EVALUATION OF CENTRAL NERVOUS SYSTEM ACTIVITIES OF PLUMBAGO ZEYLANICA L. LEAF EXTRACT

Vishnukanta*, A.C. Rana

Department of Pharmacology
B. N. College of Pharmacy, Udaipur-313001 (Rajasthan)- India

Summary

Hydroalcoholic extract of Plumbago zeylanica leaf were evaluated for central nervous system activities. It was found that extract showed significant CNS depressant activity, with muscle relaxant properties. It also showed anxiolytic activity.

* Correspondent author: Mrs. Vishnu kanta, Telphone: 09828140391 (0294) 2413182; E.mail: vishnukanta2003@yahoo.co.in
  Present address: Department of Pharmacology, B.N College of Pharmacy, Udaipur, Rajasthan.
Introduction

Herbal products are extensively used globally for the treatment of many diseases where allopathic fails or has severe side effects. Psycho neural drugs are also have very serious side effects like physical dependence, tolerance, deterioration of cognitive function and affect on respiratory, digestive and immune system. So in this contest the treatment through natural source is seen with the hope that they have the lesser side effects than that observed with synthetic drugs.

Plumbago zeylanica L. commonly known as white chitrak (Family: Plumbaginaceae) is a perennial herb that is grown in most parts of India and is used in the traditional system of Indian medicine against a number of ailments including skin diseases, diarrhoea and leprosy\(^1\).

The pharmacological studies carried out by several workers indicate that Plumbago zeylanica L. possesses antibacterial, antifungal, anticarcinogenic\(^2\) and radiomodifying properties\(^3\). It is also reported to have antitumor activity\(^4\). The roots of this plant has been reported to be a powerful poison when given orally or applied to ostium uteri, causes abortion\(^5\).

Methods

Plant collection and preparation of extract:

Fresh leaves of Plumbago zeylanica L. were collected from Rajasthan Agriculture College Campus, Udaipur Rajasthan, in the month of March. The plant was authenticated by Dr. S. S. Katewa, Dept. of Botany, College of Science, MLSU, Udaipur. Leaves were dried in shade, moderately grinded and macerated with hydroalcoholic solvent (70:30) for 7 days with intermittent shaking. On 8\(^{th}\) day the macerate was filtered through muslin cloth and solvent was evaporated at room temperature\(^6\).
The residue obtained, was lyophilized (lyophilizer-Step Origin Electric, Lonavala) and freeze-dried (Freeze dryer, Allied Frost) to provide dry hydroalcoholic extract of *Plumbago zeylanica* L. leaves (HEPZL) with the practical yield of 17 % W/W.

**Animals:**

Mature Albino Wistar rats of either sex in the weight range of 150-200 g and mice of 20-25 g were used in the study. Institution Animal Ethic Committee approved all the experimental procedures (approval no. 03/ACR/BNCP-06/IAEC). All the animals were maintained under standard husbandry conditions with food (Chakan mill, Sangali, Maharashtra) and water ad libitum.

**Acute oral toxicity:**

It was determined using OECD/OCDE guideline\textsuperscript{7} 425, main test was performed and LD50 was found to be 5000mg/kg the dose selected was 1/10\textsuperscript{th} and 1/20\textsuperscript{th} of LD50.

**CNS depressant activity:**

For the evaluation of CNS depressant activity, twenty four mature albino wistar rats of either sex, weighing between, 150-200 g were divided into four groups containing six in each. Group one, received 1% Carboxy Methyl Cellulose; served as control, group two, received diazepam (4mg/kg bw i.p.), group three, received HEPZL 250mg/kg bw, and group four, received HEPZL 500mg/kg bw.

All the animals were placed individually in the actophotometer (INCO, Ambala) for 10 minutes and basal activity was recorded. After one hour of oral treatment (with extract) or thirty minutes after i.p. treatment (with the standard), spontaneous motor activity of each animal was recorded individually for 10 minutes. Percent change in activity was calculated\textsuperscript{8,9}. 
Skeletal muscle relaxant activity:

For the evaluation of skeletal muscle relaxant activity; twenty four mature albino mice of either sex, weighing between, and 20-25g were divided into four groups containing six in each. Group one, received 1% Carboxy Methyl Cellulose; served as control, group two, received diazepam (4mg/kg bw i.p.), group three received HEPZL 250mg/kg bw, and group four received HEPZL 500mg/kg bw.

Animals were placed one by one on the rotating rod of Rota-Rod apparatus (INCO, Ambala) and fall off time (sec.) when mice falls from the rotating rod were noted. After one hour of oral treatment (with extract) or thirty minutes of i.p. treatment (with the standard), again the animals were placed one by one on the rotating rod and fall off time was recorded. The fall off time before and after drug treatment was compared and percent change in activity was calculated\(^{10}\).

Anti-anxiety activity:

For the evaluation of anti-anxiety activity; twenty four mature albino wistar rats of either sex, weighing between, 150-200 g were divided into four groups containing six in each. Group one, received 1% Carboxy Methyl Cellulose; served as control, group two, received diazepam (4mg/kg bw i.p.), group three, received HEPZL 250mg/kg bw, and group four, received HEPZL 500mg/kg bw.

After one hour of oral treatment (with extract) or thirty minutes after i.p. treatment (with the standard), the animal was placed at the center of the maze, facing one of the enclosed arm. During 5 minute test period the following measures were taken:

- The number of entries into open arms
- The number of entries into closed arms
- Time spent in the open arms
Arm entries were counted when the animal had placed all of its four paws in it. The procedure was conducted in a sound attenuated room, with observation made from an adjacent room. These measures were compared with the control group\(^8,11\).

**Statistical Analysis:**
The data was analysed by using one-way ANOVA followed by Tukey multiple comparison test. A p value <0.05 was considered to be significant.

**Results**

**CNS depressant activity:**
Both the doses of HEPZL produced significant (p<0.01) reduction in locomotor activity as compared to the control group and 35.14% and 48.91% decrease in activity was observed. The diazepam treated group also revealed a statistically significant (p<0.01), 85.5% decrease in locomotor activity (Table 1).

**Skeletal muscle relaxant activity:**
Extract and standard drug diazepam showed significant (p<0.01) reduction in the time (sec.) spent by the animals on revolving rod when compared to control. HEPZL showed 31.10% and 46.69% decrease in time on revolving rod (Table 2).

**Antianxiety activity:**
The standard drug diazepam, significantly (p<0.01) reduced the number of entries into closed arm, increased the time spent in open arm and number of entries into open arm. Low dose of HEPZL did not show any significant changes but at higher dose, it significantly (p<0.01) increased the time spent and number of entries into open arm and significantly (p<0.05) reduced the number of entries into closed arm (Table 3).
Table 1: CNS depressant activity of HEPZL by Actophotometer method

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose mg/kg</th>
<th>Locomotion activity (scores) in 10 minutes</th>
<th>Percent decrease in activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Before treatment</td>
<td>After treatment</td>
</tr>
<tr>
<td>I</td>
<td>Control (vehicle,1%CMC)</td>
<td>-</td>
<td>151.16±4.02</td>
<td>149.66±4.20</td>
</tr>
<tr>
<td>II</td>
<td>Diazepam 4, i.p.</td>
<td>4, i.p.</td>
<td>142.00±3.10</td>
<td>20.50±2.43**</td>
</tr>
<tr>
<td>III</td>
<td>HEPZL 250, p.o.</td>
<td>250, p.o.</td>
<td>138.50±3.67</td>
<td>89.83±8.35**</td>
</tr>
<tr>
<td>IV</td>
<td>HEPZL 500, p.o.</td>
<td>500, p.o.</td>
<td>146.50±7.44</td>
<td>74.83±5.43**</td>
</tr>
</tbody>
</table>

Data are analysed using one way ANOVA followed by Dunnet’s test. Values are Mean ± SEM, n=6 ** p<0.01, compared with control group.
Table 2: Skeletal muscle relaxant activity of HEPZL by Rota Rod method

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose mg/kg</th>
<th>Fall of time (Sec.)</th>
<th>Percent decrease in time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Before treatment</td>
<td>After treatment</td>
</tr>
<tr>
<td>I</td>
<td>Control (vehicle, 1% CMC)</td>
<td>-</td>
<td>317.83±7.32</td>
<td>307.00±9.76</td>
</tr>
<tr>
<td>II</td>
<td>Diazepam 4, i.p.</td>
<td>311.83±7.09</td>
<td>16.50±2.37**</td>
<td>94.7</td>
</tr>
<tr>
<td>III</td>
<td>HEPZL 250, p.o.</td>
<td>309.16±12.00</td>
<td>211.50±11.9**</td>
<td>31.10</td>
</tr>
<tr>
<td>IV</td>
<td>HEPZL 500, p.o.</td>
<td>287.5±14.24</td>
<td>163.66±10.15**</td>
<td>46.69</td>
</tr>
</tbody>
</table>

Data are analysed using one way ANOVA followed by Dunnet’s test. Values are mean ± SEM, n=6 ** p<0.01, compared with control group.
Table 3: Antianxiety activity of HEPZL by Elevated Plus Maze method

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose mg/kg</th>
<th>Number of entries into</th>
<th>Time spent in open arm (sec.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Closed arm</td>
<td>Open arm</td>
</tr>
<tr>
<td>I</td>
<td>Control (vehicle, 1% CMC)</td>
<td>-</td>
<td>13.00±1.20</td>
<td>6.16±1.01</td>
</tr>
<tr>
<td>II</td>
<td>Diazepam 4, i.p.</td>
<td>6.50±0.56**</td>
<td>15.33±0.66**</td>
<td>210.83±5.96**</td>
</tr>
<tr>
<td>III</td>
<td>HEPZL 250, p.o.</td>
<td>11.33±0.95</td>
<td>7.66±0.61</td>
<td>72.17±9.59</td>
</tr>
<tr>
<td>IV</td>
<td>HEPZL 500, p.o.</td>
<td>9.83±0.60*</td>
<td>10.33±0.80**</td>
<td>114.16±9.61**</td>
</tr>
</tbody>
</table>

Data are analysed using one way ANOVA followed by Dunnet’s test. Values are Mean ± SEM, n=6  *p<0.05, **p<0.01, compared with control group.
Discussion

Most of the CNS acting drugs influence the locomotor activities in man and animals. The CNS depressant drugs such as barbiturates and alcohol reduce the motor activity while the stimulants such as caffeine and amphetamine increase the activity. Rota rod test is used to evaluate the activity of drugs interfering with motor coordination. An antianxiety agent of benzodiazepine class of drug has muscle relaxing property. The skeletal muscle relaxation together with taming or calming effect of these agents reduces anxiety and tension. The loss of muscle grip is an indication of muscle relaxation. Elevated Plus- Maze test is used to evaluate psychomotor performance and emotional aspects of rodents. Rodents have aversion for high and open space and prefer enclosed arm therefore, spend greater amount of time in enclosed arm. When animals enter open arm, they freeze, become immobile, defecate and show fear-like movements\textsuperscript{12}.

CNS depressant drugs act at the GABA-BZD receptor-chloride channel complex and potentiate the GABAergic inhibition by increasing the life time of chloride channel opening induced by GABA. When GABA binds with its receptor, the chloride channel opens and as a result the neuron is hyperpolarized. Another mechanism is that, drugs also interfere with the sodium and potassium permeability of the neuronal membrane, leading to difficulty in excitation of the neuron\textsuperscript{13}.

CNS depressant drugs, mainly act on limbic system (anxiolysis), reticular activating system in brain stem (hypnosis), lower brain stem and spinal cord (muscle relaxation) and cerebellum (ataxia due to benzodiazepine)\textsuperscript{14}.

Locomotor activity is considered as an index of alertness and a decrease indicates sedative effect of the drug. The study on spontaneous motor activity showed that the extract decreases the frequency of movement and reduced the time spent on revolving rod by mice in rotarod test.
This represents that extract may have muscle relaxant activity which could be due to CNS depressant activity\textsuperscript{15}.

The decrease in aversion to the open arm is the result of an anxiolytic effect, expressed by the increased time spent and entries in the open arm. HEPZL (500mg/kg) increased the time spent and number of entries in the open arm, with decrease in the closed arm entries.

The study shows that the extract posses significant and dose-dependent sedative, antianxiety and muscle relaxant activity. These effects may be due to potentiation of GABAergic inhibition in brain or may be change in sodium and potassium permeability of the neuronal membrane.

Preliminary phytochemical studies revealed the presence of glycosides and flavonoids in HEPZL, which may be responsible for this CNS depressant activity\textsuperscript{11}.

References