INTRODUCTION

The family Asteraceae (compositae) comprises of herbs 1, shrubs, rarely trees found throughout the world, especially in the mountain tracts. This is the largest family of the flowering plants and comprises about one tenth of the phanerogamic flora of the world; it, however, furnishes only a few plants of economic importance. Several plants of this family are cultivated for ornamental purposes.

Natural products contribute to a great extent to fight against pathogenic micro organisms. Many plants or their parts are used in food as spices and are thought to provide a natural preservation by inhibiting the microbial growth. Varieties of herbs and spices have been used traditionally in food preservation to extend shelf life2. All the three plants were belonging to Asteraceae, are commonly growing as a weed.

Drugs of plants origin are used in India and other countries for the treatment of disease in traditional, system of medicine, people used to prepare herbal drugs in the ancient times as they do in the modern era too since these drugs are less expensive, have negligible side effects and not only eliminate the disease from the patients body but also enhance the vigor and immunity besides playing and appreciable role towards suppressing untoward immune reactions. Through the half of

ABSTRACT

Comparative study on analgesic activity was carried using ethanol extracts in animal models. *Wedelia trilobata (EEWT), Wedelia biflora (EEWB) and Eclipta alba (EEEA) were evaluated by acetic acid induced writhing method and hot plate assay to assess analgesic activity in mice. It was found that the extract caused an inhibition on the writhing response induced by acetic acid in a dose dependent manner. Dose of 500 mg/kg EEWT, EEWB, EEEA and Aspirin could block the writhing response by 49.17 %, 49.45, %, 55.23 % and 68.68 % (p<0.001) respectively. It was also indicated that the EEEA showed significant antinociceptive action in hot plate reaction time method in mice. This effect was comparable to that of standard drug morphine treated controls. The results reflects that analgesic effects and therapeutic efficacy of the extract on animal models which are comparable with those of standard drugs such as Aspirin and Morphine.

Key words: *Wedelia trilobata, Wedelia biflora and Eclipta alba; Analgesic activity- Acetic acid induced writhing method and Hot plate assay.
this centenary many herbs were considered conventional medicines for instance.\textsuperscript{3,4}

\textit{Wedelia} is a genus of scabrid, pubescent or hirsute herbs or under shrubs, found in the tropics and sub-tropics. And consisting of approximately 65 species is distributed in tropical and warm temperate regions including India, Burma, Ceylon, China and Japan. \textit{Wedelia trilobata} (L) flowers and leaf part of the plant were used in the ladies for the purpose of amenorrhea\textsuperscript{4} and childbirth.\textsuperscript{5} From the literature review reveals that the fresh entire plant is used as molluscicidal activity\textsuperscript{6}, antibacterial and anti-mycobacterial activity.\textsuperscript{7} \textit{Wedelia biflora} (DC) were used to relive of headache, stomachache, as diuretic and laxatives.\textsuperscript{9} The fresh leaf effectively used for malarial fever\textsuperscript{10} and its juice used to treat tropical sores, wounds, scabies, cuts, diarrhoea and dysentery.\textsuperscript{11} The leaves contain a fair amount of protein and alkaloids, but have a high content of fiber. \textit{Eclipta alba} (Linn) is one of the ten auspicious herbs. It posses multiple medicinal proper tics such as jaundice and fevers,\textsuperscript{12} inflammations eye diseases, leucoderma, uterine pains after delivery used in formulation of Ayurveda.\textsuperscript{13} However, no detailed pharmacology on analgesic activity has been studied of these plants. Hence, the present investigation undertaken to evaluate the effect of analgesic activity by using standard animal models.

\section*{MATERIALS AND METHODS}

\subsection*{Collection of plant materials}
The plants \textit{Wedelia trilobata} and \textit{Eclipta alba} (Asteraceae) are collected in Salem, Tamilnadu and \textit{Wedelia biflora} is from Andaman Nicobar Island, Andaman, India, in the month of August 2005. The plants were authenticated by Dr. S. Jayaraman, Director, Medicinal Plant Research Unit and Plant Anatomy Research Centre, Chennai, India.

\subsection*{Preparation of the extract}
The coarsely powdered materials of the selected plants were subjected to hot continuous Soxhlet extraction using ethanol after defatting with Petroleum ether (60-80°C). The solvent was distilled under reduced pressure using vacuo and the extracts keep it in the refrigeration for the further study. The extracts at the different doses of 250 and 500 mg/kg was suspended in aqueous Tween 80 solution (2 \%) and Indomethacin (10 mg/kg) in saline was used for the present study.

\section*{Phytochemical profile}
The phytochemical profile was performed as described by Wagner\textsuperscript{14}. The plant extracts were subjected to preliminary phytochemical screening for the detection of various plant phytoconstituents such as alkaloids, glycosides, carbohydrates, proteins and amino acids, steroids, saponins, flavonoids, tannins, phenol, triterpenoids and fixed oils by qualitative chemical tests.

\section*{Toxicological studies\textsuperscript{15}}

\subsection*{Model I: Acute toxicity study}

\textbf{Animals}
Swiss Albino Wistar Rats of the either sex (180-200 g) or albino mice of the either sex (18-22) were used for the present study. They were maintained under standard environmental conditions and were fed with standard pellet diet with water ad libitum. Toxicity studies conducted by Litchfield and Wilcoxon per internationally accepted protocol drawn under OECD guidelines in Wistar mice at a dose level of fractions up to 3000 mg/kg. Mice were fasted for overnight and maintained with water \textit{ad libitum}.

\subsection*{Model II: Acetic acid induced Writhing Response in mice}
Swiss albino mice of either sex weighing between 18-22 g were used for the present study. They were maintained under standard environmental conditions and were fed with standard pellet diet with water \textit{ad libitum}. The mice were divided into 8 groups of six animals each. The group I received solvent alone and served as solvent control. Group II received Aspirin 200 mg/kg (Turner, 1960) intraperitoneal (i.p.) 1h prior to the injection of acetic acid. Group III and IV received 250 and 500 mg/kg b.w. of the extract of \textit{W.trilobata}, Group V and VI received 250 and 500 mg/kg b.w. of the extract of \textit{W.biflora}, Group VII and VIII received 250 and 500 mg/kg b.w. of the extract of \textit{E.alba}. Writing was induced by 0.6% solution of acetic acid (10 ml/kg, i.p.). Ten minutes after acetic acid injection, the mice were placed in a transparent box and the number of writhes was counted for a period of 10
minutes. Writhing movement was accepted as contraction of the abdominal muscle accompanied by stretching of hind limbs. A significant reduction in the number of writhes by drug treatments as compared to vehicle treatment animals, which was considered as positive analgesic response and the percentage inhibition of writhing was calculated and evaluated statistically.

**Hot plate reaction time in mice**

The analgesic activities of the extracts were also evaluated by hot plate method by using mice. The temperature of the metal surface was maintained at 55 ±1ºC. Latency to discomfort reaction (forepaw licking or jumping) was determined as per standard method. The prolongation of the latency time compared with values of the control was used for statistical comparison. Morphine (5mg/kg, i.p) was used as a reference standard.

**Statistical analysis**

The results were calculated as mean ± SEM. The statistical analysis was performed by ANOVA test. Followed by student's t-test<0.05 was considered as statistically significant.

**RESULTS**

The presence of alkaloid (Dragendroff reagent and Mayer's reagent), flavonoids (Shinoda test), steroids (Liberman Burchard test) and terpenes (Vanillin-sulfuric acid reagent) were analyzed. The extract was subjected to silica gel in thin layer chromatography using increasing polarity of the solvent. The chromatograms were sprayed with various reagents to detect the presence of various classes of compounds. Each spot in the preparative TLC was identified on the basis of relative mobility. The qualitative chemical tests revealed the presence of flavonoids, phenolic compounds, steroids, tannins and terpenes in the tested extracts of all species. Alkaloids, lactones and proteins found to be absent in the extracts of Wedelia trilobata. Where, saponins are present in all the extracts except in Wedelia biflora.

The animals were observed for the behavioral pattern and the observation parameters consisted of body position, locomotion, rearing, respiration, righting reflex and lacrimation. The result of behavior studies indicated that there were no significant alterations in the lower doses (250 mg/}

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose(mg/kg)</th>
<th>No. of Writhing</th>
<th>% of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>36.4±2.36</td>
<td>-</td>
</tr>
<tr>
<td>Aspirin</td>
<td>200</td>
<td>11.6±1.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>68.68</td>
</tr>
<tr>
<td>Ethanolextract of <em>Wedelia trilobata</em> (EEWT)</td>
<td>250</td>
<td>23.4±2.14&lt;sup&gt;b&lt;/sup&gt;</td>
<td>35.35</td>
</tr>
<tr>
<td>Ethanolextract of <em>Wedelia trilobata</em> (EEWT)</td>
<td>500</td>
<td>18.4±1.52&lt;sup&gt;a&lt;/sup&gt;</td>
<td>49.17</td>
</tr>
<tr>
<td>Ethanolextract of <em>Wedelia biflora</em> (EEWB)</td>
<td>250</td>
<td>21.7±1.19&lt;sup&gt;b&lt;/sup&gt;</td>
<td>40.38</td>
</tr>
<tr>
<td>Ethanolextract of <em>Wedelia biflora</em> (EEWB)</td>
<td>500</td>
<td>18.4±0.98&lt;sup&gt;a&lt;/sup&gt;</td>
<td>49.45</td>
</tr>
<tr>
<td>Ethanolextract of <em>Eclipta alba</em> (EEEA)</td>
<td>250</td>
<td>32.1±2.6</td>
<td>38.42</td>
</tr>
<tr>
<td>Ethanolextract of <em>Eclipta alba</em> (EEEA)</td>
<td>500</td>
<td>22.3±1.5</td>
<td>55.23</td>
</tr>
</tbody>
</table>

Values shown are mean ±SEM (n=6) a, p<0.01; b, p<0.05, Experimental groups were compared with control.
kg, b.w.,) administered to the animals. However, no mortality was observed in the acute toxicity study at the higher dose levels showing safety of all the tested extracts.

The results presented in table-1, Wedelia biflora, Wedelia trilobata shows that, at the doses of 500 mg/kg exhibited significant activity in acetic acid induced writhing in mice.

From the data obtained in acetic acid induced writhing test model, the ethanol extracts of Eclipta alpa showed maximum percentage inhibition of 55.23 % (P<0.05) which is compared to that of standard drug Aspirin (68.68%, P<0.01) as well as the crude extract of Wedelia biflora, Wedelia trilobata (49.45 %, 49.17%, P<0.01 ) at the dose of 500 mg/kg.

In hot plate reaction time in mice, latency to discomfort reaction (fore paw licking or jumping) of the three extracts showed significant analgesic action in hot plate reaction time method in mice. This effect was comparable to that of standard drug Morphine treated controls, suggesting that central activity of ethanol extracts of Wedelia trilobata (3.69 ± 0.27 vs 12.12 ± 1.32 sec, P< 0.001) ,Wedelia biflora (3.67 ± 0.29 vs. 10.31± 1.02 sec) and Eclipta alpa (3.74 ± 0.32 vs. 11.06 ± 1.12 sec). There was a significant, dose-dependent inhibition of the three extracts treated, which is comparable to that of central acting drug Morphine (3.35 ± 0.28 vs. 7.58 ± 0.58 sec).

**DISCUSSION**

The results of the screening studies conducted to determine the analgesic activities of different plant extracts are shown significant activity with (% of inhibition).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Reaction Time (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15 min</td>
<td>30 min</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>2.43±0.23</td>
</tr>
<tr>
<td>Morphine</td>
<td>2</td>
<td>3.35±0.28</td>
</tr>
<tr>
<td>Ethanol extract of W. trilobata (EEWT)</td>
<td>250</td>
<td>3.57±0.14</td>
</tr>
<tr>
<td>Ethanol extract of W. trilobata (EEWT)</td>
<td>500</td>
<td>3.69±0.27</td>
</tr>
<tr>
<td>Ethanol extract of W. biflora (EEWB)</td>
<td>250</td>
<td>3.49±0.22</td>
</tr>
<tr>
<td>Ethanol extract of W. biflora (EEWB)</td>
<td>500</td>
<td>3.67±0.29</td>
</tr>
<tr>
<td>Ethanol extract of E. alba (EEEA)</td>
<td>250</td>
<td>3.54±0.32</td>
</tr>
<tr>
<td>Ethanol extract of E. alba (EEEA)</td>
<td>500</td>
<td>3.74±0.32</td>
</tr>
</tbody>
</table>

Values shown are mean ±SEM (n=6) a, p<0.001b, p<0.01, Experimental groups were compared with control.

The present study proves the analgesic activity of *Wedelia trilobata* (EEWT), *Wedelia biflora* (EEWB) and *Eclipta alba* (EEEA) extracts in standard experimental animal models. Of the various extracts tested doses were showed significant analgesic activity. The analgesic test used in the present study was chosen in order to examine different nociceptive stimuli, namely cutaneous thermic (hot plate) and chemical visceral (writhing) stimuli. In acetic acid Induced abdominal writhing it causes analgesia by liberating endogenous substances and many others excite pain to the never ending. Based on the percentage of inhibition on the number of writhes obtained with different doses of *Wedelia trilobata* and *Eclipta alba*, it was found that the intensity of the analgesic effect was similar to that of the aspirin. Aspirin and related drugs can inhibit cyclooxygenase in peripheral tissues, thus interfering with mechanism transduction in primary afferent nociceptors.

Results of the present study show that all the doses of the EEWT and EEEA produce significant antinociceptive, effect which may be due to blockade or release of endogenous substances that stimulate pain never endings similar to aspirin and other NSAIDs. The hot plate method originally was described by Woolfe and Mac Donald. This test has been found to be suitable for evaluating centrally but not peripherally acting analgesics.

The validity of this test has been shown even in the presence of substantial impairment of motor performance. The present study findings indicate that the EEWT and EEEA may be centrally acting.

So, we can concluded that the present study shows that *Wedelia trilobata* and *Eclipta alba* extract exhibit significant analgesic activity. This plant which contains natural products such as flavonoids, terpenoids and steroids etc, has received considerable attention in recent years due to its diverse pharmacological properties including antioxidant activity. We propose that the additive and synergistic antioxidant activity of phytochemicals such as flavonoids, triterpenoids, steroids, etc present in *Wedelia trilobata* and *Eclipta alba* are responsible for the analgesic activity. Further detailed investigation is underway to determine the exact phytoconstituents responsible for the analgesic activity.

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