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Analgesic activities of the medicinal plants of *Wedelia trilobata, Wedelia biflora* and *Eclipta alba* in standard experimental animal models

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ABSTRACT

Comparative study on analgesic activity was carried using ethanol extracts in animal models. *Wedelia trilobata (EEWT), Wedelia biflora* (*EEWB)* and *Eclipta alba (EEEA)* was 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.

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 organism. 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 life[2]. 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

this centenary many herbs were considered conventional medicines for instance [3, 4].

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. *Wedelia trilobata* (L) flowers and leaf part of the plant were used in the ladies for the purpose of amenorrhea [4] and childbirth.[5] From the literature review reveals that the fresh entire plant is used as molluscicidal activity[6], antibacterial and anti-mycobacterial activity.[7] *Wedelia biflora* (DC) were used to relive of headache, stomachache, as diuretic and laxatives[9]. The fresh leaf effectively used for malarial fever [10] and its juice used to treat tropical sores, wounds, scabies, cuts, diarrhoea and dysentery. [11] The leaves contain a fair amount of protein and alkaloids, but have a high content of fiber. *Eclipta alba* (Linn) is one of the ten auspicious herbs. It posses multiple medicinal proper tics such as jaundice and fevers, [12] inflammations eye diseases, leucoderma, uterine pains after delivery used in formulation of Ayurveda [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.

MATERIALS AND METHODS

Collection of plant materials

The plants *Wedelia trilobata* and *Eclipta alba* (Asteraceae) are collected in Salem, Tamilnadu and *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.

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.

Phytochemical profile

The phytochemcial profile was performed as described by Wagner [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.

Toxicological studies [15]

Model I: Acute toxicity study

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 Litchfied 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 *ad libitum*.

Evaluation of Analgesic activity [16]

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 *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) intraperitoneally (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 *W.trilobata*, Group V and VI received 250 and 500 mg/kg b.w. of the extract of *W.biflora*, Group VII and VIII received 250 and 500 mg/kg b.w. of the extract of *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 ±1°C. 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 - 1: Analgesic activity of ethanol extracts of *Wedelia trilobata, Wedelia biflora and Eclipta alba* on acetic acid induced writhing test

Treatment	Dose(mg/kg)	No. of Writhing	% ofinhibition
Control	-	36.4±2.36	-
Aspirin	200	11.6±1.02-[a]	68.68
Ethanolextract of *Wedelia trilobata(* EEWT)	250	23.4±2.14[b]	35.35
Ethanolextract of *Wedelia trilobata(* EEWT)	500	18.4±1.52[a]	49.17
Ethanolextract of *Wedelia biflora* EEWB	250	21.7±1.19[b]	40.38
Ethanolextract of *Wedelia biflora (*EEWB)	500	18.4±0.98[a]	49.45
Ethanolextract of *Eclipta alba*(EEEA)	250	32.1±2.6	38.42
Ethanolextract of *Eclipta alba*(EEEA)	500	22.3±1.5	55.23

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 *Wedeliabiflora*, *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

Table - 2: Analgesic activity of ethanol extracts of *Wedelia trilobata, Wedelia biflora and Eclipta alba* on hot plate method

Treatment	Dose (mg/kg)	Reaction Time (sec) 15 min	30 min	60 min	90 min
Control	-	2.43±0.23	3.05±0.27	2.86±0.22	3.69±0.22
Morphine	2	3.35±0.28	5.29±0.46	7.22±0.68[a]	7.58±0.58[a]
Ethanol extract of *W. trilobata*(EEWT)	250	3.57±0.14	5.48±0.49	8.45±0.69[b]	10.64± 1.90[b]
Ethanol extract of *W. trilobata* (EEWT)	500	3.69±0.27	6.35±0.56[a]	9.22±0.44[b]	12.12± 1.32[b]
Ethanol extract of *W. biflora (*EEWB)	250	3.49±0.22	5.42±0.52	8.66±0.58[b]	9.14±1.85[b]
Ethanol extract of *W. biflora* (EEWB)	500	3.67±0.29	6.18±0.45[a]	9.05±0.47[b]	10.31±1.02[b]
Ethanol extract of *E. alba* (EEEA)	250	3.54±0.32	5.62±0.43	8.55±0.58[b]	10.78±1.45[b]
Ethanol extract of *E. alba* (EEEA)	500	3.74±0.32	6.42±0.84[a]	9.33±0.43[b]	11.06±1.12[b]

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

± 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).

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 [13]. In acetic acid Induced abdominal writhing and it causes analgesia 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 *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[15].

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 [16]. 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 [17]. 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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REFERENCES

1. Ramnath chopra , Rattan lal Badhwar, Sudhamoy ghosh , Poisonous Plants of India, Vol. **1,** Academic Publishers, Rasta sangheeji son's high way Jaipur India, 557(1984).
2. J.R. L. Walker, Natural antimicrobial systems and food preservation Wallingford, Oxon, U K, CAB Int. 181- 204 (1994).
3. Riedlinger J.E., Herbal supplements, a diabetic approach for today's Pharmacist, Pharm. D.R.Ph.herbal supplement, 2 (2000).
4. Anno Hara M. Kiefer D. Kim M.D. Farrel M.D., A Review 12 commonly used Medicinal Plants, MDH. Arch, Fam. Ed**,** 7, 523 (1998).
5. Ayensu E. S., Medicinal Plants of the West Indies unpublished manuscript, 10 (1978).
6. Coee F.G. Anderson G., *J. Ethnobotony* of the Garifuna of eastern Nicaragua, *Econ bot.,* **50,** 71 (1996).
7. Medina F.R. Woodbury R., Terrestrial Plants Molluscicidal to Lymnaeid hosts of fasciliasis Hepatica in Puerto Rico *J. AGR univ Puerto Rico* ,**63**, 66 (1979).
8. Koheil M.A., Study of the essential oil of flower heads of *Wedelia trilobata* Azhar *J.pharm Sci*., **26,** 288 (2000) .
9. Dagar H.S. Daggar J.C., Ethanobotanical observations among the onge tribe of little Andaman Bull Med *Ethhno. Res,* **10,** 1(1987).
10. Holdsworth D., Traditional Medicinal Plants used in the treatment of malaria and fevers

in Papua New Guinea, *Papua New Guinea Med J*, **18,** 142 (1975) .

11. Holdsworth D., Traditional Medicinal Plants of the north solomons province Papua New Guinea Q *J. Crude Drugs Res,* **18,** 33 (1980).
12. Alyar K.N. Kolammal M., Dashapuspam. In Pharmacognosy of Ayurvedic Drugs Series., **1 -5**, 116 (1962).
13. Kirutikar K.R and Basu B.D., Indian Medicinal Plants, Vol. **3,** Sri Satguru Publications, Delhi, 1364 (1975).
14. Wagner H, Bladt S, Zgainski E M, Plant drug analysis. Berlin, Heidelberg, New York, Tokyo: Springer-Verlag, 298,(1984).
15. Handa S. S., *Pharma Times ,* **23,** 13 (1991**).**
16. Winter C. A and Poster C. C., Effect of alteration in side chain up on anti-inflammatory and liver glycogen activities in hydrocartisone ester. *J Amer Pharmacol Soc,* **46,** 515-19 (1957).
17. Lavine J. Taiwo Y., Inflammatory pain. In: Wall P. D. , Melzack R. (Eds.) Textbook of Pain. Churchill Living Stone, New York, 45-56 (1994).