Evaluation of Analgesic, Neuropharmacological and Anti-diarrheal Potential of *Jatropha gossypifolia* (Linn.) Leaves in Mice

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The aim of the study was to evaluate the possible analgesic, neuropharmacological and anti-diarrheal activities of methanol extract of leaves of *Jatropha gossypifolia* Linn. (Euphorbiaceae) in mice. Acetic acid induced writhing inhibition test was used to measure the analgesic activity of the extract. The neuropharmacological activity of the extract was assessed by hole cross, hole board and elevated plus-maze tests and the anti-diarrheal activity was evaluated by castor oil induced diarrhea inhibition test. The extract demonstrated highly significant (p<0.001) analgesic activity with % inhibitions of writhing response at doses 200 and 400 mg kg⁻¹ b.wt. were 67.56 and 65.14%, respectively. In hole cross test, the extract at both doses showed significant (p<0.05) sedative effect in mice. In hole board test, the extract showed highly significant (p<0.001) anxiolytic activity at dose 200 mg kg⁻¹ b.wt. whereas, the same activity was observed at dose 400 mg kg⁻¹ b.wt. in elevated plus-maze test. The extract also showed highly significant (p<0.001) anti-diarrheal activity in castor oil induced diarrhea inhibition test. The present findings suggest that the plant possess significant analgesic, neuropharmacological and anti-diarrheal properties and could be a prominent source of medicinally importance phytoconstituents.

**Key words:** *Jatropha gossypifolia*, analgesic, anxiolytic, sedative, anti-diarrheal
INTRODUCTION

Jatropha gossypifolia Linn. (Euphorbiaceae) is a bushy, extraneous shrub of about 1.8 m in height and commonly known as Bellyache bush (English) (Oduola et al., 2005a), Lal Bhander, Laljeol, Aar koche (Bengali), Karachi (Marma), Kander (Garo) (Uddin, 2006). The shrub is native to tropical America (Bullangpoti et al., 2012) but it is also commonly seen in road sides and fallow lands in Bangladesh.

J. gossypifolia is a medicinally important shrub as it is rich source of diverse phytoconstituents. The bark of the shrub contains jatrophone (an alkaloid) (Oduola et al., 2005b) whereas the stem contains jatroiden (a lignin) (Oduola et al., 2005a). The phytoconstituents previously reported to be found in the plant were saponin, lignin, tanin, phenolic compounds, flavonoid, curcum, triterpenes, diterpene, jatrophone, jatropholones A and B, jatrophatrine, apigenin, cyclogossine A (Khumrungsee et al., 2009). J. gossypifolia is traditionally being used for the treatment of various ailments. In Latin America and the Caribbean, the leaves are traditionally being used in boils, carbuncles, eczema, itching and venereal diseases (Parvathi et al., 2012). The leaves are also used as febrifuge (Dhale and Birari, 2010). The bark is used as emmenagogue (Dhale and Birari, 2010) while seeds are used as emetic, purgative and also used for cancer and body pain (Dhale and Birari, 2010). The use of roots of the shrub to treat leprosy is well known to date (Dhale and Birari, 2010). J. gossypifolia has also been reported for its antiallergic, molluscicidal, antimicrobial, insect repellent (Khumrungsee et al., 2009), larvicidal (Bullangpoti et al., 2012), coagulating and anti-coagulating (Parvathi et al., 2012) activities by various researchers.

The present investigations were carried out to identify the possible analgesic, neuropharmacological and anti-diarrhoeal activities of methanol extract of J. gossypifolia leaves available in Bangladesh.

MATERIALS AND METHODS

Plant collection and identification: The leaves of J. gossypifolia were collected in August, 2011 from Sadhuhati, Bangladesh. The collected plant part was identified by a taxonomist (Dr. Bushra Khan, Principal Scientific Officer) of Bangladesh National Herbarium, Mirpur, Dhaka where a voucher specimen of the plant (DACB Accession No. 35937) has been deposited for future reference.

Preparation of methanol extract: The collected leaves were thoroughly washed with water to remove the adhering dirt and sun dried for 15 days. The dried, coarsely powdered material (1 kg) was extracted by cold extraction process using methanol (2.5 L) as the solvent at room temperature. The extract was then filtered and concentrated with a rotary evaporator (IKA, Germany) at low temperature (45°C) and reduced pressure to get dry extract (12.6% w/w). The dry extract was stored at 4°C until use.

Experimental animals: Twenty Swiss albino mice of either sex, purchased from the Animal Research Branch of the International Center for Diarrheal Disease and Research, Bangladesh (ICDDR, B), were taken for the tests. Mice were housed under standard laboratory conditions (room temperature 25±2°C, relative humidity 55-65% and 12 h light: dark cycle). They were fed with standard food (ICDDR, B formulated) and had free access to tap water. The experiments were performed on an isolated and noiseless room. The time interval between the tests was two weeks. The experimental protocols were in accordance with the principles and guidelines adopted by the Animal Experimentation Ethics Committee (AEBC) of East West University.

Analgesic activity: Acetic acid induced writhing inhibition test was used to assess the analgesic activity of methanol leaves extract of J. gossypifolia. The test was performed according to the method described by Al-Amin et al. (2011). According to the method, mice were fasted overnight with water ad libitum and randomly divided into four groups comprising of five mice each. Each group received a particular treatment i.e., control group received 1% (w/v) Tween-80 (Merck, Germany) in normal saline (Beximo Infusion Ltd., Bangladesh) at a dose of 0.5 mL per kg body weight. Test groups received methanol extract at the dose of 200 and 400 mg per kg body weight, respectively. A 0.7% v/v acetic acid (Merck, Germany) solution was administered (0.1 mL/10 g) intraperitoneally to each mouse to generate pain, 30 min after oral administration of control and test groups. The positive control was administered orally 15 min prior to administration of acetic acid. Just 5 min after the administration of acetic acid solution, the number of writhing by each mouse was counted individually for a period of 20 min. Mice did not always accomplish full writhing. This incomplete writhing was taken as a half-writhing and two half-writhings were counted as one full writhing (Al-Amin et al., 2011; Zulfiker et al., 2010). Analgesic activity was expressed as writhing inhibition (%) and was calculated by using the following formula:
where, \( W_c \) is the mean number of writhing of control and \( W_t \) is the mean number of writhing of the test sample.

**Neuropharmacological activities:** The neuropharmacological activities of *J. gossypifolia* leaves was evaluated by performing hole cross, hole board and elevated plus-maze tests. Mice were divided into four groups each comprising of 5 mice during each experiment. The groups received particular treatments:

- **Group 1:** Control (1% v/v tween-80 in normal saline, 0.5 mL mice\(^{-1}\))
- **Group 2:** Positive control (diazepam, Square Pharmaceuticals Ltd., Bangladesh, 1 mg kg\(^{-1}\) b.wt.)
- **Group 3:** Test sample I (methanol extract at the dose of 200 mg kg\(^{-1}\) b.wt.)
- **Group 4:** Test sample II (methanol extract at the dose of 400 mg kg\(^{-1}\) b.wt.)

**Hole cross test:** The sedative activity of the extract was assessed by using hole cross test, as described by Subhan et al. (2008). A wooden box partitioned in the middle and having a dimension of 30×20×14 cm was taken for the test. A hole of 3 cm diameter was made in the center partition of the box at a height of 7.5 cm. The spontaneous movement of the mice from one chamber to other through the hole was observed immediately after oral administration of test drugs. The observation was carried out for 3 min at 30, 60, 90 and 120 min.

**Hole board test:** The hole board test is the widely used for evaluating anxiolytic and/or anxiogenic activity of mice (Takeda et al., 1998). The test was done according to the method described by Somani et al. (2010). A wooden hole board apparatus (20×40 cm) with sixteen evenly spaced holes and elevated to a height of 25 cm was used for the test. The diameter of each hole was 3 cm. Each mouse was placed on the center of the board after 30 min of the oral administration of particular treatment and the number of head dipping and the latency until the first entry was calculated during a period of 5 min.

**Elevated plus-maze test:** Elevated plus Maze (EPM) test was performed according to the method described by Thippeswamy et al. (2011). The EPM apparatus was consisted of two open arms (16×5 cm) and two closed arms (16×5×12 cm) which emerged from a central platform (5×5 cm) and elevated to a height of 40 cm. The apparatus was set up in a dimly illuminated and noiseless room. Mice were placed on the central platform of the maze facing towards one of the open arms after 30 min of oral administration of treatments. For each mouse both the total exploratory activity (number of entries in both arms) and other ethologically derived measures (such as grooming, rearing and stretch-attend postures) were recorded by using digitized video camera for a period of 5 min.

**Anti-diarrheal activity:** Castor oil induced diarrhea inhibition test, described by Shoba and Thomas (2011), was used to evaluate the anti-diarrheal activity of the methanol extract. In this method, mice were fasted overnight and randomly divided into four groups having five mice in each. Each group received a particular treatment i.e., control (1% v/v tween-80 in normal saline, 0.5 mL mice\(^{-1}\)), positive control (loperamide, Square Pharmaceuticals Ltd., Bangladesh, 2 mg kg\(^{-1}\) b.wt.) and test samples (200 and 400 mg kg\(^{-1}\) of b.wt.) A 0.2 mL castor oil (BDH Chemicals Ltd., UK) was fed to each mouse to induce diarrhea, 30 min after the treatments. After that mice were placed in separate beakers lined with filter papers for observation. During an observation period of 2 h, a numbers of parameters were recorded: (1) Onset of dry stool (2) No. of wet stool (3) Weight of wet stool (4) Total weight of fecal output and (5) Onset of wet stool.

**Statistical analysis:** All the data were presented as Mean±SEM. SPSS for WINDOWS™ (version 12.0) was applied for the data analysis and statistically analyzed by one-way ANOVA followed by Dunnet t-test (2-sided). p<0.05 was taken to be the level of significance, p<0.001 was taken to be the level of highly significance.

**RESULTS**

**Analgesic activity:** In the acetic acid-induced writhing inhibition test, the extract at both doses (200 and 400 mg kg\(^{-1}\)) demonstrated highly significant (p<0.001) inhibition of writhing response induced by the acetic acid in a dose dependant manner. The percent inhibitions of the writhing response at the doses 200 and 400 mg kg\(^{-1}\) were found to be 67.56 and 65.14%, respectively (Table 1).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (g.o.)</th>
<th>No. of writhing</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.5 mL mice(^{-1})</td>
<td>78,64,29</td>
<td>-</td>
</tr>
<tr>
<td>Positive control</td>
<td>10 mg kg(^{-1})</td>
<td>1,5,0.15**</td>
<td>98.09</td>
</tr>
<tr>
<td>JGL200</td>
<td>200 mg kg(^{-1})</td>
<td>25,5,0.70**</td>
<td>67.56</td>
</tr>
<tr>
<td>JGL400</td>
<td>400 mg kg(^{-1})</td>
<td>27,4,0.33**</td>
<td>65.14</td>
</tr>
</tbody>
</table>

Values are expressed as Mean±SEM (n = 5), **p<0.001 compared to control. JGL200 and JGL400 indicate dose of 200 and 400 mg kg\(^{-1}\) b.wt., respectively. Positive control: Diclofenac sodium.
Neuropharmacological activities

Hole cross test: In the hole cross test, the extract at both doses of 200 and 400 mg kg\(^{-1}\) b.wt. showed highly significant (p<0.001) decrease in locomotion activity in the test animals at the observation periods compared to the control group (Table 2). The positive control, diazepam showed decrease in locomotion activity at all the observation periods (Table 2).

Hole board test: In the hole board test, the extract at dose 200 mg kg\(^{-1}\) b.wt. showed an increase number in head dipping (50.6±0.87) behavior compared to the control group which was statistically highly significant (p<0.001) (Table 3). But the extract at dose 400 mg kg\(^{-1}\) showed highly significant (p<0.001) decrease in head dipping (27.8±0.58) behavior and latency until the first head dipping (6.4±0.51) behavior compared with the control group (Table 3).

Elevated plus-maze test: In Elevated Plus-maze (EPM) test, the extract at dose 200 mg kg\(^{-1}\) b.wt. showed decrease in time spent in open arm compared to control. But the extract at dose 400 mg kg\(^{-1}\) b.wt. showed increase in the time spent in the open arm compared to control (Table 4). The effects of extract on mice ethologically derived measures after oral administration were shown in Fig. 1. A significant (p<0.05) decrease in rearing behavior was observed by treatment with the extract at dose of 400 mg kg\(^{-1}\) b.wt. (Fig. 1).

Anti-diarrheal activity: In castor oil-induced diarrhea inhibition test, the extract at both doses of 200 and 400 mg kg\(^{-1}\) b.wt. showed highly significant (p<0.001) decrease in mean number of stool and total weight of fecal output compared to control group (Table 5).

**DISCUSSION**

**Analgesic activity:** In the acetic acid induced writhing inhibition test in mice, the extract showed highly significant (p<0.001) inhibition of writhing response in a dose dependent manner (Table 1). The highly significant inhibition of writhing response by the extract may be due to the presence of analgesic principals in the plant such as alkaloids, flavonoids, saponins, resins and tannins which were reported by other researchers (Oduola et al., 2005b; Khumungsee et al., 2009). These phytochemicals were reported to inhibit pain sensation primarily by acting

![Fig. 1: Effect of diazepam and methanol extract of J. gossypifolia leaves (JGL) on ethologically derived measures in mice tested on the elevated plus-maze. Results are shown as Mean±SEM, n = 5, *p<0.05, compared to control; JGL200 and JGL400 indicate dose of 200 and 400 mg kg\(^{-1}\) b.wt., respectively. Diazepam: Positive control](image-url)
Table 5: Effect of methanol extract of J. gossypifolia leaves (JGL) and loperamide on castor oil induced diarrhea in mice

<table>
<thead>
<tr>
<th>Treatment (dose)</th>
<th>Dose (p.o.)</th>
<th>Latent period (min)</th>
<th>No. of stools</th>
<th>Total weight of fecal output</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.5 mL/mice</td>
<td>42.2±1.16</td>
<td>14.4±1.75</td>
<td>0.94±0.02</td>
</tr>
<tr>
<td>Positive control</td>
<td>2 mg kg⁻¹</td>
<td>95.2±1.71**</td>
<td>8.6±0.75**</td>
<td>0.73±0.03**</td>
</tr>
<tr>
<td>JGL200</td>
<td>200 mg kg⁻¹</td>
<td>7.2±0.66**</td>
<td>7.6±0.51**</td>
<td>0.48±0.03**</td>
</tr>
<tr>
<td>JGL400</td>
<td>400 mg kg⁻¹</td>
<td>53.6±3.56**</td>
<td>9.2±0.66**</td>
<td>0.30±0.02**</td>
</tr>
</tbody>
</table>

Values are expressed as Mean±SEM (n=5), **p<0.05, ***p<0.001 compared to control; JGL200 and JGL400 indicate dose of 200 and 400 mg kg⁻¹ b.wt, respectively. Positive control: Loperamide

with prostaglandin pathways (Rahman, 2012). Further detailed investigations are needed to determine the actual mechanism by which the plant shows its non-narcotic analgesic activity.

Neuropharmacological activities

**Hole cross test:** The extract at both doses showed decrease in spontaneous motor activity in the hole cross test (Table 2). The decrease in number of hole cross indicates the sedative activity of the plant. Drug acting on CNS mainly exert their action through GABA_A receptor as Gamma-amino-butyric Acid (GABA) is the major inhibitory neurotransmitter in the CNS (Dolai et al., 2012). The observed sedative effect of the extract may be due to CNS hyperpolarization via interaction with GABA_A receptor. The positive control showed decrease in number of hole cross compare to control which may be due to the dose (1 mg kg⁻¹) used in the test that can produce sedation in mice (Apu et al., 2013).

**Hole board test:** The hole board experiment is a widely accepted test to measure the anxiolytic and anxiogenic behaviour of mice (Takeda et al., 1998). The extract showed highly significant (p<0.001) anxiolytic and anxiogenic behavior at dose 200 and 400 mg kg⁻¹ b.wt respectively (Table 3). Preliminary qualitative phytochemical screening reveals the presence of alkaloids flavonoids, saponins and tannins (Oduola et al., 2005a; Khunrungsee et al., 2009) in J. gossypifolia which are well known to have activity against many CNS disorders (Adeyemi et al., 2006). So the observed anxiolytic activity at lower doses (200 mg kg⁻¹ b.wt.) may be due to the binding of any of the phytochemicals to the GABA_A-BZDs complex; however further studies are needed to assurance this. The highly significant (p<0.001) decrease in head-dipping behavior observed in diazepam treated mice compared to control may be due to the sedative dose (1 mg kg⁻¹) used in the test (Apu et al., 2013).

**Elevated plus-maze test:** The elevated plus maze test is considered to be an etiologically valid animal model of testing psychomotor performance and emotional aspects of mice. In the elevated plus-maze test (Table 4, Fig. 1), the extract at higher dose (400 mg kg⁻¹ b.wt.) showed increased in the time spent in open arm which indicates the anxiolytic activity of the plant, whereas anxiogenic effect was observed at dose (200 mg kg⁻¹ b.wt.) as described by Apu et al. (2013). The observed anxiolytic activity of the extract may be due to the presence of various phytoconstituents such as sterols, flavonoids, saponins, tannins in the plant (Gadakar et al., 2011).

**Anti-diarrheal activity:** Castor oil develops diarrhea upon oral administration in mice due to the presence of recinoleic acid which can cause intestinal stimulation of motility and secretion (Apu et al., 2013). It is well evident that recinoleic acid showed the effects by irritation and inflammation of the intestinal mucosa, leading to the release of prostaglandins (Ezeonwumelu et al., 2012). Di Carlo et al. (1993) reported the activity of flavonoids in diarrhea by inhibition of release of autacoids and prostaglandins, which result in inhibition of motility and secretion induced by castor oil. Tannins are also reported to have anti-diarrheal property since these phytoconstituents may precipitate proteins of the enterocytes; reduce peristaltic movement and intestinal secretions (Okudo et al., 1989). So, the observed anti-diarrheal activity (Table 5) of the methanol extract may be due to the presence of these phytochemicals.

**CONCLUSION**

Finally, it can be concluded from the results of the study that methanol leaves extract of J. gossypifolia has significant analgesic, neuropharmacological and anti-diarrheal activities at the investigated doses. However extensive studies can be carried out to identify the phytoconstituent(s) with their mechanism of actions responsible for the observed pharmacological activities.

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**REFERENCES**


