HEMIDESMUS INDICUS L.: EVALUATION OF SEDATIVE & HYPNOTIC EFFECT IN THE ELEVATED PLUS-MAZE APPARATUS

Javed Khan Pathan1*, Girendra Gautam2 and Arun Kumar Gupta3

1Research Scholar, Bhagwant University, Ajmer (Raj.).
2Bhagwant University, Ajmer (Raj.).
3Chamelidevi Institute of Pharmacy, Indore (M.P.).

*Corresponding Author: Javed Khan Pathan
Research Scholar, Bhagwant University, Ajmer (Raj.).

ABSTRACT
This is the preliminary study of *Hemidesmus indicus* L. for sedative and hypnotic activity. The plant is used as folk medicine. In present work sedative & hypnotic like properties were evaluated for ethanolic & aqueous extract of *H. indicus* L. Stem & Leaves. Thiopental Sodium has been used as an index for the hypnotic effect. Loss of the righting reflex was used to determine start of sleep. The sleep latency and sleeping time were evaluated in the present study. The results showed that extracts could obviously shorten the sleep latency and prolong the sleeping time of mice produced by thiopental sodium (60 mg/kg, i.p.). Stem & Leaves extracts were produced significant sedative & hypnotic effect in elevated plus-maze. The dose-effect relationship is remarkable. **Aim of Study:** The goal is to use the evidentiary strengths that both systems (Ayurvedic and Western paradigms) of thought bring to the table, taking care not to evaluate one system using the lenses of the other. So we are not to evaluate one system using the lenses of the other. **Aim of Study:** The goal is to use the evidentiary strengths that both systems (Ayurvedic and Western paradigms) of thought bring to the table, taking care not to evaluate one system using the lenses of the other. Presently increasing demand of herbal products acquired in global markets, as well as medicinal plants collected for personal consume are a known modern tendency. In the present study, ethnomedicinal use of *H. indicus* L. stem & leaves as sedative and hypnotic has been studied & evaluated.

KEYWORDS: *Hemidesmus indicus*, *H. indicus*, sedative & hypnotic, thiopental sodium, sleeping time.

INTRODUCTION
The medicinal herb *Hemidesmus indicus*, also named anantamul has been growing in demand in the U.S. marketplace. Although numerous freely available herbal resources of anantamul which shows its traditional benefits and supportive Ayurvedic theory. Literature searches using terms ‘anantamul’ or ‘anantamul’ or *Hemidesmus indicus* or ‘Indian sarsaparilla’ were conducted in various databases including PubMed, Academic Search Complete and others. Secondary searches were conducted using Google and Google Scholar and scoured for additional information regarding this plant.

A review of literatures revealed a lack of formal clinical trials. The current status of biomedical research on this herb is not well known. However, more than 100 animal and laboratory studies have been conducted since the 1960s and together these studies show many traditional medical actions of plant.[5][6]

A preliminary study of pharmacologic properties, bioactive and therapeutic potential of anantamul was described.[2][3] For safety parameter there is a lack of reported adverse effects of in available literatures.[4]

Moreover, for nervous disorders, it’s sweet and cooling effect theoretically nourishes sadhaka pitta and the mind, makes particularly better for disturbed orangy or irritated emotions due to high pitta which aggravates the equilibrium of the mind.[5] Anantamul has also been combined with gotu kola, rose, and brahmi for hot emotions and disturbed pitta. There is a preliminary evidence suggesting that plant may possess acetylcholinesterase inhibitory activity.[6]

MATERIALS AND METHODS
Collection & Preparation of Plant Parts
Plant stem & leaves were procured from Elixer distributors, Kanpur (U.P.). Then proper identification of plant sample was done by an expert Botanist Dr. S. N. Dewedi, Professor and Head, Department of Botany, Janta PG College, APS University, Rewa, M.P. (Voucher No. J/BOT/H-238). Leaves of the plant were sun dried for several days. The plant materials were then oven dried for 24 hours at considerably low temperature for better grinding. The dried stem & leaves were then ground in coarse powder using high capacity grinding machine.
Washing and Drying of Plant Parts
At first leaves were thoroughly washed with tap water to remove dust, soil, bird’s droppings etc. within them. The leaves were then dried under sunlight for several days. But, due to rainy season sun drying was avoided. Instead, the leaves were dried in hot air oven at 50°C for 2 hours.

Grinding and Storage of Dried Samples
The dried parts were ground to coarse powder with the help of home blender machine. This process breaks the plant parts into smaller pieces thus exposing internal tissues and cells to solvents and facilitating their easy penetration into the cells to extract the constituents. Then the powdered sample was kept in clean closed glass containers till extraction. During grinding of sample, the grinder was thoroughly cleaned to avoid contamination with any remnant of previously ground material or other extraneous matters deposited on the grinder.

Extraction of the Dried Powdered Sample
The fine powder of *H. indicus* stem & leaves was dissolved in ethanol and it was thoroughly shaken to dissolve the powder into the solvent. Then it was kept in a closely covered glass jar for 7 days and shaken several times during the process for more interaction between the powdered particles and the solvent. This process is termed as maceration. The cover of the jar was closed properly to resist the entrance of air in the jar.

Filtration of the extracts
After the extraction process the plant extracts was filtered with sterilized cotton filter and filter paper. The filtrate was collected in a beaker. The filtration process was repeated three times by using cotton and filter paper. Then the filtrate was taken into a volumetric flask and it was thoroughly shaken to get more dried extract. All beakers were covered with aluminum foil. The extract was then collected and stored in a cool (4°C) dry place for further assay.

Evaporation and Condensation of extracts
The extracts were transferred to the round bottle flask of rotary evaporator. Then excess amount of solvents in the extracts were removed by rotary evaporator, with reduced pressure which was done by using a vacuum pump. The temperature of the rotary evaporator was set 50°C. It run for 1 hours 10 minutes and the RPM was set 80 for evaporation process. After evaporation extract was transferred in a beaker. Rest of the extract was removed from the round bottle flask. Then extract was kept in hot air oven to get more dried extract. All beakers were covered with aluminum foil. The extract was then collected and stored in a cool (4°C) dry place for further assay.

Experimental Protocols
Protocol has been approved by Institutional Animal Ethical Committee (IAEC) prior to the initiation of the experiment. Protocol approval reference number (PBRI/IAEC/PN-17047a) Swiss albino mice of 3-4 weeks of age, weighing between 20–25 gram was taken for pharmacological activity and females of the same strain for LD<sub>50</sub> calculation. The mice were kept in animal house at a standard environmental condition (temperature-22 ± 1°C relative humidity-55 ± 5% and 12h light/12 h dark cycle). Animals were fed *ad libitum* with standard food and water except when fasting was required in the course of study. The animals were acquired from the local market. Animals were kept in standard environmental conditions and had free access to feed and water.

Method for Identification of Animals
Each group consists of six animals. It was difficult to observe the biological response of six mice at a time receiving same treatment. It is quite necessary to identify individual animal of groups during treatment. The animals were individualized by marking lines on tail.

Acute toxicity study
Acute oral toxicity studies have been conducted separately followed by using OECD guideline 423. The method used defined doses of 5, 50, 300, 2000 mg/kg p.o. body weight. Results were allowed substance rank and classify according to the Globally Harmonized System (GHS) for classification of chemicals which causes acute toxicity. From LD<sub>50</sub> determination, 1/10<sup>6</sup> of the dose was focused as the medial for pharmacological screening. Since all animals were alive & no toxicity and no significant changes in the body weight between the control and treated group were demonstrated at doses up to 2000 mg.[1]

Pharmacological Evaluations
The activity of ethanolic & aqueous extract from *H. indicus* stem & leaves the central nervous system was studied, using a battery of behavioral tests used in psychopharmacology. We analyzed the effect of different doses of the ethanolic and aqueous extracts (200mg/kg and 400 mg/kg, p.o.) from *H. indicus* for their sedative and hypnotic activities.

Agents used in activity
- Distilled water (0.1 ml per mouse) as negative control.

Standard drugs in activity
- Diazepam (3 mg/kg) *i.p.* as positive control.

Doses in activity
- Ethanolic & Aqueous extracts of *H.indicus* stem & leavesat a dose of 200mg/kg & 400mg/kg were administered orally. Distilled water has been used as a vehicle extracts for preparing different doses.
Determination of sleep induced by Thiopental Sodium<sup>(8,9)</sup>

Thiopental sodium (60 mg/kg i.p., sub-hypnotic dose) has been injected, 30 minutes after administration of plant extracts. After these 30 minutes and after 15 minutes of diazepam treatment, thiopental sodium was administered to each animal. The control group (n = 6) has been given distilled water 10 ml/kg p.o. & positive control group (n = 6) has been treated with diazepam (3 mg/kg i.p.) Then the animals were observed for the time to lose their righting reflex. The effects have been recorded for disappearance (latency) and reappearance (duration) of the righting reflex. Hypnotic sleeping time has been considered to be the time interval between disappearance and reappearance of the righting reflex.

Table 1: Sedative & Hypnotic Effect of <i>H. indicus</i> by Thiopental Sodium induced sleeping time in mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment (mg/kg), p.o.</th>
<th>Onset of Sleep (min.)</th>
<th>Duration of Sleep (min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normo Control</td>
<td>Distilled water (0.1 ml per mouse)</td>
<td>12.50 ± 0.40</td>
<td>85.45</td>
</tr>
<tr>
<td>Positive Control</td>
<td>Diazepam (3 mg/kg) i.p.</td>
<td>6.80 ± 0.10</td>
<td>180.55</td>
</tr>
<tr>
<td>Test 1 200 mg/kg</td>
<td>5% W/V Stem extract (ethanol)</td>
<td>9.20 ± 0.20</td>
<td>120.35</td>
</tr>
<tr>
<td>Test 2 400 mg/kg</td>
<td>10% W/V Stem extract (ethanol)</td>
<td>8.50 ± 0.25</td>
<td>175.35</td>
</tr>
<tr>
<td>Test 3 200 mg/kg</td>
<td>5% W/V Stem extract (Aqueous)</td>
<td>10.00 ± 0.51</td>
<td>110.30</td>
</tr>
<tr>
<td>Test 4 400 mg/kg</td>
<td>10% W/V Stem extract (Aqueous)</td>
<td>9.60 ± 0.70</td>
<td>130.30</td>
</tr>
<tr>
<td>Test 5 200 mg/kg</td>
<td>5% W/V Leaves extract (ethanol)</td>
<td>9.80 ± 0.25</td>
<td>130.40</td>
</tr>
<tr>
<td>Test 6 400 mg/kg</td>
<td>10% W/V Leaves extract (ethanol)</td>
<td>8.30 ± 0.25</td>
<td>170.35</td>
</tr>
<tr>
<td>Test 7 200 mg/kg</td>
<td>5% W/V Leaves extract (Aqueous)</td>
<td>9.20 ± 0.50</td>
<td>105.30</td>
</tr>
<tr>
<td>Test 8 400 mg/kg</td>
<td>10% W/V Leaves extract (Aqueous)</td>
<td>9.10 ± 0.70</td>
<td>170.30</td>
</tr>
</tbody>
</table>

DISCUSSION

For the production of commercial drugs or development of lead compounds, medicinal plants are good reservoir. Majorly drugs used for depression affects the quality life of patient. On the other hand, herbal medicines are less toxic, good absorptive capacity and has lower side effects. So, this has been used since ancient times. Hence, it’s required to put-on efforts to represent new medicinal plants for production of cheaper & less toxic drugs. As a result, number of experiments have been done on the plant extracts of <i>H.indicus</i> to determine its sedative - hypnotic activity on mice. <i>H. indicus</i> showed Sedative - Hypnotic effect better in 400 mg/kg p.o as compare to its half of the dose 200 mg/kg p.o. Diazepam was used as standard drug in the study as compared with the control and extracts. There is increase in duration of sleeping time after administration of 400 mg/kg extracts. This duration has been compared to that of the standard drug diazepam at a dose of 3 mg/kgip and control (normal water). The activity indicates that the increase in sleeping time decreases emotional state of animals and shows sedation or depression state. By abovementioned, it was observed that plant extracts exhibit positive effects.

None the less, from our best knowledge, this is the first report of CNS depressant or Sedative - Hypnotic on <i>H.indicus</i> L. stem & leaves.

CONCLUSION

Our results revealed that the ethanolic extract & aqueous extract of <i>H. indicus</i> L. stem & leaves appears to contain some chemicals that shows edation. <i>H. indicus</i> L. has medicinal value on psychological aspects that shows potent depressive effect. The crude extracts dose showed significant result when it is evaluated by elevated plus-maze method. The reduction found to be significant when it is compared to negative control. The effect of the extracts is comparable to that of the standard drug, diazepam 3mg/kgip.

Now, on the basis of results it can be concluded that the plant may be useful as CNS depressant agent. <i>H.indicus</i> L. stem & leaves possess potent Sedative - Hypnotic effect in both 200 mg/kg po and 400 mg/kgpo.

Although our work was only preliminary effort. It will require additional detailed advanced investigation. Future studies will be focused on the neurobiological mechanisms of action and a possible interaction with the phytoconstituents responsible for the observed central effects has to be isolated and identified.

BIBLIOGRAPHY


