

A study of the analgesic effect of aqueous extract of the whole plant of *ageratum conyzoides* Linn. in experiments on animal models

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Abstract

A study of the analgesic effects of aqueous extract of the whole *Ageratum conyzoides* (AC) plant in suitable experimental animal models. The whole plant of *Ageratum conyzoides* was extracted with distilled water using Soxhlet apparatus. The extract thus obtained was then screened for analgesic activity using the tail-flick and writhing methods in albino rats and albino mice respectively. The plant's aqueous extract had produced significant increases in the reaction times at 30, 60 and 120 minutes as compared to respective controls in albino rats ($p < 0.05-0.001$). The extract of AC in the doses of 500mg/kg, 1000mg/kg and 2000mg/kg produced 11.5% ($p < 0.01$), 11.5% ($p < 0.01$), 24.73% ($p < 0.001$) inhibition of writhing movement respectively. The standard drug Aspirin (100mg/kg) produced 60.63% inhibition from writhing movement. The study demonstrates significant analgesic effects of the aqueous extract of *Ageratum conyzoides* plant. However, the study found that the extract was less effective than the standard drug (Aspirin) used.

Keywords: *Ageratum conyzoides* (AC), Analgesic, Flick, Pethidine, Writhing, Aspirin.

Introduction

Plants have been used for medicinal purposes for a long time, since before recorded history. Primitive men had observed and appreciated the great diversity of plants that was available to them. Much of the medicinal use of plants seems to develop through observations of their use by wild animals and also by trial and error. The effectiveness of plant remedies, their easy availability, low cost and plants comparatively being devoid of adverse effects popularised them.¹

Ageratum conyzoides L. belonging to the Family Asteraceae (khongzai napi in local dialect) is selected for the study. *Ageratum conyzoides* was originally introduced as a garden plant (and probably as a contaminant with other garden plant seeds) and is utilised in traditional medicine systems wherever it grows.²

Ageratum conyzoides L. is an annual herbaceous plant. The name '*Ageratum*' is derived from the Greek word '*ageras*', which means 'non-ageing', referring to the longevity of the plant. The name '*Conyzoides*' on the other hand is derived from '*Konyz*', the Greek name of '*Inula Helenium*' which the plant resembles. The stem is erect, cylindrical and decumbent and covered with fine, white hairs. Leaves are opposite, acute at apex, acute to obtuse or sub-cordately rounded at base, ovate and triangular, and pubescent with long petiole (1.5-2.0 cm or even up to 3.2 cm), soft and bearing trichomes on both the surfaces, glandular dorsally. Flowers are arranged in a terminal inflorescence- head corymb, involucre subglabrous, bracts acute-acuminate and are white, fruits are cypsel blackish brown and linear-oblong. The plant has a shallow tap root system and grows commonly in the proximity of human habitation, thrives in garden soil and is very common in waste dumping places and ruined sites.^{3,4}

The whole plant possesses various biological activities for wound dressing, curing various skin diseases, ophthalmic,

colic, ulcers treatment, as purgative and a febrifuge. The decoction of the plant is given in stomach ailments such as diarrhoea, dysentery, intestinal colic, flatulence, rheumatism, fever and in gynaecological diseases as a tonic. It has a quick and effective action in burn wounds and is also recommended as an anti-rheumatic.⁵

Therefore, the present study has been undertaken to evaluate the analgesic properties in suitable animal experimental models.

Materials and Methods

The fresh whole plant of *Ageratum conyzoides* L. were collected in the month of July-August 2016 from Senapati District. The plant was then identified and authenticated by the Department of Life Sciences, Manipur University. The aqueous extract preparation of the whole AC (*Ageratum conyzoides* L.) was done by utilization of the method Verma and Aggarwal et al⁶ with slight modification. The dried powder 50gm (approx.) of the whole plant *Ageratum conyzoides* L. was extracted in a soxhlet apparatus with a yield of 29% at the end of extraction process.

Experimental Animals

Healthy albino rats of either sex weighing 100-250gms and albino mice weighing 25-30gms were recruited from the Animal House of JNIMS, Imphal and kept in the Departmental polypropylene cages and acclimatized for 10 days in laboratory atmosphere. The animals were maintained on balance diet (consisting of Bengal gram, maize and cabbage in sufficient quantities) and water *ad libitum* during the entire period of the experiment. The animals were kept in a temperature of 24-28°C with a 12 hours dark-light cycle. The animals were kept fasted for 18 hours prior to the experiment and care was taken to prevent coprophagy. All the experimental protocols were given prior approval by the Institutional Animal Ethics Committee.

Acute Toxicity Study

Acute toxicity study for the extract of the whole plant was carried out using OECD/OCED guideline 425⁷. The test procedure minimizes the number of animals required to estimate the oral toxicity. Six healthy young adult albino rats (200-250gm) and mice (25-30gm) were used for this study. Animals were fasted (water provided) prior to dosing. The fasted body weight of each animals was determined, and the dose was calculated according to the body weight (mg /kg) of the animals.

No adverse effect or mortality was detected in albino rats fed up to 2gm/kg p.o of aqueous extract of the plant, AC during the 24 hours observation period.

Drugs

The following chemicals and drugs were used – Pethidine hydrochloride, Gum acacia and Aspirin.

Analgesic Activity of AEAC

Tail-flick Test in Albino Rats

The method of D' Armor and Smith⁸ as modified by Medhabati M et al⁹ was followed to evaluate the analgesic activity of the aqueous extract of *Ageratum conyzoides* (AEAC) by tail flick method using an analgesiometer. The animals were divided into five groups with six animals in each of the group.

Group	Drugs
Group A	2% Gum acacia in distilled water
Group B	(10ml/kg body weight) p.o
Group C	Aqueous extract of AC (500mg/kg) p.o
Group D	Aqueous extract of AC (1000mg/kg) p.o
Group E	Aqueous extract of AC (2000mg/kg) p.o
	Pethidine (5mg /kg) i.p

The test drug was suspended in distilled water using 2% gum acacia and administered orally. The volume of medicaments was kept constant at 10 ml/kg body weight of the animals used. The Tail flick latencies (reaction time) of the animals was assessed by an analgesiometer. The strength of the current passing through the naked nichrome wire was kept constant at 6 amperes. The distance between the heat source and the tail skin was kept at 1.5 cm and the site of application of the radiant heat had been fixed at 2.5cm, measured from the root of the tail of all rats. The time taken by the animals to withdraw (flick) their tail from the hot wire was noted and taken as the reaction time. The cut off reaction time was fixed at 10 seconds to avoid any tissue damage. The reaction time was recorded at 30, 60 and 120 minutes after the drug administration. The average values of reaction time after each time interval were calculated.

Writhing Method

The method of Witkin LB et al¹⁰ Acetic acid induced writhing test on albino mice as modified by Sawant SB et al¹¹ was followed to evaluate the analgesic effect.

Healthy albino mice of either sex weighing 25-30 gms were recruited from Animal House of JNIMS. The animals

were then divided into five groups with six animals in each group.

The extract was suspended with distilled water and mixed with 2% gum acacia and administered orally. The volume of the medicaments was kept constant at 10 ml/kg body weight of the animal.

Group	Drugs
Group A	2% gum acacia in distilled water (10ml/kg
Group B	body weight)
Group C	Aqueous extract of AC (500mg/kg)
Group D	Aqueous extract of AC (1000mg/kg)
Group E	Aqueous extract of AC (2000mg/kg)
	Aspirin (100mg/kg)

*AC (*Ageratum conyzoides*)

Writhing was induced 20 minutes later in each mouse by administration of 10 ml/kg body weight of 3% acetic acid in distilled water intraperitoneally. Each mouse was kept in a beaker and the total number of writhes was counted 5 minutes after the administration of the extract for the treated animals for 20 minutes.

One way 'ANOVA' followed by Dunnett's 't' test were used to statistically analyse the data obtained for the different groups.

The percentage protection at each dose level is calculated as follows % protection = (1 - No. of writhes in treated group/ No. of writhes in Control group) × 100.

Statistical Analysis

For descriptive studies, Mean and Standard Deviations and Standard Error were used for statistical analysis, ANOVA (Analysis of Variance) was applied and followed by post-hoc Dunnett's 't' test. A probability level of p (<0.05) was considered significant.

Results

Analgesic Effect

Tail-flick Method

The mean reaction time in seconds after 30 minutes, 60 minutes and 120 minutes of drug injection B (500mg/kg) were 4.50±0.55, 6.17±0.41 and 6.67±0.52 respectively.

With the extract dose C (1000 mg/kg), the mean reaction time in seconds after 30minutes, 60minutes and 120minutes of its injection were 6.00±0.89 (p<0.05), 7.00±0.89 (p<0.05) and 7.67±0.52 (p<0.01) respectively.

The mean reaction time for extract D (2000mg/kg) in seconds after 30minutes, 60minutes and 120minutes of drug injection were 6.00±0.63 (p<0.05), 7.00±0.63 (p<0.05) and 7.67±0.52 (p<0.01) respectively.

The mean reaction time for the standard drug pethidine (5mg/kg) in seconds after 30minutes, 60minutes and 120minutes of drug injection were 6.67±0.52 (p<0.001), 7.67±0.51 (p<0.001) and 8.33±0.52 (p<0.001) respectively.

Dose dependent increased reaction times at various intervals of the observations were produced by the extract AC in the doses of 500 mg/kg, 1000 mg/kg and 2000 mg/kg.

Table 1: Analgesic effect of the aqueous extract of *Ageratum conyzoides* L. (n=6 in each group, values are Mean ± SEM, *p<0.05 **p<0.01 *p<0.001)**

Group	Drug Dose (mg/kg i.p)	Pre-Drug Reaction Time (In Seconds)	Reaction Time (In Seconds) After Treatment		
			30 mins	60 mins	120 mins
(A) Control	10ml/kg	4.67±0.52	5.00±0.00	6.00±0.00	6.33±0.52
(B) EAC	500	3.67±0.82	4.50±0.55	6.17±0.41	6.67±0.52
(C) EAC	1000	5.00±0.89	6.00±0.89*	7.00±0.89*	7.67±0.52**
(D) EAC	2000	4.83±0.75	6.00±0.63*	7.00±0.63*	7.67±0.52**
(E) Standard	5ml/kg	6.17±0.75**	6.67±0.52***	7.67±0.52***	8.33±0.52***

One Way F = 5.224 12.830 8.751 15.000
Anova df = 4, 25 4, 25 4, 25 4,25

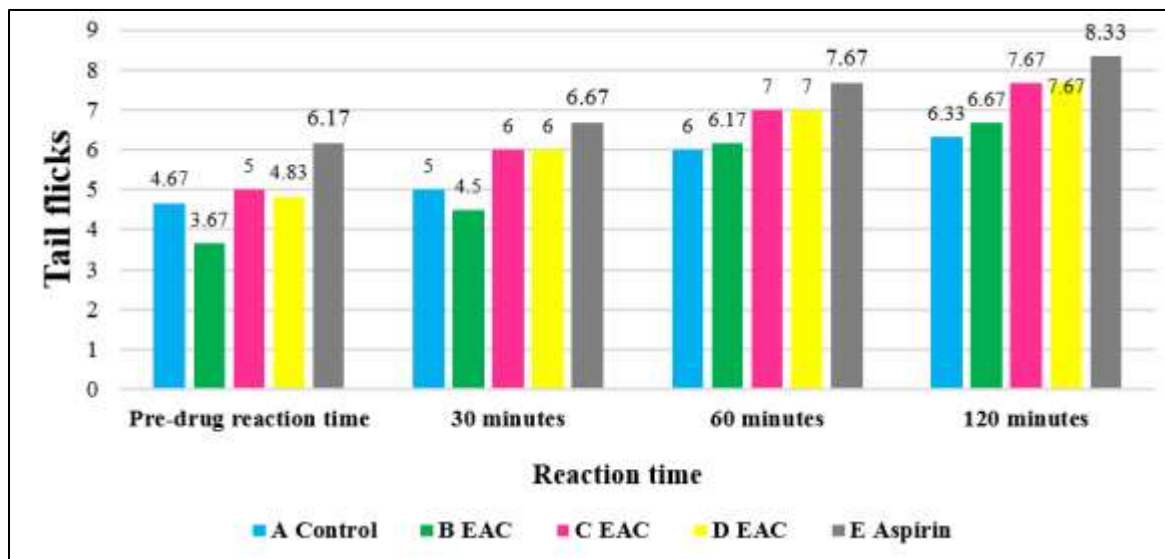


Fig. 1: Analgesic activity of the aqueous extract of *Ageratum conyzoides* L. on tail flick response

Acetic Acid Induced Writhing

Table 2 Analgesic effect of aqueous extract of *Ageratum conyzoides* L. on acetic acid induced writhing test in albino mice. The number of writhes in the various treated groups of animals were observed to determine the analgesic activity.

Table 2

Group	Drug Dose (mg/kg) p.o	No. of Writhing Movement (MEAN±SD)	% of Protection
(A) Control	10ml/kg	47.83±2.78	-
(B) EAC	500	42.33±1.75*	11.50
(C) EAC	1000	42.33±1.94*	11.50
(D) EAC	2000	36.00±4.80**	24.73
(E) Aspirin	100	18.83±3.06**	60.63

One Way Anova F = 69.835, DF = (4,25), p<0.01
Values are Mean ± SD, n = 6 in each group, *p<0.01, **p<0.001 when compared to control

(EAC- aqueous Extract of *Ageratum conyzoides*)

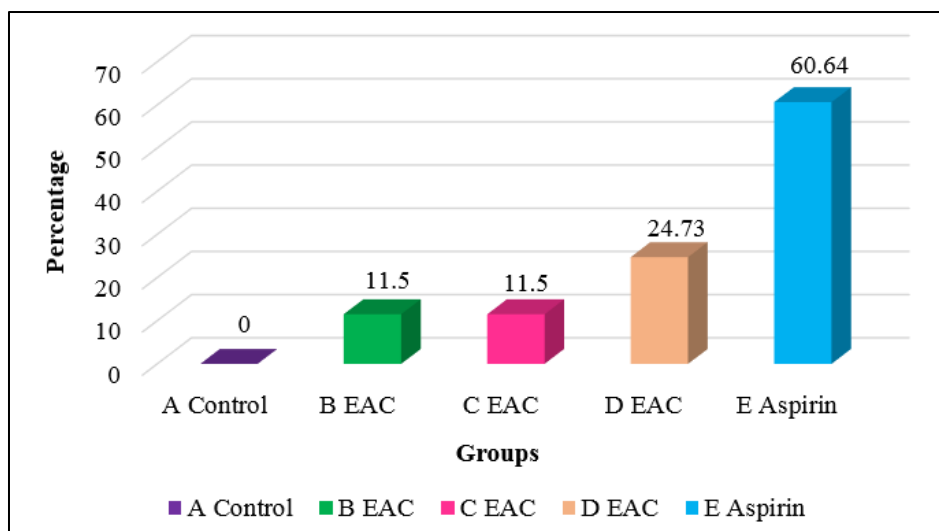


Fig. 2: Analgesic activity of the aqueous extract of *Ageratum conyzoides* L. on acetic acid induced writhing effect

The mean number of writhing movements in the Control group was 47.83 ± 2.78 , extract B (500mg/kg) 42.33 ± 1.75 ($p < 0.01$), extract C (1000mg/kg) 42.33 ± 1.94 ($p < 0.01$), extract D (2000mg/kg) 36.00 ± 4.80 ($p < 0.001$) and Aspirin (100mg/kg) 18.83 ± 3.06 ($p < 0.001$) respectively. The extract in doses of 500mg/kg, 1000mg/kg and 2000mg/kg produces 11.5%, 11.5% and 24.73% inhibition of writhing movements.

The standard drug Aspirin in a dose of 100mg/kg produced 60.63% inhibition of writhing movement. In both the extract and standard groups the number of writhing was significantly reduced in comparison to the control group.

Discussion

The Tail flick test is used for screening centrally acting analgesics whereas Acetic acid induced writhing test is used for screening both centrally and peripherally acting analgesics.

The Tail flick method is the standard model for evaluating analgesic activity of drugs in albino rats. In this study, the number of writhes was counted for 20 minutes and the reaction time was recorded at 30 minutes, 60 minutes and 120 minutes after the extract administration. Pethidine (5 mg/kg) was used as the standard drug in this study.

There was no significant difference between the pre-drug reaction time of different groups. The animals having reaction time of 3-4 seconds were recorded as the normal reaction time in this study. The reaction time was recorded at 30 minutes, 60 minutes and 120 minutes after the extract and standard were administered. The extract (EAC) with the doses of 500 mg/kg, 1000 mg/kg and 2000 mg/kg were able to increase the pain threshold significantly ($p < 0.05-0.001$) at the interval of 30 minutes, 60 minutes and 120 minutes of administration in a dose dependent manner when compared to the pain threshold of the control group at the respective time intervals. The standard drug pethidine 5mg/kg increased the pain threshold significantly at the interval of 30 minutes, 60 minutes and 120 minutes of observations. The observations of pain threshold produced by the control and

standard groups can support the reports of Medhabati M et al.⁹

The possible partial opioid agonistic effect of the aqueous extract of the *Ageratum conyzoides* (AC) may be the cause of increase in the pain threshold. The stress tolerance capacity of the animals can be increased by the aqueous extract of AC (EAC) and also indicates the involvement of a higher centre. The standard drug pethidine did its action through the μ receptors indicating narcotic involvement.¹²

The observations of this present study show that there is a significant decrease in the number of writhes in a dose dependent manner which is produced by the aqueous extract of AC. The EAC at doses 500 mg/kg, 1000 mg/kg and 2000 mg/kg when given orally exhibited a decrease in writhing movements by 11.5%, 11.5% and 24.73% respectively while the standard drug Aspirin had inhibition of 60.63%. The number of writhing movements during 20 minutes of observation in the control group was 2.70 per minute (47 ± 2.78) which corresponds to the findings of Sawant SB et al¹¹ as 3.05 per minute.

The EAC when administered orally at the doses of 1000 mg/kg ($p < 0.01$) and 2000 mg/kg ($p < 0.001$) significantly inhibited the acetic acid induced writhings in mice. Pain sensation in acetic acid induced writhing paradigm is elicited due to production of localized inflammatory response which is through the release of free arachidonic acid from tissue phospholipids via cyclooxygenase and lipoxygenase i.e. specially PGE_2 and PGF_2 . These PGs cause inflammation by increasing capillary permeability. The substance which can inhibit the writhing will have analgesic effect preferably by inhibition of PG synthesis, a peripheral mechanism of pain inhibition. The abdominal constriction of the animal is related to the sensitization of nociceptive receptors by PGs.¹³

The intraperitoneal injection of acetic acid can produce an abdominal writhing response due to sensitization of chemo-sensitive nociceptors by PGs.¹⁴ The analgesic effect of the extract may therefore be proposed either to its action on visceral receptors sensitive to acetic acid, the inhibition of the production of algogenic substances or the inhibition of the

release of the neurotransmitter at the central level of the transmission of painful messages. The results strongly suggested that the mechanism of action of the aqueous extract of *Ageratum conyzoides* may be linked partly to the inhibition of local peritoneal response.

Thus, the present study can propose possible mechanism of action of the analgesic activity of the aqueous extract of the *Ageratum conyzoides* L. through both peripheral and central chemo-transmitters and autacoids.

Conclusion

The aqueous extract of the whole plant *Ageratum conyzoides* L. (EAC) at doses of 500 mg/kg, 1000 mg/kg and 2000 mg/kg increased the pain threshold significantly on acetic acid induced writhing movement in albino mice and the reaction time in tail flick experiment when measured with an analgesiometer in albino rats. The number of writhes was significantly reduced with doses of 500 mg/kg, 1000 mg/kg and 2000 mg/kg of the aqueous extract in a dose dependent manner. The reaction time was significantly increased by different doses of the extract (EAC) at different interval of observation.

Conflict of Interest: None.

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