Investigations of Sedative and Antidiarrheal Activity of *Commelina appendiculata* in Swiss Albino Mice

Pritesh Ranjan Dash¹,a*, Md. Sohel Rana¹,b and Mohammad Shawkat Ali²,c

¹Department of Pharmacy, Jahangirnagar University, Savar, Dhaka, Bangladesh
²Department of Clinical Pharmacy and Pharmacology, University of Dhaka, Dhaka, Bangladesh

ªpritesh.ju@gmail.com, bprofdr.sohelrana.ju@gmail.com, cprofdr.shawkat@gmail.com

**Keywords:** *Commelina appendiculata*, thiopental sodium induced sleeping time test, hole cross test, open field test, castor oil, magnesium sulphate.

**Abstract.** The aim of the present study was to investigate the sedative and antidiarrheal activity of ethanol extract of *C. appendiculata* at the doses of 100 and 200 mg/kg body weight per oral (p.o) in *Swiss albino* mice. Sedative activity of the extract (100 and 200 mg/kg, p.o.) was assessed utilizing thiopental sodium-induced sleeping time model to evaluate sedative potential of the extract, hole cross to evaluate motor activity and open field test to evaluate exploratory behaviour. The extract also produced rapid onset and maximized the duration of sleeping time when administered with thiopental sodium. The extract fundamentally (p < 0.05-0.001) diminished motor action and exploratory conduct of mice in hole cross and open field test separately. In antidiarrheal activity study, the concentrate (200 mg/kg) likewise demonstrated most extreme inhibition of defecation (90.64% and 80.95%) in both models individually. The present study demonstrates that the ethanol extract of *C. appendiculata* have central nervous system (CNS) depressant and antidiarrheal properties. In this manner, the plant may be further investigated to find for its pharmacological active natural products.

**Introduction**

*Commelina appendiculata* (*C.appendiculata*) locally called as Kanda Loa, under the family of commelinaceae is an annual herb distributed in Eastern India and Sri Lanka. In Bangladesh, it occurs in Tangail, Mymensingh and Sylhet district. It is used in the Sunamganj district as a folk medicine for the treatment of cats, dogs and snake bites [1]. A portion of the type of *Commelina* are utilized in Chinese medicine for the treatment of wind-heat type common cold, sore throat, fever and dropspy [2]. For instance, *C. communis* L., a species of the *Commelina* genus, widespread in the world especially in the tropics and subtropics, has long been used in folk medicine in China, and modern pharmaceutical investigations has revealed its anti-inflammatory, antiviral, and anti-hyperglycemic effects [3]. It has been reported that many plants of this genus are used in medicine for the treatment of inflammation, pain, fever, dysentery, diarrhea, neurological disorders, cancer, toxicity, heart diseases, asthma, oxidative stress etc [3, 4]. The plant is accounted for to have pain relieving and cytotoxic action [5]. Past phytochemical examinations of the Commelina genus were accounted for on C. undulata R.Br., C. benghalensis L. furthermore, C. communis L. from which a few sorts of compounds, for example, alkaloids, steroids, terpenoids, iridoids, flavonoids, lignans, aliphatic alcohols, polyols, and phenolic acids were obtained [6-14]. Moreover, the plant of *C. appendiculata* was reported to contain carbohydrates, tannins, flavonoids, glycoside and alkaloids [5]. Literatures survey revealed no previous report on the sedative and antidiarrheal activity of *C. appendiculata*. Subsequently, as a piece of our proceeding with studies [5, 15] on characteristic items for their pharmacological properties interestingly we explored the ethanolic extract of *C. appendiculata* for its sedative and antidiarrheal activity.
Materials and Methods

Collection of the Plant

The plant of *C. appendiculata* was gathered from neighborhood Sylhet sadar in January 2014. The gathered plant was then recognized by the taxonomist of Jahangirnagar University Herbarium, Savar and a voucher example has been kept (DACB: 39,321) for further reference.

Extraction of the Plant Material

After shade drying, the entire plant was decreased to coarsely powder utilizing a grinding mill. The plant was extracted by a cold extraction strategy. The dried and coarse powder (106 g) was extracted with ethanol (500 ml) in an air proof, clean level bottomed holder for 3 days at room temperature with incidental mixing. The extract was then separated by filtration and evaporated using rotary evaporator under reduced pressure to got 18 gm (yield = 16.98%) crude extract which was utilized for conducting the study regarding sedative and antidiarrheal activity.

Chemicals

Magnesium sulfate (Merck, Germany), castor oil (Well's Health Care, Spain). Diazepam and Loperamide (Square Pharmaceuticals Ltd., Bangladesh), thiopental sodium (Gonoshasthaya Pharmaceuticals Ltd., Bangladesh) Normal saline arrangement (Orion Infusion Ltd., Bangladesh), were acquired and utilized as a part of the examination. All chemicals in this examination were of analytical reagent grade.

Animals

Swiss albino mice of either sex, 3-4 weeks of age, weighing between 25-30 gm were utilized for as a part of vivo pharmacological screening. Mice were gathered from the animal research branch of the International Center for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B). They were housed in standard ecological conditions and sustained with rat eating routine and water not indispensable. The departmental animal look into moral board of trustees endorsed the test convention.

Drugs and Treatment

After reconstituted in distilled water the extract was controlled to the mice at 100 and 200mg/kg per orally by gavage. The water (5ml/kg) was controlled by gavage to control group. All medications, utilized as standard, were dissolved in 0.9% saline and directed intraperitoneally (i.p). Diazepam (2mg/kg i.p) and Loperamide (3mg/kg) were utilized as standard CNS depressant and antidiarrheal sedate, separately.

Acute Toxicity Study

Mice were isolated into control and test bunches (n=6). Diverse measurements of plant concentrates up to 2000 mg/kg, p.o. was managed and test animals were watched for the initial 3 hours of administration and mortality rate was determined within 72 hours [16].

Neuropharmacological Activity

Thiopental Sodium Induced Sleeping Time Test

The animals were haphazardly partitioned into four gatherings comprising of six mice each. The test groups got of *C. appendiculata* extracts at the measurements of 200 and 400 mg/kg while positive control was treated with diazepam (2 mg/kg i.p.) and control with water. After thirty minutes, pentobarbitone (40 mg/kg, i.p.) was regulated to every mouse to actuate rest. The animals were watched for the time between pentobarbitone administration to loss of correcting reflex and term of time between the loss and recuperation of correcting reflex [17]. Rate of effect in percentage was computed utilizing the following formula:
Hole Cross Test

The most solid behavioural change is a hyperemotional reaction to novel ecological. The technique was embraced as depicted by Takagi et al. [18]. The point of this study was to describe the passionate conduct of rodents utilizing the hole cross test. The quantity of section of a mouse through the gap from one chamber to the next was meant a time of 3 min at 0, 30, 60, 90 and 120 min after oral administration of standard Diazepam (2 mg/kg) and ethanol separate at measurements of 100 and 200 mg/kg body weight. Percentage inhibition of movements was computed utilizing the following equation:

\[
\text{Movements inhibition (\%) } = \frac{\text{Mean No. of movements (control) } - \text{Mean No. of movements (test)}}{\text{Mean No. of movements (control)}} \times 100
\]

Open Field Test

This investigation additionally assesses a range of anxiety-induced, locomotor activity and exploratory activities of rodents. The animals were dealt with as the past expressed way and at same dosages. The test was performed by procedure portrayed by Gupta et al. [19]. The quantity of squares went by the animals was computed for 3 min, at 0, 30, 60, 90 and 120 min ensuing to oral administration of the test crude extracts. Percentage inhibition of movements was computed utilizing a similar formula utilized as a part of hole cross test.

Antidiarrheal Activity

Castor Oil Induced Diarrhea

Test animals were arbitrarily chosen and isolated into four groups meant as control, standard and test tests (100 and 200 mg/kg) and comprising of 6 mice in every group. This trial depicted by Shoba and Thomas, (2001) [20]. Mice were fasted for 18h preceding the test with free access to water. Control (water 5ml/kg), standard (Loperamide 3mg/kg) and test tests (100 and 200 mg/kg) were managed orally. At that point 1 h later, 0.3ml castor oil was regulated orally to every mouse to prompt the runs. Each animal was put in an individual confine, the floor of which was fixed with white smearing paper. The papers were changed each hour. The aggregate quantities of both dry and wet defecation discharged by the animals were noted each hour for a time of 4 h. The aggregate number of diarrheal faeces of the control group was viewed as 100%. Percentage of inhibition of defecation was calculated using the following formula:

\[
\text{Inhibition of defecation (\%) } = \frac{\text{Mean No. of defecation by castor oil } - \text{Mean No. of defecation by drug or extract}}{\text{Mean No. of defecation caused by castor oil}} \times 100
\]

Magnesium Sulphate-Induced Diarrhea

The examination was done by technique depicted by Doherty (1981) [21]. Loose bowels was initiated by oral administration of magnesium sulfate at the measurements of 2 g/kg. The mice were screened and partitioned into various gatherings. Mice were treated with magnesium sulfate (2 g/kg, p.o.) after 30 min of the administration of the vehicle, medication or concentrates. Subsequent to watching the diarrheal droppings for the following 4 h, the percent (%) restraint of defecation was computed utilizing a similar equation utilized as a part of castor-oil initiated looseness of the bowels test.
Statistical Analysis

The statistical examination for animal trial was completed utilizing one-way ANOVA followed by Dunnett's various correlations. The outcomes got were contrasted with the control group. P < 0.05 and P < 0.001 were thought to be measurably noteworthy.

Results

Acute Toxicity

Oral administration of *C. appendiculata* at the measurements of 500–2000 mg/kg did not deliver any mortality or perceptible behavioral changes in mice within 72 hr perception period. Accordingly, it can be proposed that *C. appendiculata* have low poisonous quality profile with LD50 more noteworthy than 2000 mg/kg.

Neuropharmacological Activity

Thiopental Sodium Induced Sleeping Time Test

In thiopental sodium instigated dozing time test, the concentrate at the measurements of 100 and 200 mg/kg altogether incited the rest at a prior stage furthermore drawn out the length of dozing time in guinea pigs when contrasted with control (Table 1). The outcome at the dosage of 200 mg/kg was observed to be measurably huge (p < 0.001). In this test, EECA at the doses of 100 and 200mg/kg showed maximum 147.48% and 195.98% effect in duration of loss of righting reflex respectively, whereas the standard drug Diazepam (2mg/kg) produced 200 percent effect.

| Table 1. Effect of EECA on thiopental sodium induced sleeping time test in mice. |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
| **Group**                       | **Dose (mg/kg)** | **Onset of Sleep (minutes)** | **Duration of Sleep (minutes)** | **Percent effect** |
| Control                         | 5 ml/kg         | 2.06±0.64        | 66.33±8.04      | 100             |
| Diazepam                        | 2               | 1.73±0.20        | 133±8.31*       | 200.00          |
| EECA                            | 100             | 2.47±0.55        | 97.83±14.94     | 147.48          |
|                                | 200             | 1.10±0. 21       | 130±13.59**     | 195.98          |

Control group received water 5ml/kg (p.o.), standard group received Diazepam 2mg/kg body weight (i.p.), test group EECA was treated with 100 and 200 mg/kg body weight of the extract (p.o.) respectively. Values are mean ±SEM, (n=6); * p < 0.05, **p < 0.001, Dunnett t-test as compared to control. EECA=Ethanolic extract of *Commelina appendiculata*.

Hole Cross Test

In hole cross test, the EECA demonstrated detectable abatement in the locomotor movement from second perception (30 min) to fifth perception (120 min) at the dosages of 100 and 200 mg/kg which speak to the CNS depressant of the concentrate (Table 2). Most extreme depressant impact was seen from third (30 min) to fifth (120 min) perception period. The impact was dose dependent and factually huge (p < 0.05, 0.001) compared to control. In hole cross test, maximum 89.02%, and 96.7% suppression of locomotor activity were exhibited with the EECA at the doses of 100 and 200 mg/kg respectively. In this study Diazepam (63.42% suppression) was used as standard.
Table 2. Effect of EECA on hole cross test in mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>Number of movements (% of Number of movements inhibition)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td>Control</td>
<td>5ml/kg</td>
<td>15.5±1.84</td>
</tr>
<tr>
<td>Diazepam</td>
<td>2</td>
<td>12.83±2.18</td>
</tr>
<tr>
<td>EECA</td>
<td>100</td>
<td>8.17±2.53</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>9±2.59</td>
</tr>
</tbody>
</table>

Control group received water 5ml/kg body weight (p.o.), standard group received Diazepam 2mg/kg body weight (i.p.), test group EECA was treated with 100 and 200 mg/kg body weight of the extract (p.o.) respectively. Values are mean ±SEM, (n=6); * p < 0.05, **p < 0.001, Dunnett t-test as compared to control. EECA=Ethanolic extract of Commelina appendiculata.

Open Field Test

The abatement in velocity was likewise obvious from the consequences of open field test. The concentrate delivered dose dependent and measurably critical (p < 0.05, 0.001) CNS depressant impact (Table 3). The most extreme impact was seen from third perception (30min) to fifth perception (120 min) period. In open field test, maximum 84.37%, and 90.26% suppression of locomotor activity were exhibited with EECA (100 and 200 mg/kg) respectively, whereas the standard drug Diazepam displayed 65.81% suppression.

Table 3. Effect of EECA on open field test in mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>Number of movements (% of Number of movements inhibition)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td>Control</td>
<td>5ml/kg</td>
<td>112.83±6.24</td>
</tr>
<tr>
<td>Diazepam</td>
<td>2</td>
<td>131.33±7.71</td>
</tr>
<tr>
<td>EECA</td>
<td>100</td>
<td>52.83±11.37</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>51.5±10.81</td>
</tr>
</tbody>
</table>

Control group received water 5ml/kg body weight (p.o.), standard group received Diazepam 2mg/kg body weight (i.p.), test group EECA was treated with 100 and 200 mg/kg body weight of the extract (p.o.) respectively. Values are mean ±SEM, (n=6); * p < 0.05, **p < 0.001, Dunnett t-test as compared to control. EECA=Ethanolic extract of Commelina appendiculata.

Antidiarrheal Activity

Castor Oil-Induced Diarrhea

In the antidiarrheal action, the extract showed noteworthy (p< 0.001) and a dose dependent reduction in the aggregate number of fecal dropping in castor oil incited the runs in mice. The percentage of inhibition of castor oil-induced diarrhoea in the extract-treated mice was 74.64% and...
90.64% at the doses of 100 and 200 mg/kg respectively. In this study standard drug Loperamide (3 mg/kg) produced 54.64% inhibition of defecation.

**Table 4.** Effects of EECA on castor oil-induced diarrhea in mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>No. of faeces in 4hr</th>
<th>% Inhibition of defecation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5 ml/kg</td>
<td>12.5±1.56</td>
<td>---</td>
</tr>
<tr>
<td>Loperamide</td>
<td>3</td>
<td>5.67±1.40*</td>
<td>54.64</td>
</tr>
<tr>
<td>EECA</td>
<td>100</td>
<td>3.17±1.51**</td>
<td>74.64</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>1.17±0.67**</td>
<td>90.64</td>
</tr>
</tbody>
</table>

Control group received water 5ml/kg (p.o.), standard group received Loperamide 3mg/kg body weight (p.o.), test group EECA was treated with 100 and 200 mg/kg body weight of the extract (p.o.) respectively. Values are mean ±SEM, (n=6); * p < 0.05, **p < 0.001, Dunnett t-test as compared to control. EECA=Ethanolic extract of *Commelina appendiculata*.

**Magnesium Sulphate-Induced Diarrhea**

In magnesium sulfate-prompted diarrhea, the concentrates demonstrated a dose dependent diminishment in fecal dropping in a comparative way like in castor oil-initiated loose bowels (Table 5). Critical (p < 0.001) diminishment in fecal dropping was appeared by the ethanol extract at 200 mg/kg (80.95%) measurements. At 100 mg/kg measurement likewise demonstrated 65.00% hindrance of diarrheal droppings, (p < 0.001).

**Table 5.** Effects of EECA on magnesium sulphate-induced diarrhoea in mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>No. of faeces in 4hr</th>
<th>% Inhibition of defecation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5ml/kg</td>
<td>10.5±0.92</td>
<td>---</td>
</tr>
<tr>
<td>Loperamide</td>
<td>3</td>
<td>5.16±1.22*</td>
<td>50.85</td>
</tr>
<tr>
<td>EECA</td>
<td>100</td>
<td>4±1.35**</td>
<td>65.00</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>2±0.57**</td>
<td>80.95</td>
</tr>
</tbody>
</table>

Control group received water 5ml/kg (p.o.), standard group received Loperamide 3mg/kg body weight (p.o.), test group EECA was treated with 100 and 200 mg/kg body weight of the extract (p.o.) respectively. Values are mean ±SEM, (n=6); * p < 0.05, **p < 0.001, Dunnett t-test as compared to control. EECA= Ethanolic extract of *Commelina appendiculata*.

**Discussion**

It is normally acknowledged that the sedative impacts of drugs can be assessed by estimation of pentobarbitone sleeping time in laboratory animals [22, 23]. Prolongation of pentobarbitone-initiated mesmerizing is suggestive of central depressant movement of a compound [24]. The impact on the CNS of the EECA (200 mg/kg) were delivered a critical (p < 0.001) increment in the mesmerizing impact initiated by the phenobabitone, in a dose dependent way, in this manner proposing a profile of sedative action. Locomotor action considered as an expansion in readiness and lessening in locomotor action showed sedative effect [25]. In hole cross and open field test, the extract demonstrated discernible diminishing in the locomotor action from second perception (30 min) to fifth perception (120 min) at the measurements of 100 and 200 mg/kg which speak to the CNS depressant of the extract (Table 2 and 3). Greatest depressant impact was seen from third (30 min) to fifth (120 min) perception period. The impact was dose dependent and statistically noteworthy (p < 0.05, 0.001) contrasted with control. In hole cross test, most extreme 89.02% and 96.70% concealment of locomotor movement were shown with the dosages of 100 and 200 mg/kg of EECA, separately. In this study, Diazepam (63.3%) was utilized as standard. In open field test, greatest 84.37% and 90.26% concealment of locomotor action were shown with the measurements...
of 100 and 200 mg/kg of CA individually, while the standard medication Diazepam created 65.81% concealment. Concentrate of CA diminished locomotor action shows its CNS depressant action. Gamma-amino-butyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system. Diverse anxiolytic, muscle relaxant, narcotic trancelike medications are clarification their activity through GABAA, subsequently it is conceivable that concentrate of C. appendiculata may acts by potentiating GABAergic hindrance in the CNS by means of membrane hyperpolarization which prompts to a diminishing in the firing rate of basic neurons in the mind or might be because of direct enactment of GABA receptor by the concentrate [26]. Many research demonstrated that plant containing flavonoids, saponins and tannins are helpful in numerous CNS issue [27]. Prior examination on phytoconstituents and plants proposes that numerous flavonoids and neuroactive steroids were observed to be ligands for the GABAA receptors in the central nervous system, which prompted to the accept that they can go about as benzodiazepine like molecules [25]. Tannins have additionally been accounted for to demonstrate non-particular CNS discouragement in mice [28]. Phytochemical examinations likewise demonstrated the presence of alkaloids, flavonoids, sugars and tannins in EECA [5]. So may be this phytoconstituents are in charge of its CNS depressant movement. The restraint of loose bowels in mice, initiated by castor oil and magnesium sulfate, is utilized to decide the anti diarrheal action of plant extracts. Castor oil fortifies the arrival of prostaglandin E in the colon [29], diminishes Na+, K+ ATPase movement [30], and adjusts the intestinal histology and penetrability [31]. These decrease or reverse absorption of water and electrolytes from the intestinal lumen and colon and cause secretory loose bowels in mice. Magnesium sulfate causes expanded loss of intestinal substance because of the lessening in reabsorption of water and cholecystokinin discharge from the duodenal mucosa. These expansion the secretion and motility of small intestine, keep the reabsorption of sodium chloride and water, and instigate diarrhea [32, 33]. In this study, the concentrates demonstrated a dose dependent diminishment in fecal droppings in both castor oil-and magnesium sulfate-prompted looseness of the bowels (Table 4 and 5). Tannins may grant antidiarrheal impact potentially by hindering the intestinal motility [32] and diminishing the intestinal secretion [34]. It might be conceivable that the antidiarrheal movement is because of the tannins [35], alkaloids [36], glycosides [37] and different phytoconstituents.

Conclusion

The consequences of the present study shows the anti-diarrhoeal and CNS depressant action of C. appendiculata which deserve further studies to build up its helpful esteem and additionally its mechanism of action.

Competing Interests

The authors declare that they have no competing interests.

Authors’ Contributions

All authors are equally contributed.

Acknowledgments

The authors are acknowledge to the director of Animal Research Division of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B) for supplying mice and Jahangirnagar University Herbarium for identifying the plant sample.
References


