ABSTRACT: The plant *Wedelia chinensis* was found to be used by different traditional systems and folklore for the treatment of various disorders. The aim of the present study is to investigate the effect on central nervous system (CNS) of the ethanol extract of *Wedelia chinensis* whole plant in Swiss albino mice and Wistar rats. The CNS effects were evaluated by general behaviour, exploratory behaviour, muscle relaxant activity and phenobarbitone sodium–induced sleeping time using standard procedures in experimental animal models. The results revealed that the ethanol extract at 200 and 300 mg/kg caused a significant reduction in the spontaneous activity (general behavioural profile), exploratory behavioural pattern (Y–maze and head dip test), muscle relaxant activity (rotarod and traction tests), and significantly potentiated phenobarbitone sodium–induced sleeping time. The results conclude that the extract exhibit CNS depressant activity in tested animal models.

KEYWORDS: *Wedelia chinensis*; muscle relaxant; phenobarbitone-induced sleeping time; CNS depressant activity

Introduction

*Wedelia chinensis* (Asteraceae) is a perennial herb of about 0.3 to 0.9 cm height. Leaves are fleshy, usually 4-9 cm long and 2-5 cm wide, irregularly toothed or serrate, usually with a pair of lateral lobes and obviate in shape. Flowers are yellow, tubular in terminal or axillary head and 4-5 cm in diameter.

Traditionally the fruits, leaves and stem are used in childbirth and in the treatment of bites and stings, fever and infection. The leaves are used in the treatment of kidney dysfunction, cold, wounds and amenorrhea (Mathew, 1983). The leaves are also used for dyeing hair and for promoting their growth. The tonic of the leaves is used in cough and cephalalgia. Decoction of the plant is used in menorrhagia and skin diseases (Kirtikar and Basu, 1975; Saxena et al., 1986). The plant has also found its use in inflammations, helminthic diseases and liver disorders (Anonymous, 1983). The decoction of the plant was extensively used by the tribes in Kolli Hills of Namakkal District, Tamilnadu, India, to reduce mental tension and also to induce sleep and the plant affects CNS (Anonymous, 1948). The plant has been used as astringent, bitter, acrid, anti-inflammatory andcardiotonic, and treatment of wounds, seminal weakness and viral-hepatitis (Chopra, 1956; Vaidyaratnam, 1997).

An ethanolic (5 %) extract inhibits the growth of Ehrlich’s ascites carcinoma. The extracts of this plant have been tested in experimental animal models for their hepatoprotective effect (Aperset al., 2002), analgesic and anti-inflammatory activity(Sureshkumar et al., 2006) and androgen suppressing activity (Lin et al., 2007). The alcoholic extract of the leaves was found to possess wound healing properties (Verma et al., 2008; Mishra et al., 2009), antioxidant (Verma and Khosa, 2008) and lipid peroxidation inhibitory activity (Verma and Khosa, 2009) in rats.

The plant is traditionally used to reduce mental tension and to induce sleep and scientifically reported to possess antioxidant property which indicates its usefulness in reducing anxiety and stress in emotional conditions. Therefore, in the light of the traditional and reported uses, the present study was undertaken to investigate the CNS activity of the ethanol extract of *Wedelia chinensis* whole plant in various experimental animal models.

Materials and Methods

Plant material and extraction

The plant was collected from Kolli hills in Salem district, Tamil Nadu, India in December 2006 and identified by a Taxonomist from Botanical Survey of India, Coimbatore, Tamilnadu, India and the specimens were deposited in the herbarium of Department of Pharmacognosy, JKK Munirajah Medical Research Foundation College of Pharmacy, Komarapalayam, Tamilnadu, India. One kg of coarsely powdered plant material was successively extracted with three volumes of 95% ethanol for 72 h at room temperature. The whole extract was collected in a 5 litre conical flask, filtered, and the solvent was evaporated to dryness under reduced pressure in rotary evaporator.
groups of animals were administered with the extract at the doses of 100 and 200 mg/kg and pethidine (5mg/kg) were administered intraperitoneally. The last group received the same dose of vehicle under the same conditions. Injections were normally made intraperitoneally until otherwise mentioned. All procedures described were reviewed and approved by the Institutional Animal Ethics Committee (IAEC).

**Chemicals**

Chlorpromazine hydrochloride (Indus Pharmaceuticals Limited, India), diazepam (Lupin Laboratories Limited, India), phenobarbitone sodium (Rhone-Poulenc India Limited, India), pethidine (Ranbaxy Laboratories Limited, India), aspirin (USV, Mumbai, India) and propylene glycol (SRL Laboratories, India) were procured and used in the study. All other chemicals of highest available purity were obtained from Merck, Mumbai, India.

**Acute toxicity in animals**

For acute toxicity studies, the test extract in the doses 100, 200, 400, 800 and 1600 mg/kg were administered in five groups of 10 mice each. The mortality rates were observed after 72 hours. The LD50 was determined using the graphical methods of Litchfield and Wilcoxon (1949).

**General behavioural methods**

Evaluation of general behavioural profile was performed by the method of Dixit and Varma (1976). Fifty adult albino mice were divided into five groups. The first three groups of animals were administered with the extract at the doses of 100, 200 and 300mg/kg intraperitoneally. The last two groups receive either chlorpromazine (5mg/kg) as standard drug or propylene glycol (5ml/kg) as vehicle control. The animals were under observation for the first hour and at one hour intervals for next 4 h for the following parameters (Mukherjee et al., 1996; Murugesan et al., 1999).

**Awareness, alertness and spontaneous activity**

The awareness and alertness were recorded by visual measure of the animal’s response when placed in different positions and its ability to orient itself without bumps or falls (Turner, 1965). The normal behaviour at resting position was scored as 0. Similarly little activity (+), moderate flexibility (++), strong response (+++) and abnormal restlessness (++++) were recorded. The spontaneous activity of mice was recorded by placing the animal in a bell jar. It usually shows a moderate degree of inquisitive behaviour. Less or moderate activity was scored as + and strong activity as ++. If there is slight or little motion, the score was + while the animal sleeps, the score was -. Excessive or very strong inquisitive activity like constant walking or running was scored as ++++. A similar test was performed with the same scoring, when the animal is removed from the jar and placed on a table (Turner, 1965; Mukherjee et al., 1996).

**Touch, pain and sound responses**

The touch response was recorded by touching the mice with a pencil or forceps at a various parts of the body (i.e. on the side of the neck, abdomen and groin). The pain response was graded when a small artery clamp was attached to the base of the tail, and response was noted. Albino mice normally utter no sound, so that vocalization may indicate noxious stimulus.

**Analgesic activity**

Analgesic activity was studied by using tail immersion and tail-flick methods.

**Tail immersion test**

Swiss albino mice of either sex were divided into four groups of 10 animals each. Propylene glycol (5ml/kg), extract at the doses of 100 and 200 mg/kg and pethidine (5mg/kg) were administered intraperitoneally. The tail (up to 5cm) was then dipped into a pool of water maintained at 5±0.5°C. The time in seconds to withdraw the tail out of water was taken as the reaction time. The reading was taken after 30 minutes of the administration of the test drug (Gosh, 1984).

**Tail flick test**

Wistar rats of either sex weighing 150 - 180g were divided into 4 groups of 10 animals each. The tail of the rat was placed on the nichrome wire of an analgesiometer (Techno, Lucknow, India) and the time taken by the animal to
withdraw (flick) its tail from the hot wire was taken as the reaction time. Extract at the doses of 100 and 200 mg/kg and pethidine (5mg/kg) were administered intraperitoneally. Propylene glycol (5ml/kg) was served as a control. Analgesic activity was measured after 30 min of administration of test and standard drugs (Gosh, 1984).

**Effect on phenobarbitone sodium induced sleeping time**

Mice were divided into 4 groups of 10 animals each. Animals received 40mg/kg i.p. phenobarbitone sodium 30 min after the injection of propylene glycol (5 ml/kg) and the extract at the doses of 100, 200 and 300 mg/kg. The sleeping time was recorded, and measured as the time interval between the loss and remaining of the light reflux (Dhandiya and Collumbine, 1959; Mandalet al., 2001).

**Exploratory behaviour**

Exploratory behaviour of the animals was evaluated using Y-maze and head dip tests.

**Y-maze test**

The test was performed in 4 groups of 10 albino rats at 30, 60, 90 and 120 min after injection of either propylene glycol (5ml/kg), extract (100 and 200 mg/kg) and diazepam(10 mg/kg) respectively. The rats were placed individually in a symmetrical Y-shaped runway (33 × 38 × 13cm) for 3 min and the number of times a rat entered in the arm of the maze with all 4ft (an ‘entry’) were counted (Rushton et al., 1961; Mandalet al., 2001).

**Head dip test**

Five groups of female albino mice (n=10) were placed on the top of a wooden box with 16evenly spaced holes, 30min after injection of the extract (100, 200 and 300mg/kg, vehicle (5ml/kg, propylene glycol) and diazepam (10mg/kg) respectively. The number of times that each animal dipped the head into the hole was counted for a period of 3 min (Dorr et al., 1971).

**Muscle relaxant activity**

The effect of extract on muscle relaxant activity was studied by usingtraction and rotarod tests.

**Traction test**

The screening of the animals was done by placing the forepaws of the male mice in a small twisted wire rigidly supported above a bench top. Normally the mice grasp the wire with the forepaws, and place at least one hind foot on the wire within the 5sec when allowed to hang free. The test was conducted on five group of animals (n=10) which were previously screened, 30 min after the injection of the extract (100, 200 and 300mg/kg), vehicle (5 ml/kg, propylene glycol) and diazepam (10mg/kg) respectively. The inability to put at least one hind foot was considered as failure in the traction test (Rudziket al., 1973).

**Rotarod test**

Mice were placed on a horizontal steel rod (32mm diameter) rotating at the speed of 25 rpm. The mice capable of remaining on the top for 3 min or more, in three successive trails were selected for the study. The selected animals were divided into five groups (n=10). Groups were injected intraperitoneally with the extract at 100, 200 and 300mg/kg, propylene glycol (5ml/kg) and diazepam (10mg/kg) respectively. Each group of animals was then placed on the rod at an interval of 30, 60, 90, 120 and 150 min. The animals failed more than once to remain on the rotating rod for 3 min were considered as positive for muscle relaxation (Dunham and Miya, 1957).

**Statistical analysis**

The results were expressed as mean ± S.E.M. Statistical analysis of difference between groups was evaluated by ANOVA followed by Dunnnett’s posthoc test. The Chi-square test was used for calculating the percentage muscle relaxant activity. A p-value less than 0.05 was considered significant.

**Results and Discussion**

The preliminary phytochemical screening of *Wedelia chinensis* ethanol extract was performed by standard methods and the results indicated the presence of tannins, terpenoids, flavonoids, steroids and reducing sugars.

**Toxicity study**

The plant extract was found to be non-toxic up to doses of 1.6 g/kg and did not cause any death of the animals tested. This indicates that the LD$_{50}$ value of the extract was more than 1.6 g/kg.

**Effect on behavioural profiles**

The results obtained from the experiments are presented in Table 1. The extract affected spontaneous activity, sound and touch responses at a dose of above 200mg/kg and produced moderate or slight depression relating to awareness and alertness. However, the standard drug chlorpromazine hydrochloride caused significant depression of all these responses compared with ethanol extract. The results indicate that the extract influences general behavioural profiles, as evidence in the spontaneous activity, touch, sound and pain responses.
Analgesic activity
The results of the analgesic activity by tail immersion and tail flick methods were presented in Table 2. In both the tests the reaction time was significantly increased in treated animals when compared to control. This indicates that the extract have shown significant analgesic activity compared with the control in a dose dependent manner. The activity may be due to its action on central nervous system, similar to that of pethidine.

Exploratory behaviour potentials
In the Y-maze test, the animals treated with the extract in tested doses have shown a marked decrease in exploratory behaviour compared with controls (Table 3). In head dip test, there was a significant reduction in the head tip responses occurred in mice treated with the extract, compared with the control (Table 4).

Effect on phenobarbitone sodium-induced sleeping time
The extract significantly potentiated the phenobarbitone sodium-induced sleeping time at the doses studied, with respect to the control (Table 5). The potentiation of phenobarbitone sodium-induced sleeping time is possibly through a CNS depressant action (Dunham NW al., 1957) or a tranquilizing action (Mandal et al., 2001).

Table 1 Effect of ethanol extract of Wedeliachinensis on general behavioural profiles in mice and rats (n=10).

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Wedeliachinensis extract (mg/kg)</th>
<th>Chlorpromazine (5 mg/kg)</th>
<th>Propylene glycol (5 ml/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100</td>
<td>200</td>
<td>300</td>
</tr>
<tr>
<td>Spontaneous activity</td>
<td>+</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Alertness</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Awareness</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Sound response</td>
<td>+</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Touch response</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Pain response</td>
<td>+</td>
<td>+</td>
<td>+++</td>
</tr>
</tbody>
</table>

Depression levels: -, no effect; +, slight; ++, moderate; +++, strong; ++++, very strong

Table 2 Analgesic effect of Wedeliachinensis on tail flick and tail immersion tests in mice and rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose</th>
<th>Tail flick test (Reaction time in sec)</th>
<th>Tail immersion test (Reaction time in sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propylene glycol</td>
<td>5 ml/kg</td>
<td>2.25±0.14</td>
<td>2.32±0.16</td>
</tr>
<tr>
<td>Aspirin</td>
<td>100 mg/kg</td>
<td>4.20±0.18</td>
<td>4.48±0.12</td>
</tr>
<tr>
<td>W. chinensis extract</td>
<td>100 mg/kg</td>
<td>2.50±0.13</td>
<td>2.48±0.02</td>
</tr>
<tr>
<td></td>
<td>200 mg/kg</td>
<td>3.02±0.06</td>
<td>3.05±0.02</td>
</tr>
</tbody>
</table>

Values are mean ± S.E., n=10. All the data are significant at P<0.001 vs. control, Students t-test.

Table 3 Effect of Wedeliachinensis on exploratory behaviour (Y-maze test) in rats.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Dose</th>
<th>Number of entries after treatment (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30</td>
<td>60</td>
</tr>
<tr>
<td>Propylene glycol</td>
<td>5 ml/kg</td>
<td>9.2 ± 0.2</td>
</tr>
<tr>
<td>Diazepam</td>
<td>10 mg/kg</td>
<td>3.0 ± 0.1</td>
</tr>
<tr>
<td>W. chinensis extract</td>
<td>100 mg/kg</td>
<td>6.4 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>200 mg/kg</td>
<td>5.0 ± 0.2</td>
</tr>
</tbody>
</table>

Values are the number of entries in 3 min (mean ± S.E., n=10). All the data are significant at P<0.001 compared with control.
Table 4 Effect of *Wedelia chinensis* on exploratory behaviour (head dip test) in mice.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Dose</th>
<th>Head dip test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propylene glycol</td>
<td>5 ml/kg</td>
<td>99 ± 1.0</td>
</tr>
<tr>
<td>Diazepam</td>
<td>10 mg/kg</td>
<td>32 ± 2.0***</td>
</tr>
<tr>
<td><em>W. chinensis</em> extract</td>
<td>100 mg/kg</td>
<td>71 ± 3.0*</td>
</tr>
<tr>
<td></td>
<td>200 mg/kg</td>
<td>60 ± 2.0**</td>
</tr>
<tr>
<td></td>
<td>300 mg/kg</td>
<td>33 ± 1.0***</td>
</tr>
</tbody>
</table>

Values are number of head dips in 3 min (mean ± S.E., n=10)

*P < 0.05, **P<0.01 and ***P<0.001 compared with control

Table 5 Effect of *Wedelia chinensis* on phenobarbitone sodium-induced sleeping time.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Dose</th>
<th>Sleeping time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propylene glycol</td>
<td>5 ml/kg</td>
<td>64 ± 0</td>
</tr>
<tr>
<td><em>W. chinensis</em> extract</td>
<td>100 mg/kg</td>
<td>70 ± 2.0*</td>
</tr>
<tr>
<td></td>
<td>200 mg/kg</td>
<td>82 ± 3.0*</td>
</tr>
<tr>
<td></td>
<td>300 mg/kg</td>
<td>119 ± 3.0*</td>
</tr>
</tbody>
</table>

Values are mean ± S.E., n=10. P<0.001 compared with control

Effect on muscle relaxant activity

In the traction test, the mice treated with the extract showed a significant failure in traction at all the doses tested. The result from the rotarod test showed that the extract significantly reduced the motor co-ordination of the tested animals.

All these results support the use of this plant’s decoction by the tribes in Kolli Hills of Namakkal District, Tamilnadu, India, to reduce mental tension and also to induce sleep (Anonymous, 1948).

Conclusion

The possible CNS activity of ethanolic extract of *Wedelia chinensis* was investigated by common psychopharmacological tests. The reduction in exploratory behaviour in animals is similar with the action of other CNS depressant agents. A significant lack in motor co-ordination and muscle relaxant activity was also noted in animals treated with crude extract. The results altogether indicates that the extract shows CNS depressant activity.

References


