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Original Article

Investigating the Anti-Inflammatory and Analgesic Activity of Leaves of *Wedelia chinensis* (Osbeck) Merr. In Standard Experimental Animal

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Abstract

The ethanolic extract of *Wedelia chinensis* (EEWC) belonging to the family of Asteraceae was evaluated by hot plate and acetic acid induced writhing methods to assess its analgesic activity. The extract was also evaluated for its by using on carrageenan, mediators such as histamine and serotonin induced paw oedema, and cotton pellet induced granuloma tests for its effect on acute and chronic phase inflammation models in rats, as well as 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 EEWC and aspirin could block the writhing response by 51.92 % and 68.68 % ($p < 0.001$), respectively. It was also indicated that the EEWC showed significant antinociceptive action in hot plate reaction time method in mice. This effect was comparable to that of standard drug morphine treated controls, suggesting the central activity of EEWC. Maximum inhibition (56.14 %) was obtained at the dose of 500 mg/kg after 3 h of drug treatment in carrageenan induced paw oedema, whereas indomethacin (standard drug) produced 61.65 % of inhibition. In the chronic model (cotton pellet induced granuloma) the EEWC (125,250 and 500 mg/kg) and standard drug showed decreased formation of granuloma tissue by 56.69,34.57,43.30 % and 55.23 % respectively. The results indicate the potent analgesic and anti-inflammatory effects and therapeutic efficacy of *Wedelia chinensis* extract on animal models which are comparable with those of standard drugs such as Aspirin, Morphine and Indomethacin respectively.

Keywords: *Wedelia chinensis*; Analgesic activity; anti-inflammatory effect; cotton pellet-induced granuloma.

Introduction

A scientific evaluation of plants and the traditional methods of their use in disease management can permit their incorporation into the officinal health systems in India and in developing countries elsewhere. The genus wedelia consisting of approximately 65 species is distributed in tropical and warm temperature regions including India, Burma, Ceylon, China and Japan. This is a perennial herb, with light camphor like odour. It grows in wet places. Leaves are linear oblong, heads solitary, bright yellow (1). The leaves of *W.Chinensis* are used for dyeing hair and for promoting their growth. It's roots yield a black dye. The tonic of the leaves is used in cough, cephalagia and alopecia. Decoction of herb is used in menorrhagia and skin diseases (2, 3). It affects CNS (1) and has been used as astringent,

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bitter, acrid anti –inflammatory, and cardio tonic, I that also been used for the treatment of wounds, seminal weakness and viral-hepatitis (4, 5). 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 anti-hepatoprotective effect (6). Previous phytochemical profile investigation of *Wedelia chinensis* has revealed that the expressed juice of the herb contained oil soluble black dye, tannin, carotene, saponin, phytosterol, waxy compound, and resin (1, 2). The leaves were also found to contain isoflavanoid and wedelolactone. The latter is the lactone of 5:6-dihydroxy-2-(2,6-dihydroxy-4-methoxyphenyl)-benzofuron-3-corboxlic acid and analogous in structure to coumestrol (1). Recently few new oligoglycosidic compounds were isolated from the plant *W.chinensis* (7). However, fewer reports are available with respect to the pharmacological properties of the plant. Keeping this in view, the present study has been undertaken to investigate the anti-inflammatory and analgesic effects of ethanol extract of *Wedelia chinensis* (EEWC) in standard animal models.

Experimental

Plant Material

The plant species of *Wedelia chinensis* were collected in the month of December 2004 in Namakkal District of Tamilnadu, India. Dr. S. Jayaram, (Director, Medicinal plant research unit and plant anatomy research center, Chennai, Tamilnadu, India) kindly authenticated the plant and a specimen was preserved in the Department of Pharmacognosy in our institutions for future references.

Chemicals and Reagents

The chemicals used in the present study were carrageenan (S. D. Fine Chemicals Limited, Bombay), histamine (Sigma, USA), 5-hydroxy tryptamine hydrochloride (serotonin) (Sigma, USA), and indomethacin (IPCA, Bombay).

Preparation of Extract

The dried powdered plant material was extracted with ethanol in a Soxhlet extraction apparatus. The solvent was removed under reduced pressure and semi solid mass was obtained (yield 18.7 %). The extract showed positive test for alkaloids, steroids, and tannins. The extract at the different doses of 125, 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.

Phytochemical profile

The phytochemcial profile was performed as described by Wagner (8). The presence of alkaloid (Dragendroff reagent and Mayer's reagent), flavonoids (shinoda test), steroids (liberman Burchard test) and terpenes (Vanillin –sulfuric acid reagent) were assessed. 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.

Animals

Swiss albino mice of either sex weighing between (18-22 g) or Albino Wistar Rats of the either sex (180-200 g) 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 Study

An acute toxicity study relating to the determination of LD_{50} was performed. Albino Swiss mice of either sex weighing 20-25g were used. The animals were fed standard pellet diet (Hindustan Lever Ltd., Calcutta) and given water *ad libitum*. The animals were kept fasting for 18 h before the commencement of experiments. The test compounds (dissolved in propylene glycol) at different doses were injected intraperitoneally to different groups of mice, each group containing 10 animals. The animals were observed and the number of deaths recorded after 24 and 72 h. The LD_{50} , the dose killing half of the animals, was determined by the conventional graphic method of Litchfield and Wilcoxon (9). The ratio of the median lethal dose to the median effective dose ($LD_{50:}$ ED_{50}) is known as the therapeutic index.

Evaluation of analgesic activity

Acetic Acid Induced Writhing Response In Mice

Swiss albino mice of either sex were used and divided into five groups of seven animals. Writhing test determined according to the method of Turner with slight modification (10). The animals were treated with 125, 250 and 500 mg/kg of the extract and Aspirin (200 mg/kg) injected intraperitoneally 1 h prior to the injection of acetic acid while control group received vehicle. Writhing was induced by 10 ml/kg of acetic acid solution (0.6 %) intraperitoneally (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 muscles 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 a positive analgesic response and the percentage inhibition of writhing was calculated and evaluated statistically. Standard drug aspirin was used as reference standard.

Hot Plate Reaction Time In Mice

The hot-plate test was assessed on groups of 7 mice. The temperature of metal surface was maintained at 55 ± 1°C. Latency to discomfort reaction (forepaw licking or jumping) was determined by the method of Turner (10). The cut-off time was 20 seconds. The latency was recorded before and after the EEWC (125**,** 250 and 500 mg/kg) and standard drug administered by i.p. route. The prolongation of the latency times compared with values of the control was used for statistical comparison. Morphine (5 mg/kg, i.p.) was used as reference standard.

Investigation of Anti-inflammatory effects:

Carragenan Induced Paw odema (11)

The rats were divided into 5 groups (n = 6). The extract and the standard used for this study were prepared in the same manner as mentioned earlier. Animals were deprived of food and water for 18 h before the experiment. On the day of the experiment they were assigned to 5 groups of six animals each. They were marked and numbered for identification. The test compounds and standard drugs were administered orally, after 60 mins of administration of extracts and standard drugs, 0.9% saline was injected to the lateral mallelolus on the sub plantar region of the right hind paw of the rats and paw volume was measured plethysmometrically, at 1, 2, 3 and 4 hours after injection respectively. The percentage of inhibition of inflammation was calculated for comparison.

The ratio of the anti-inflammatory effect of EEWC was calculated by the following equation:

$$\text{Anti-inflammatory activity (\%)} = (1\text{-}D/C) \times 100$$

Where D represents the percentage difference in paw volume after EEWC was administered to the rats, and C represents the percentage difference of volume in the control groups.

Mediator induced inflammation

The anti-inflammatory activity of the extract was measured with phlogistic agents (*viz*. histamine, 5-HT) which act as mediator of inflammation. The paw oedema was induced in rats by sub plantar injection of freshly prepared histamine (1 mg/kg) and serotonin (1 mg/kg) solutions respectively and the paw oedema was measured as mentioned earlier (11).

Cotton pellets-induced granuloma

The rats were divided into four groups (n = 6). The cotton pellet granuloma model investigated the proliferation phase of inflammation (12). The extract at different doses (125,250, and 500 mg/kg) and Indomethacin at 10 mg/kg body weight were given to the animals orally. After 30 min the animals were anesthetized. And after shaving the fur 10 mg of sterile cotton pellets were inserted, one in each axilla. The extracts were administered daily for a period of seven days. The rats were sacrificed after a high dose of anesthesia on the eighth day and the pellets were removed surgically and made free from extraneous tissues. The pellets were incubated at 37°C for 24 h and dried at 60°C to constant weight. Increment in the dry weight of the pellets was taken as measure of granuloma formation.

Statistical Analysis

The experimental results were expressed as mean ± SEM. Data were assessed by ANOVA method followed by student's *t*-test. $p<0.05$ was considered as statistically significant.

Result and Discussion

Acute toxicity study

When the mice were observed for the behavioral changes after intraperitoneally

administration of a single dose of the extract, none of the mice exhibited any abnormal behavioral responses at lower doses. These include inactiveness, loss of appetite, slow movement, and dizziness, erection of hairs and hypothermia. But, those mice, which received higher dose showed slight toxic symptoms, which are, reflect the LD_{50}. However, the greater a drug's therapeutic index, the less likely is the fatalities that will follow an accidental overdose.

Phytochemical profile study

The active part of the alcoholic extract was subjected to chemical analysis to determine the classes of compounds present in it. It was determined by identification reactions based on the chemical group to be determined or thin layer chromatography.

According to the TLC pattern of the extract, seven spots over siliga gel were identified. The phytochemical study indicated that it contains alkaloids, tannin, flavonoids, triterpenoids, steroids, *etc*. Due to the importance of the isolated compounds, the crude extract of *W.chinensis* was chosen for the present study.

Analgesic studies

The effect of the EEWC on writhing response of mice is shown in table 1. It was found that the extract caused an inhibition on the writhing response induced by acetic acid in a dose dependent manner. Doses of 500 mg/kg EEWC and aspirin could block the writhing response by 51.92 % and 68.68 %, respectively. As shown in figure 1, EEWC showed significant antinociceptive action in hot plate reaction time method in mice. This effect was comparable to that of standard drug morphine treated controls, suggesting the central activity of EEWC.

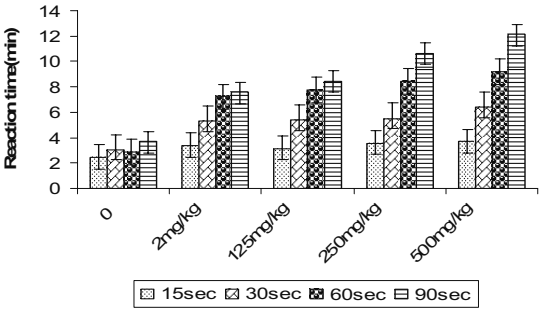

Figure 1. Analgesic activity of ethanol extract of Wedelia chinensis on hot plat method.

Anti-inflammatory studies

EEWC (125, 250 and 500 mg/kg) in various experimental animal models exhibited significant ($p < 0.05$) anti-inflammatory activity. The effects of EEWC and indomethacin on the inflammation induced by carrageenan, histamine and cotton pellet induced granuloma are summarized in table 2-4.

As shown in table 1, EEWC showed maximum inhibition of 56.14% at the dose of 500 mg/kg after 3 h of treatment in carrageenan induced paw oedema, whereas the standard drug (indomethacin at 10 mg/kg) showed 61.65 % of inhibition ($p < 0.05$). EEWC showed 51.83 and 51.83 % ($p < 0.001$) of inhibition at the dose of 500 mg/kg whereas indomethacin showed 61.17, and 58.49 % of inhibition in histamine and serotonin induced paw oedema. In the chronic model (cotton pellet induced granuloma), EEWC (125, 250 and 500 mg/kg)

Table 1. Effect of ethanol extract of Wedelia chinensis extract on acetic acid induced writhing test.

Treatment	Dose (mg/kg)	Number of writhing	Percentage of inhibition
Control	-	36.4 ± 2.36	-
Aspirin	200	11.6 ± 1.02[a]	68.68
EEWC	125	29.4 ± 2.48 [b]	19.23
EEWC	250	22.8 ± 1.19 [b]	37.36
EEWC	500	17.5 ± 0.98 [a]	51.92

Values shown are mean ± SEM (n= 7). [a] $p < 0.01$, [b] $p < 0.05$, experimental groups were compared with control

Table 2. Effect of the *Wedelia chinensis* extract on carrageenan and induced pedal oedema**.**

Treatment	Dose (mg/kg)	Paw volume (ml)	Percentage of inhibition
Carrageenan control	0	0.732 ± 0.071	-
Indomethacin	10	0.281 ± 0.016	61.65
EEWC	125	0.455 ± 0.034	37.84
EEWC	250	0.393 ± 0.031	46.31
EEWC	500	0.321 ± 0.026	56.14

Values are mean ± SEM (n = 6). Experimental groups were compared with control $p < 0.001$.

and indomethacin showed 56.69, 34.57, 43.30 % and 55.23 % decrease in formation of granuloma tissue respectively.

The present study proves the analgesic and anti-inflammatory activity of *Wedelia chinensis* extract in standard experimental animal models. EEWC at the dose sof 125, 250 and 500 mg/kg showed significant analgesic and anti-inflammatory 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 (13). In acetic acid induced abdominal writhing and it causes algesia by liberating endogenous substances and many others excite pain to the never ending (14). Based on the percentage of inhibition on the number of writhes obtained with different doses of EEWC, 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 (15). The ostaglandins amplify the pain mechanism of and enhance vascular permeability whilst the leukotrienes contract smooth muscles of blood vessels. Prostaglandins also enhance the vascular permeability and mediate proinflammatory and allergic response (16, 17). Results of the present study show that all the doses of the EEWC 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 (18). 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 (19). The present study findings indicate that the EEWC may be centrally acting.

The present study also establishes the anti-inflammatory activity of ethanol extract of *Wedelia chinensis*. It is evident that carrageenan is commonly used to induce acute inflammation and is believe to be bi-phasic. Based on this, it could be argued that the suppression of the first phase may be due to inhibition of the release of early mediators, such as histamine and serotonin, and the action in the second phase may be explained by an inhibition of cyclo-oxygenase.

Table 3. Effect of Wedelia chinensis extract on mediators like histamine and 5-HT induced pedal oedema in rats

Treatment	Dose (mg/kg)	Paw volume (ml)	Percentage of inhibition
Histamine control	0	0.544 ± 0.048	-
Indomethacin	10	0.208 ± 0.020	61.17
EEWC	125	0.364 ± 0.032	33.08
EEWC	250	0.326 ± 0.031	43.97
EEWC	500	0.262 ± 0.019	51.83
Serotonin control	0	0.624 ± 0.056	-
Indomethacin	10	0.259 ± 0.015	58.49
EEWC	125	0.495 ± 0.048	36.59
EEWC	250	0.357 ± 0.037	42.78
EEWC	500	0.293 ± 0.024	52.96

Values are mean ± SEM (n = 6). Experimental groups were compared with control $p < 0.001$.

Table 4. Effect of the Wedelia chinensis extract on cotton-pellets induced granuloma in rats.

Treatment	Dose (mg/kg)	Weight of cotton pellet (mg)	Percentage of inhibition
Control	0	47.8 ± 3.2	-
Indomethacin	10	20.7 ± 1.3	56.69
EEWC	125	31.3 ± 2.6	34.57
EEWC	250	27.1 ± 2.4	43.30
EEWC	500	21.4 ±1.9	55.23

Values are mean ± SEM (n = 6). Experimental groups were compared with control $p < 0.001$.

These mediators take part in the inflammatory response and are able to stimulate nociceptor and thus induce pain. It has been reported that second phase of oedema is sensitive to most clinically effective anti-inflammatory drugs, which has been frequently used to access the anti-oedematous effect of natural products (20,21). Based on these reports, it can be inferred that the inhibitory effect of the extract of *Wedelia chinensis* on carrageenin-induced inflammation in rats may by due to inhibition of the mediators responsible for inflammation.

Histamine is one of the important inflammation mediators and it is a potent vasodilator substance and increase the vascular permeability (22, 23) .The study showed that EEWC effectively suppressed the odema produced by histamine, which indicates that the extract exerts its anti-inflammatory action by means of either inhibiting the synthesis, release or action of inflammatory mediators such as.histamine, serotonin and prostaglandins. So, it maybe suggested that its anti-inflammatory activity is associated with its anti-histaminic activity.

The extract exhibited significant anti-inflammatory activity on the cotton pellet test. The cotton pellet granuloma widely used to evaluate the transudative and proliferative components of the chronic inflammation. The moist weight of the pellets correlates with transuda, the dry weight of the pellet correlates with the amount of granulumatous tissues (24, 25). Chronic inflammation occurs by means of the development of proliferate cells. These cells can exist exist either in spreaded form or in granuloma form. Non-sterodal anti-inflammatory drugs decrease the size of granuloma, which results from cellular reaction by inhibiting granulocyte infiltration / inflammation, preventing generation of collagen fibers and suppressing mucopolysaccharides (26,27). The *Wedelia chinensis* showed significant anti-inflammatory activity in cotton-pellet induced granuloma and thus found to be effective in chronic inflammatory conditions, which reflected its efficacy in inhibiting the increase in the number of fibroblasts and synthesis of collagen and mucopolysaccharides during granuloma tissue formation.

So, we can conclud that the present study shows that *Wedelia chinensis* extract exhibit significant analgesic and anti-inflammatory activity against early phase (acute paw edema), late phase (cotton pellet granuloma) of inflammation models. This plant which contains natural products such as flavonoids, terpenoids and steroids etc. have 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 chinensis* are responsible for the analgesic and anti-inflammatory activity. Further detailed investigation is underway to determine the exact phytoconstituents responsible for the analgesic and anti-inflammatory activity.

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