ANALGESIC ACTIVITY OF MUCUNA PRUIENS LINN


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Abstract
The current study was aimed to investigate the Analgesic properties of the leaf extracts (n-hexane, dichloromethane, and methanol) of Mucuna pruriens Linn. (Family: Fabaceae, Bengali name: Alkushi). The phytochemical screening of the plant confirmed the presence of several chemical groups including- steroids, alkaloids, flavonoids, tannins, gums, and saponins in the methanolic extract of the leaf. The cytotoxic activity on brine shrimp nauplii, increase in mortality with increased concentration of the extracts which suggests possible antitumor, antibacterial and pesticidal agents. It was observed that Acetic Acid Induced Writhing Test of methanolic extract of the leaf inhibited 61.92 % and 87.62 % of writhing with lower and higher dose respectively. Whereas the positive control Diclofenac-Na inhibited 72.14 % of writhing at a dose of 50mg/kg body weight. The results of Tail Flick test of the leaf extract on mice were not so impressive. Thus, the obtained results in this project work provide a support for the use of this plant for medicinal purposes and encourage further investigations for more fruitful results.

Keywords: Analgesic, Alkushi, phytochemical screening, Writhing Test.

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INTRODUCTION

The origin and historical distribution of *Mucuna pruriens* L. is India. *Mucuna pruriens* Linn. is grown all over the tropics and sub-tropics but by far the greatest diversity of cultivars can be found in eastern India, China, Carribian. It is widely believed that these are the areas where the primitive forms of the species were first cultivated. The genus Mucuna contains approximately 100 species distributed throughout tropical and subtropical regions. Numerous crosses of *Mucuna pruriens* L. and the native Mucuna species have contributed to producing a wide range of floral forms of this species.[1,2]

![Fig. 1: Plant of Mucuna pruriens L](image)

**Growth Habit:**
An Annual, much-branched, 15 cm. climbing woody vines called lianas, homboid leaves and hanging white lavender or purple color of flower, commonly planted as an medicinal purposes in gardens or yard as well as hilly area, or specific area of the country. [3]

**Growing Environment:**
- **Sun Exposure:** Full Sun
- **Soil pH requirements:** < 5.0 – 8.0
- **Watering needed:** Moderate water. Need good drainage system, and moist soil.

**Propagation:**
The normal method of propagating Mucuna is by cuttings that root easily within a month or so. Taking healthy shoot tip cuttings from current season’s growth or softwood cuttings, the former being most appropriate for an upright single stem if a person preferred a standard specimen eventually. It can also be propagated by air-layering, grafting and even raised from seeds.

**Characteristics of this plant family:**
- **Leaves:** The leaves are tripinnate, ovate, reverse ovate, rhombus-shaped or widely ovate. The sides of the leaves are often heavily grooved and the tips are pointy. In young *M. pruriens* plants,
both sides of the leaves have hairs. The stems of the leaflets are two to three millimeters long. Additional adjacent leaves are present and are about 5 mm long.

● **Flowers:** The flower heads take the form of axially arrayed panicles. They are 15 to 32 cm long and have two or three, or many flowers. The accompanying leaves are about 12.5 mm long; the flower stand axes are from 2.5 to 5 mm. The bell is 7.5 to 9 mm long and silky. The sepals are longer or of the same length as the shuttles. The crown is purplish or white. The flag is 1.5 mm long. The wings are 2.5 to 3.8 cm long.

● **Fruits:**
In the fruit ripening stage, a 4 to 13 cm-long, 1 to 2 cm-wide, unwinged, leguminous fruit develops. There is a ridge along the length of the fruit. The husk is very hairy and carries up to seven seeds. The seeds are flattened uniform ellipsoids, 1 to 1.9 cm long, 0.8 to 1.3 cm wide and 4 to 6.5 cm thick. [4]

**Therapeutic use of Mucuna pruriens Linn.:**
- Leaf paste is applied to ulcers.
- Infusion of root mixed with honey prescribed for cholera.
- Hairs of the pod use as anthelmintic.
- Seed regarded as Nervine tonic, snake bite remedy, edema, and most potential for the treatment of Parkinson’s disease.

**MATERIALS AND METHODS**

**Identification of the Plant:**
The plant leaves were collected from Dhaka, Bangladesh. Later the plant was identified by Bangladesh National Herbarium Institute, Mirpur, Dhaka. An accession number were given from there and the number is given below.

<table>
<thead>
<tr>
<th>Table 1: Local Name, Botanical Name and Accession Code of the Plant:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Local Name</td>
</tr>
<tr>
<td>-------------</td>
</tr>
<tr>
<td>Alkushi</td>
</tr>
</tbody>
</table>

**Collection, Drying and Pulverization of Plant:**

**Plant Collection:**
*Mucuna pruriens* Line. was collected from Botanical Garden, Dhaka, Bangladesh. The time of collection was August, 2010.
Drying

*Mucuna pruriens* Linn. Leaves were first separated from undesirable materials. They were dried for one week in a shaded place.

Grinding

After drying, the plant part was grinded by Blender Machine (NOWAKE, JAPAN). Coarse powder was obtained after grinding.

Extraction of Plant Materials

The shade dried and powdered plant material was separately extracted to exhaustion in a Soxhlet apparatus with n-hexane, Dichloromethane and methanol. The extractive was filtered through fresh cotton bed and finally with Whatman no-1 filters paper. The volume of the filter was concentrated with a rotary evaporator at low temperature (40-50°C) and reduced pressure.

Overall Extraction Process

![Flow Chart of the Overall Extraction Process of Mucuna pruriens L.](image-url)
RESULT AND DISCUSSION

Analgesic drugs which are currently in use are either narcotics or non-narcotics which have proven side and toxic effects. To develop new synthetic compounds in this category is an expensive venture and again may have problems of side effects. On the contrary, many medicines of plant origin had been used and are in use successfully since long time without any serious effects [5]. Pain has been officially defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage. Pain acts as a warning signal against disturbances of the body and has a proactive function. Analgesic means a drug that selectively relieves pain by acting in the CNS or on peripheral pain mechanisms, without significantly altering consciousness. So, analgesic activity means capacity of a substance to neutralize the pain sensation [6]. The lack of potent analgesic and anti-inflammatory drugs now actually in use prompted the present study, in which leaf of *Mucuna pruriens* Linn. had been selected for its reported biological activities in indigenous system of medicine.

![Diagram of Prostaglandins and Leukotrienes](image)

**Fig. 3: Synthesis of Prostaglandins and Leukotrienes**

**Experimental Animal**

Young *Swiss albino* mice aged 3-4 weeks, average weight 18-20 gm. were used for the experiment. For this experiment, four groups (I, II, III, and IV) of mice was used and each group contains 3 mice.
Preparation of the Test Materials
To prepare solution of the plant extract at a doses of 200mg/kg and 400mg/kg body weight, 48 mg extract was measured and added with it 2.4 ml of distilled water and mixing with the help of vortex apparatus. From this solution 0.20ml was taken for 200mg/kg and 0.40ml for 400mg/kg dose.

Mechanism of Writhing Test
The acetic acid induced writhing method is an analgesic behavioral observation assessment method that demonstrates a noxious stimulation in mice. The test consists of injecting the 0.7% acetic acid solution intraperitoneally and then observing the animal for specific contraction of body referred as 'writhing'. A comparison of writhing was made between positive control (Diclofenac), control and test sample given orally 30 minutes prior to acetic acid injection.

Fig. 6: Schematic representation of acetic acid induced writhing of mice for investigation of analgesic activity
If the sample possesses analgesic activity, the animal that received the sample, will give lower number of writhing than the control, i.e. the sample having analgesic activity will inhibit writhing. Diclofenac-Na is used as reference standard drug.

**Study Design**

Experimental animals were randomly selected and divided into four groups denoted as group-I, group-II, group-III and group-IV, consisting of 3 mice in each group. Each group received a particular treatment i.e. control, positive control and the two doses of the extract. Each mouse was weighed properly and the doses of the test samples and control materials were adjusted accordingly.

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Table 2: Experiment Profile to assess the effect of crude extract of Mucuna pruriens L. on acetic acid induced writhing of mice

<table>
<thead>
<tr>
<th>Animal Group</th>
<th>Treatment</th>
<th>No. of Animals</th>
<th>Dose</th>
<th>Route of Administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Placebo (Water and Tween 80)</td>
<td>5</td>
<td>0.50 ml</td>
<td>Oral</td>
</tr>
<tr>
<td>Positive Control</td>
<td>Diclofenac-Na</td>
<td>5</td>
<td>50 mg/kg</td>
<td>Oral</td>
</tr>
<tr>
<td>Group-1</td>
<td>Extract of Mucuna pruriens (200 mg/Kg dose)</td>
<td>5</td>
<td>200 mg/kg</td>
<td>Oral</td>
</tr>
<tr>
<td>Group-2</td>
<td>Extract of Mucuna pruriens (400mg/Kg dose)</td>
<td>5</td>
<td>400 mg/kg</td>
<td>Oral</td>
</tr>
</tbody>
</table>

Counting of Writhing:
After inducing the plant extract and control every mice of all groups was observed carefully to count the number of writhing which made within 15 minutes.

![Fig. 12: Half Writhing](image1)
![Fig. 13: Full Writhin](image2)

Result:

Data of Analgesic Activity by Writhing Test of Leaf Extract:
All the experimental data on Analgesic Activity by writhing are presented in Table

Table 3: Analgesic Activity of the Leaves of Mucuna pruriens L. on Mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Average Body Weight of Mice (gm.)</th>
<th>Writhing Counting</th>
<th>Mean</th>
<th>% of Writhing</th>
<th>SD</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>M-1</td>
<td>M-2</td>
<td>M-3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>18-20</td>
<td>35</td>
<td>30</td>
<td>32</td>
<td>32.3</td>
<td>100</td>
</tr>
<tr>
<td>Positive control</td>
<td></td>
<td>6</td>
<td>8</td>
<td>13</td>
<td>9</td>
<td>27.86</td>
</tr>
<tr>
<td>Group-1</td>
<td></td>
<td>9</td>
<td>15</td>
<td>13</td>
<td>12.3</td>
<td>38.08</td>
</tr>
<tr>
<td>Group-2</td>
<td></td>
<td>3</td>
<td>5</td>
<td>4</td>
<td>4</td>
<td>12.38</td>
</tr>
</tbody>
</table>

Control: Tween-80 + Water, Positive Control: Diclofenac-Na (50 mg/kg),
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Group-1: Extract (200mg/kg) and Group-2: Extract (400mg/kg)

Table 4: Results with percent of inhibition

<table>
<thead>
<tr>
<th>Administered Substance</th>
<th>SEM</th>
<th>Mean ± SEM</th>
<th>% of Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.45</td>
<td>32.3±1.45</td>
<td>0.00</td>
</tr>
<tr>
<td>Positive control</td>
<td>2.07</td>
<td>9±2.07</td>
<td>72.14</td>
</tr>
<tr>
<td>Group-1</td>
<td>1.77</td>
<td>12.3±1.77</td>
<td>61.92</td>
</tr>
<tr>
<td>Group-2</td>
<td>0.58</td>
<td>4±0.58</td>
<td>87.62</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ±SEM (n=3)

Graphical Presentation:

Percent of Inhibition of Writhing for Leaf Extract:

Experimental data on Analgesic Activity by writhing are presented graphically in Figure.

Fig. 14: Bar Diagram showing the result of Writhing Test for Leaf Extract

Analgesic Activity by Tail-flick Test

There are two variants of the Tail-flick Test. One consists of applying radiant heat to a small surface of the tail. The other involves immersing the tail in water at a predetermined temperature. Although apparently similar, these two alternatives are actually quite different at a physical level [7].

Principle

Immersion of an animal's tail in hot water provokes an abrupt movement of the tail and sometimes the recoiling of the whole body. Again, it is the reaction time that is the time to flick the tail from hot water which is monitored. [8] If a sample contains any analgesic principle it increases the ability of the mice to retain its tail in the hot water which is reflected in the increase in the tail flicking time.

Procedure

The Tail-flick test was used with modification described by Dambisyo et al., (1990). The screening cut-off time was 5 sec, while the test cut-off time was 10sec. The extract was administered orally at two doses (200, 400 mg/kg body weight) using Diclofenac Sodium as
standard. The post drug reaction times were measured at 0, 30, 60 and 90 minutes later [9]. The tail of the mouse was immersed to a constant level (3 cm) in a water bath maintained at 55 ± 0.5°. The time to flick the tail from water (reaction time) was recorded. A maximum immersion time of 10 sec. was maintained to prevent thermal injury to the animals. A significant increase in reaction time compared with control animals was considered a positive analgesic response.

Result

The Analgesic Activity Tests were carried out in the laboratory on four groups of mice by Tail-flick Method. Time interval for the test was 30 minutes. The acquired results of the tests are presented both in the Tabular Form and Graphical Form in the following chapters.

Data Analysis of Analgesic Activity by Tail-flick Method:

The Analgesic Activity Tests results by Tail-flick Method are given in the following Tables:

<table>
<thead>
<tr>
<th>Group</th>
<th>Time</th>
<th>Tolerance Time</th>
<th>Mean</th>
<th>SD</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>M-1</td>
<td>M-2</td>
<td>M-3</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0 min</td>
<td>1.57</td>
<td>1.34</td>
<td>1.3</td>
<td>1.40</td>
</tr>
<tr>
<td></td>
<td>30 min</td>
<td>2.00</td>
<td>1.90</td>
<td>1.94</td>
<td>1.94</td>
</tr>
<tr>
<td></td>
<td>60 min</td>
<td>1.97</td>
<td>1.86</td>
<td>2.45</td>
<td>2.00</td>
</tr>
<tr>
<td></td>
<td>90 min</td>
<td>2.30</td>
<td>1.56</td>
<td>1.9</td>
<td>1.92</td>
</tr>
<tr>
<td>Positive</td>
<td>0 min</td>
<td>3.00</td>
<td>2.10</td>
<td>3.4</td>
<td>2.83</td>
</tr>
<tr>
<td>Control</td>
<td>30 min</td>
<td>5.49</td>
<td>4.70</td>
<td>5.90</td>
<td>5.36</td>
</tr>
<tr>
<td></td>
<td>60 min</td>
<td>7.70</td>
<td>6.70</td>
<td>7.80</td>
<td>7.4</td>
</tr>
<tr>
<td></td>
<td>90 min</td>
<td>7.21</td>
<td>6.49</td>
<td>7.9</td>
<td>7.2</td>
</tr>
<tr>
<td>Group-1</td>
<td>0 min</td>
<td>3.81</td>
<td>2.98</td>
<td>2.64</td>
<td>3.14</td>
</tr>
<tr>
<td></td>
<td>30 min</td>
<td>7.31</td>
<td>3.64</td>
<td>3.90</td>
<td>4.95</td>
</tr>
<tr>
<td></td>
<td>60 min</td>
<td>3.55</td>
<td>3.39</td>
<td>3.00</td>
<td>3.31</td>
</tr>
<tr>
<td></td>
<td>90 min</td>
<td>4.52</td>
<td>2.47</td>
<td>2.39</td>
<td>3.12</td>
</tr>
<tr>
<td>Group-2</td>
<td>0 min</td>
<td>1.14</td>
<td>2.76</td>
<td>2.36</td>
<td>2.08</td>
</tr>
<tr>
<td></td>
<td>30 min</td>
<td>2.71</td>
<td>4.26</td>
<td>3.92</td>
<td>3.63</td>
</tr>
<tr>
<td></td>
<td>60 min</td>
<td>3.14</td>
<td>2.70</td>
<td>3.47</td>
<td>3.10</td>
</tr>
<tr>
<td></td>
<td>90 min</td>
<td>2.72</td>
<td>3.85</td>
<td>3.58</td>
<td>3.38</td>
</tr>
</tbody>
</table>

Control: Tween-80 + Water, Positive Control: Diclofenac-Na (50 mg/kg),

Group-1: Extract (200mg/kg) and Group-2: Extract (400mg/kg)
Table 6: Secondary Data Table (Results of Tail-flick Test for Leaf Extract)

<table>
<thead>
<tr>
<th>Test Group</th>
<th>Dose (mg/kg)</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
<th>90 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.40±0.08</td>
<td>1.94±0.02</td>
<td>2.0±0.19</td>
<td>1.92±0.21</td>
<td></td>
</tr>
<tr>
<td>Positive Control</td>
<td>2.83±0.38</td>
<td>5.36±0.35</td>
<td>7.4±0.35</td>
<td>7.2±0.40</td>
<td></td>
</tr>
<tr>
<td>Group-1</td>
<td>3.14±0.35</td>
<td>4.95±1.18</td>
<td>3.31±0.17</td>
<td>3.12±0.69</td>
<td></td>
</tr>
<tr>
<td>Group-2</td>
<td>2.08±1.00</td>
<td>3.63±0.47</td>
<td>3.10±0.23</td>
<td>3.38±0.34</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SEM, (n=3)

Graphical Representation (for Leaf Extract):

Experimental data on Analgesic Activity by Tail-flick Method are presented graphically in as shown below:

CONCLUSION

In this study, the tail flick test and acetic acid induced writhing reflex were used to elucidate central and peripheral anti-nociceptive effects. The methanolic extract of *Mucuna pruriens* Linn. Displayed a significant and dose dependent analgesic activity that of the standard whereas result of tail flick method on mice was not so impressive. The mean number of abdominal constriction after I.P. injection of acetic acid was 33.4 in vehicle treated control animals. Diclofenac sodium (50 mg/kg) treatment produced 72.14 % inhibition of writhing response. A dose dependent reduction in the number of abdominal constriction was observed in animals treated with different
concentration of methanolic extract of *Mucuna pruriens* L. At the dose of 200mg/kg and 400mg/kg, inhibition of writhing response was observed 61.92 % and 87.62 % respectively for leaf extract.

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