ANTINOCICEPTIVE ACTIVITY OF ETHANOLIC EXTRACT OF 
WEDELIA CHINENSIS

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Abstract
The objective of the present study is to evaluate the antinociceptive activity of ethanolic extract of Wedelia chinensis whole plant in Swiss albino mice. The ethanolic extract of Wedelia chinensis (WCEE) at a dose of 200, 300 and 500 mg/kg was used for the antinociceptive studies using writhing response induced by acetic acid and hot plate method in Swiss albino mice. Ethanolic extract of Wedelia chinensis at a dose of 200,300 and 500 mg/kg inhibited the writhing response induced by acetic acid in a significant and dose-dependent manner, by 7.5%, 57.7% and 78.2% respectively. Using the same doses its ethanolic extract elicited a reduction of licking time during the first phase (neurogenic) at the highest dose of its ethanolic extract. In conclusion, its ethanolic extract possesses antinociceptive properties.

Key words : Wedelia chinensis; antinociceptive; acetic-acid writhing; hot plate.

Introduction
The genus Wedelia comprises over 60 species distributed in tropical and warm temperate regions, including India, Burma, Ceylon, China and Japan of which nearly two dozen species are reported to be medicinally active (Verma and Khosa, 2015). Among these Wedelia chinensis Merrill (Syn. Wedelia calendulaceae) (Asteraceae), is a small much branched annual herb commonly known as “Pilabhamgara” or “Bhringraj’ is a reputed herbal medicine in both Ayurvedic and Unani system of medicine. The herb contains wedelolactone and demethylwedelolactone (Coumestans derivatives) possessing potent anti-hepatotoxic effect and is incorporated as a major ingredient in a number of developed potent anti-hepatotoxic phytopharmaceuticals formulations. Wedelia chinensis is used as traditional herbal medicines throughout the world and they have been reported to possess hepatoprotective, bactericidal, molluscidial, hypoglycemic and antitumor activities (Jiangsu, 1977) whereas antioxidant (Verma and Khosa, 2008) wound healing (Verma et al., 2008) antistress activity (Verma and Khosa, 2009) and hepatoprotective activity on W. chinensis have been reported by our research group in recent years (Verma et al., 2009) It is useful in the treatment of osteoporosis of knee and also possesses anti-inflammatory activity (Anonymous, 2005; Yuan et al., 2013) As it contains large amount of phenolic constituents and it is also effective in the treatment of inflammatory conditions, so its antinociceptive activity was studied in detail.

Materials and Methods
Plant material
The whole plant of Wedelia chinensis was procured from the Plant Physiology Division, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Krishi Nagar, Jabalpur, M.P. and authenticated by the taxonomic division, National Herbarium of Cultivated Plants, National Bureau of Plant Genetic and Resources, New Delhi. A voucher specimen (NHCP/NBPGR/2007/99/2225 dated 22/08/2007) was retained in our laboratory for further reference.

Plant extract
The plant material was dried under shade, reduced to moderately coarse powder and was extracted successively with petroleum ether (60-80°C) and ethanol using soxhlet apparatus. The ethanolic extract was dried under vacuum (yield 6.78%) and its qualitative analysis showed the presence of phenolic compounds, saponins,
reducing sugars and flavonoids. The ethanolic extract of *Wedelia chinensis* (WCEE) was used for the antinociceptive studies.

**Animals**

Swiss albino mice of both sexes (20±2g) were used for the present studies. They were housed in clean polypropylene cages (38X23X10 cm) with not more than six animals per cage and maintained standard laboratory condition (temperature 25±2°C) with dark and light cycle (12/12 h). They were allowed free access to standard pellet diet (Hindustan Lever, Kolkata, India) and water ad libitum. The Institutional Animal Ethics Committee, (IAEC) approved the use of animals for the present study.

**Writhing test**

The mice were pretreated with WCEE (200, 300 and 500 mg/kg i.p) and Aspirin (100mg/kg i.p). After 30 min, 0.6% (v/v) solution of acetic acid was injected i.p. (10ml/kg). The numbers of writhes were noted for 15 minutes beginning 5 minutes after acetic acid injection (Collier *et al.*, 1968; Omonkhelin and Eric 2007).

% Inhibition = \( \frac{W_C - W_T}{W_C} \times 100 \)

Where,

\( W_C \) = Mean number of writhes in control group

\( W_T \) = Number of writhes in test group

**Hot plate test**

Each mice was dropped on the heated plate (55±0.5°C), separated by 30 min interval from each other. The first trial familiarized the animals with the test procedure and second served as control reaction time (licking the paw or jumping). Animals showing a reaction time greater than 10 sec. were discarded. Immediately after the second trial (control reaction time), groups of six mice each received i.p. saline, WCEE (200,300 and 500 mg/kg) and Pentazocine (10mg/kg) (Goverdhan Puchchakayala et al 2008). Reaction time were measured at time zero (0 time), 30, 60, 120 and 180 min after compounds administration with a cut-off time of 40 sec (Turner, 1965; Goverdhan *et al.*, 2008).

**Statistical analysis**

All the data obtained were expressed as mean ± standard error. The results were analyzed using student t-test.

**Results**

Antinociceptive activity was investigated by the acetic acid induced writhing test and Hot plate method in mice. Results of writhing studies in mice are presented in (Table 1). The maximum writhes were produced by saline treated mice. The ethanolic extract of *W. chinensis* (200, 300, 500 mg/kg i.p.) showed a significant dose dependent reduction in the number of writhing with 7.5%, 57.7% and 78.2% of inhibition respectively. The maximum inhibition was observed at a dose of 500mg/kg, which was statistically similar to the standard drug, diclofenac sodium (10 mg/kg).

The ethanolic extract of *W. chinensis* (200, 300, 500 mg/kg i.p.) elicited a significant analgesic activity in the hot plate as evidenced by increase in latency time in seconds table 2 as compared with vehicle control. The increase in latency time was dose dependent. The latency time was noted at 0, 30, 60, 120 and 180 minutes after the administration of vehicle, standard and plant extract. WCEE at doses of 300 and 500 mg/kg showed significant increase in latency time in mice which is comparable with standard drug.

**Discussion**

Inhibition of acetic acid-induced writhing in mice suggests that the analgesic effect of the extract may be peripherally mediated via the inhibition of the synthesis and release of prostaglandins (Koster *et al.*, 1959) Writhes can be described as a wave of constriction and elongation passing caudally along the abdominal wall with twisting of the trunk and extension of the hind limbs in mice. This is due to the nociceptive property of acetic acid (Surender and Mafumdar 1995). The percentage of inhibition, clearly shown in Table 1, also indicates that the extract at 300 and 500 mg/kg produced a significant inhibition when compared to aspirin (100 mg/kg) a known standard analgesic drug.

**Table 1:** Effect of Ethanolic extract of *Wedelia chinensis* on the acetic acid induced writhing in mice.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Groups</th>
<th>Dose (mg/kg)</th>
<th>No. of Writhings (Mean ± SEM)</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td></td>
<td>39.83 ± 1.5584</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Aspirin</td>
<td>100</td>
<td>6.83 ± 0.4773**</td>
<td>82.85</td>
</tr>
<tr>
<td>3</td>
<td>WCEE</td>
<td>200</td>
<td>36.83 ± 0.6010*</td>
<td>7.53</td>
</tr>
<tr>
<td>4</td>
<td>WCEE</td>
<td>300</td>
<td>16.83 ± 0.7033**</td>
<td>57.74</td>
</tr>
<tr>
<td>5</td>
<td>WCEE</td>
<td>500</td>
<td>8.66 ± 0.6147**</td>
<td>78.25</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± S.E.M. n=06

* * p<0.02 vs control

** p<0.001 vs control

and release of prostaglandins (Koster *et al.*, 1959) Writhes can be described as a wave of constriction and elongation passing caudally along the abdominal wall with twisting of the trunk and extension of the hind limbs in mice. This is due to the nociceptive property of acetic acid (Surender and Mafumdar 1995). The percentage of inhibition, clearly shown in Table 1, also indicates that the extract at 300 and 500 mg/kg produced a significant inhibition when compared to aspirin (100 mg/kg) a known standard analgesic drug.

Hot plate test has been found to be suitable for evaluation of centrally but not of peripherally acting analgesics. The results shown in table 2 clearly indicates that the extract at 300 and 500 mg/kg showed significant...
Table 2: Effect of Ethanolic extract of *Wedelia chinensis* on the hot plate test in mice.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Groups</th>
<th>Dose (mg/kg)</th>
<th>0</th>
<th>30</th>
<th>60</th>
<th>120</th>
<th>180</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>6.33±0.1666</td>
<td>6.76±0.2092</td>
<td>7.28±0.1301</td>
<td>7.33±0.0918</td>
<td>7.48±0.0792</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Pentazocine</td>
<td>10</td>
<td>6.26±0.0792</td>
<td>10.76±0.1763***</td>
<td>14.71±0.3736***</td>
<td>19.41±0.5364***</td>
<td>21.36±0.6587***</td>
</tr>
<tr>
<td>3</td>
<td>WCEE</td>
<td>200</td>
<td>6.41±0.2007</td>
<td>7.16±0.1308</td>
<td>7.88±0.1905*</td>
<td>8.06±0.2275**</td>
<td>7.73±0.1706</td>
</tr>
<tr>
<td>4</td>
<td>WCEE</td>
<td>300</td>
<td>6.63±0.2044</td>
<td>8.60±0.2221***</td>
<td>9.08±0.1832***</td>
<td>9.38±0.1851***</td>
<td>9.48±0.3081***</td>
</tr>
<tr>
<td>5</td>
<td>WCEE</td>
<td>500</td>
<td>6.75±0.1544</td>
<td>9.5±0.1844***</td>
<td>10.98±0.2040***</td>
<td>14.78±0.3944***</td>
<td>19.06±0.6946***</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± S.E.M.  n=06

*     p<0.05 vs control  
**    p<0.02 vs control  
***   p<0.001 vs control

analgesic activity which is comparable with the standard drug, Pentazocine (10 mg/kg).

The fact that WCEE showed analgesic activity in both the models studied, indicated that the analgesic effect of WCEE could possess two components viz. central and peripheral (Panthong et al., 1998).

**Conflict of interest statement**

We declare that we have no conflict of interest.

**Acknowledgement**

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**References**


