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Comparative Study of Analgesic Activity of Two Indian Medicinal Plants: *Gmelina arborea* L and *Combretum indicum* L

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ABSTRACT

The present study was undertaken to examine analgesic properties in the leaves by ethanol extracts in mice. The analgesic effect of ethanol extracts was evaluated by 'Tail flick method' and 'acetic acid-induced writhing test' in mice by using Ibuprofen (30 mg/kg) as reference drugs. *Gmelina arborea Leaves* ehanol extract possess analgesic activity, but here we also used *Combretum indicum Leaves* extract to check the extent of the difference in activity. In the tail flick method with ethanol extracts of *Gmelina arborea Leaves* (400 mg/kg), the basal reaction time was increased significantly ($p<0.01$) and the percentage increase in the threshold to pain was also significant as compared to standard & *Combretum indicum Leaves* extract. The ethanol extract of *Gmelina arborea Leaves* (400 mg/kg) possessed significant analgesic activity, as in acetic acid-induced writhing test, the writhing count was reduced significantly ($p<0.01$) compared to standard and *Combretum indicum Leaves* ethanol extracts.

INTRODUCTION

Pain is an unpleasant sensation localized to a part of the body. It is both sensation and emotion. Pain usually occurs when peripheral nociceptors are stimulated in response to tissue injury, visceral distension, or other factors. In such situation, pain perception is a normal physiologic response mediated by the healthy nervous system [(1)].

Due to having adverse side effects, like gastric lesions, caused by NSAIDs and tolerance and dependence induced by opiates, the use of these drugs as analgesic agents have not been successful in all the cases. Therefore, analgesic drugs lacking those effects are being searched all over the world as alternatives to NSAIDs and opiates. During this process, the investigation of the efficacy of plant-based drugs used in the traditional medicine has been paid great attention because they are cheap, have little side effects and according to WHO still about 80% of the world population rely mainly on plant-based drugs [(2)].

The exploration of medicinal properties of plants throughout the ages was accomplished principally through careful observation, trial and error, and accidental discovery, which are beneficial from nutritive and medicinal standpoints. Most of such indigenous knowledge was handed down, through the ages, by at first orally and later in written form as papyri, baked clay tablets, parchments, manuscripts, herbal, and finally printed herbals, pharmacopeias and other works [(3),(4)]. Like the other medicinal plants, *Gmelina arborea* & *Combretum indicum* is full of different constituents which are used for different treatment purpose by the human beings.

MATERIALS AND METHODS

Animals

Healthy adult albino mice of Wistar strain weighing 20-60g were used for this study. The animals were placed randomly and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2ºC and relative humidity of 30-70%. A 12:12 light: day cycle was followed. All the animals were allowed for free access to water and fed with standard commercial pelleted mice chow (M/S Hindustan Lever Ltd. Mumbai).

The plant material

Plant material leaves of *Gmelina arborea* & *Combretum indicum r*espectively were collected from the medicinal garden of Seshachala College of Pharmacy, Puttur. The authenticated samples were used for the preparation of extract. They were put in shied drying then crushed to produce powdered material.

Preparation of extract

Dried and powdered leaves of *Gmelina arborea* & *Combretum indicum* were extracted by using ethanol in soxhlet apparatus. The total extract obtained was dried at 60°C on steam bath followed by a vacuum oven (50°C) to obtain dried extracts. The extractive Value was calculated as % w/w yield and was found to be 6.63% & 5.12%.

PHARMACOLOGICAL EVALUATION

Tail Flick Method

The tail flick test was used with modification described by Dambisya and Lee [(5)]. The extract was administered orally at two doses of *Gmelina arborea* & *Combretum indicum (*200 and 400mg/kg body weight) using Ibuprofen as standard. The post drug reaction time was measured at 0, 15, 30, 45, 60 and 90 minutes later. The tail of the mouse was immersed to a constant level (3cm) in a water bath maintained at 55°C. The time to flick the tail from water (reaction time) was recorded. A maximum immersion time of 10 seconds was maintained to prevent thermal injury to the animals.

Acetic acid induced writhing test

The acetic acid induced writhing method is an analgesic behavioral observation assessment method that demonstrates a noxious stimulation in mice [(6)]. The animals were divided into four groups, each of which containing 6 mice.

Group-1: Control group, Group-2: Mice administered with Ibuprofen, Group-3: Mice administered with *Gmelina arborea* (200mg/kg), Group-4: Mice administered with *Gmelina arborea* (400mg/kg), Group-5: Mice administered with *Combretum indicum* (200mg/kg) &

Group-6: Mice administered with *Combretum indicum* (400mg/kg). The control and the leaf extract were given orally by means of feeding needle. A thirty minutes interval was given to ensure proper absorption of the administered substances. Then the writhing inducing chemical, acetic acid solution (0.7%) was administered intraperitonally to each of the animals of a group. After an interval of fifteen minutes, this was given for absorption and no writhing was counted for 5 minutes. Then every mouse of all groups was observed carefully to count the number of writhing which made within 15 minutes. The percentage inhibition of writhing by an analgesic is calculated according to the following formula-:

$$\% \text{ Inhibition} = \frac{\text{Average withers in control group - Average withers in treated group}}{\text{Average withers in control group}} \times 100$$

Statistical Analysis

Data obtained from pharmacological experiments was expressed as Mean ± SD. Difference between the control and the treatments in these experiments were tested for significance using ANOVA followed by Dunnett's test. Values of $P<0.05$ were considered statistically significant.

RESULTS

Radiant heat tail-flick method

In radiant heat tail-flick test the crude extract produced 40.74% ($p<0.001$) and 61.48% ($p<0.001$) elongation of tail flicking time 30 minutes after oral doses of 200 and 400mg/kg body weight respectively. After 60 minutes the extract showed 31.29% ($p<0.001$) and 41.37% ($p<0.001$) elongation of tail flicking time (table – 1).

Table 1: Effect of Crude extract on tail-flick method

Group	Treatment	Dose	Basic reaction time (sec)	Reaction time in sec.			
				15min	30 min	60 min	120min
I	Control(0.5 % CMC)	1ml/kg	5.21±0.18	5.40±0.06	5.77±0.08	5.88±0.08	5.35±0.13
II	Ibuprofen	30mg/kg	6.42±0.12	9.17±0.10*	12.59±0.09**	15.61±0.11**	19.36±0.26**
III	*Gmelina arborea*	200mg/kg	5.12±0.06	6.67±0.06*	8.18±0.07*	9.33±0.10*	11.76±0.14**
IV	*Gmelina arborea*	400mg/kg	6.30±0.09	7.37±0.06*	9.57±0.06*	11.30±0.08**	14.50±0.13**
V	*Combretum indicum*	200mg/kg	5.69±0.03	6.99±0.08	8.91±0.06*	10.52±0.12*	12.46±0.10**
VI	*Combretum indicum*	400mg/kg	6.80±0.08	8.18±0.11*	10.72±0.09*	11.90±0.11**	15.32±0.09**

Values are expressed as Mean ± SEM, n=6, Data analyzed by One-way ANOVA followed by Dunnette's test * * P < 0.01, * P < 0.05,

Acetic acid induced writhing test

In the acetic acid induced writhing test the extract of *F. recemosa* (200 and 400 mg/kg body weight) showed a significant (p<0.001) reduction in the number of writhes with 48.17 % and 59.85 % of inhibition, as compare to *J. sambac* (200 and 400 mg/kg body weight no. of writhes with 45.54% and 55.88% respectively (table – 2).

Table-2: Effects of crude extract on acetic acid induced writhing response in mice

Group	Treatment	Dose	Writhing	% Inhibition
I	Solvent control	-	21.42±1.34	-
II	Ibuprofen	30mg/kg	7.92±0.40**	64.72
III	*Gmelina arborea*	200mg/kg	11.62±0.70**	45.54
IV	*Gmelina arborea*	400mg/kg	9.45±0.60**	55.88
V	*Combretum indicum*	200mg/kg	11.10±0.80**	48.17
VI	*Combretum indicum*	400mg/kg	8.60±0.60**	59.85

Values are expressed as Mean ± SEM, n=6, Data analyzed by One-way ANOVA followed by Dunnette's test * * P < 0.01, * P < 0.05.

DISCUSSION

In the acetic acid induced writhing test the extract of *Gmelina arborea* (200 and 400 mg/kg body weight) showed a significant (p<0.001) reduction in the number of writhes with 48.17% and 59.85% of inhibition, as compare to *Combretum indicum* (200 and 400 mg/kg body weight) no. of writhes with 45.54%and 55.88% respectively (table 2).

In Tail flick test the *Gmelina arborea* extract produced 32.34% (p<0.001) and 36.72% (p<0.001) elongation of tail flicking time 30 minutes after oral doses of (200 and 400 mg/kg body weight) and *Combretum indicum* extract produced 27.75% and 35.16 (p<0.001) respectively (table 3). After 60 minutes the *Gmelina arborea* extract showed 41.93% (p<0.001) and 48.31% (p<0.001) elongation of tail flicking time and *Combretum indicum* produced 40.24% & and 44.24% (p<0.001) (table 1).

The constriction response of abdomen produced by acetic acid is a sensitive procedure for

peripheral analgesic agents. This response is believed to be mediated by the prostaglandin pathways [7]. The extract of *Gmelina arborea* produced antinociceptive activity and thus indicates the presence of analgesic components that might influence the prostaglandin pathways. In the Tail flick test, the plant extract prolonged the stress tolerance capacity of the mice, indicating the possible involvement of a higher center [8].

Thus, the present study revealed that *Gmelina arborea L*. possesses significant central and peripheral analgesic activities as compare to *Combretum indicum.*

CONCLUSION

From the above investigation, it is quite apparent that ethanolic extract of *Gmelina arborea L*. leaves possesses potent analgesic effect against different stimuli as compare to leaves of *Combretum indicum*. This is evidenced by significant increase in the reaction time by stimuli in different experimental models.

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