 5, respectively.

The extract of *C. paludosa* when administered orally at 200- and 400-mg/kg b.w. did not exhibit significant glucose lowering effect when compared to control. However, the percent reduction of blood glucose level was found to be dose dependant. In this study, maximum effect was observed at the dose of

400-mg/kg b.w. (Table 3). Various studies revealed that plant extracts exert anti-hyperglycaemic effect by stimulating or regenerating β cells or by protecting these cells from destruction, by restricting glucose load as well as by inducing unrestricted endogenous insulin action (Joseph *et al.*, 2011). Anti-

hyperglycaemic effect may also be caused by insulin release from β cells or activation of the insulin receptors to absorb the blood sugar and stimulate the peripheral glucose consumption by plant extract (Khan *et al.*, 2014). In the present study, the crude methanolic extract of *C. paludosa* produced non-significant hypoglycemic effect on experimental mice. However, the percentage reduction of blood glucose of the whole plant extract was comparable with the standard drug.

During evaluation of CNS depressant activity by the open field and hole cross test, crude extract of *C. paludosa* at both the doses showed dose dependent sedative effects in comparison to the standard drug (diazepam) (Table 4 and Table 5). There was no statistically significant difference among the values of control and the two dosages in both the experiments. Increased alertness indicates increase in locomotor activity whereas decrease in locomotor activity indicates sedative effect (Crunkhorn *et al.*, 1971).

Table 3. Anti-hyperglycaemic activity of methanol extract of *C. paludosa*.

Animal group	Dose	0 min	30 min	60 min	120 min	180 min
		M \pm SEM (mmol/l)				
CTL	-	5.20 \pm 0.59	23.22 \pm 4.47	19.36 \pm 4.30	7.22 \pm 0.45	4.18 \pm 0.47
STD	5-mg/kg b.w.	5.20 \pm 0.35	16.32 \pm 1.21	11.82 \pm 1.27	4.50 \pm 0.69	2.90 \pm 0.31
CP 200	200-mg/kg b.w.	4.66 \pm 0.59	14.18 \pm 1.33	10.08 \pm 1.32	6.48 \pm 0.50	4.50 \pm 0.18
CP 400	400-mg/kg b.w.	5.06 \pm 0.32	18.98 \pm 0.64	11.58 \pm 1.87	7.80 \pm 1.42	4.78 \pm 0.57

Values are expressed as Mean \pm SEM (n=5). CTL = negative control (1% Tween 80 in water), STD = positive control (Glibenclamide at 5-mg/kg b.w.).

Table 4. Depressant effects of the methanol extract of *C. paludosa* whole plant on open field test in mice model.

Animal group	Dose	Number of movements (Mean \pm SEM)				
		0 min	30 min	60 min	90 min	120 min
CTL	-	184.20 \pm 25.12	102.00 \pm 35.43	43.00 \pm 14.32	102.00 \pm 12.45	40.00 \pm 14.58
STD	1-mg/kg	89.00 \pm 1.69**	87.40 \pm 0.91	70.00 \pm 1.84	65.00 \pm 1.58*	51.00 \pm 1.77
CP 200	200-mg/kg b.w.	212.00 \pm 20.43	100.00 \pm 25.74	72.00 \pm 16.36	56.00 \pm 22.53	52.00 \pm 20.74
CP 400	400-mg/kg b.w.	177.00 \pm 30.70	90.00 \pm 25.00	84.00 \pm 11.51*	48.00 \pm 17.10*	26.00 \pm 9.75

Values are expressed as Mean \pm SEM (n=5). *p< 0.001, **p< 0.01 compared to control. CTL = negative control (1% Tween 80 in water), STD = Positive control (Diazepam at 1-mg/kg b.w.).

Table 5. Depressant effects of the methanol extract of *C. paludosa* whole plant on hole cross test in mice.

Animal group	Dose	Number of movements (Mean \pm SEM)				
		0 min	30 min	60 min	90 min	120 min
CTL	-	2.40 \pm 1.35	3.40 \pm 1.85	2.20 \pm 1.64	1.60 \pm 1.23	4.40 \pm 1.62
STD	1-mg/kg	6.60 \pm 1.07**	5.40 \pm 1.07	3.80 \pm 0.92	3.80 \pm 1.47	3.00 \pm 1.26
CP 200	200-mg/kg b.w.	3.20 \pm 2.25	2.60 \pm 1.89	2.20 \pm 1.39	2.40 \pm 1.44	1.20 \pm 1.05
CP 400	400-mg/kg b.w.	4.40 \pm 2.17	2.40 \pm 1.40	1.40 \pm 1.23	1.00 \pm 1.12	0.60 \pm 0.74

Values are expressed as Mean \pm SEM (n=5). **p< 0.01 compared to control. CTL = negative control (1% Tween 80 in water), STD = Positive control (Diazepam at 1-mg/kg b.w.).

Sedative-hypnotic, anxiolytic and muscle relaxant drugs revealed their activity through the inhibition of gamma-aminobutyric acid (GABA) which is the

major inhibitory neurotransmitter. Open field and hole cross methods are common ways to investigate the depressant activity of crude extracts. In this study,

the methanol extract of *C. paludosa* showed dose dependant depressant activity which might be due to the direct activation of GABA receptor enhancing GABAergic inhibition in the CNS.

Conclusion

The whole plant extract of *C. paludosa* was found to be effective in analgesic, hypoglycemic and depressant activities. Therefore, further studies are needed to discover other bioactivities.

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