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Evaluation of Hydroalcoholic Extract of Melia Azedarach Linn Roots for Analgesic and Anti-Inflammatory Activity

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Abstract

Hydroalcoholic extract of Melia azedarach Linn roots were evaluated for analgesic and anti-inflammatory activity in experimental animals. In the doses of 100 and 200 mg/kg, extract inhibited 82.23 % and 88.94 % writhing induced by acetic acid respectively and reduces 15.08 % and 26.45 % paw volume in carageenan induced paw edema.

Keywords: Melia azedarach, analgesic, anti-inflammatory, wtithing

Introduction

Natural products, including plants, animals and minerals have been the basis of treatment of human diseases. *Melia azedarach* L, commonly known as mahanimba belongs to family Meliaceae. It is large evergreen tree found throughout India and very similar to neem. Traditionally it is used as anthelmintic, antilithic diuretic, astringent and stomachic [1]. Various scientific studies reported the anticancer [2], antiviral [3], antimalarial [4], and activity of this plant. The present investigation is related with analgesic and anti-inflammatory activity of roots of this plant.

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Materials and Methods

Plant collection and preparation of extract

Fresh roots of the *Melia azedarach* L. were collected from forest research farm, Banki-Sisarama, District-Udaipur, (Rajasthan) in the month of Feb. The plant was authenticated by Dr. S. S. Katewa, Dept. of Botany, College of Science, MLSU, Udaipur. Roots were dried in shade, moderately grinded and macerated with hydro alcoholic solvent (70:30) for 7 days with intermittent shaking. On 8th day, the macerate was filtered through muslin cloth; solvent was evaporated at room temperature and lyophilized

in lyophilizer (Step, origin electric, Lonavala) then freeze-dried (Freeze dryer, Allied Frost) to provide dry hydro alcoholic extract of *Melia azedarach* L. roots (HEMAR).

Animals Used

Female wistar rats weighing between 150-200 g were used for the study. Institution animal ethic committee approved all experimental procedures. All the animals were maintained under standard husbandry conditions with food (Chakan mill, Sangali, Maharashtra) and water ad libitum.

Acute oral toxicity

It was determined using OECD/OCDE guideline 425 [5], main test was performed and LD50 was found to be 1030mg/kg.

Statistical Analysis

The data was analysed by using one-way ANOVA followed by Dunnet's test. A p value <0.05was considered to be significant.

Analgesic activity

The analgesic activity was evaluated using acetic acid induced writhing method. For exploration of peripheral analgesic activity, HEMAR was orally administered in the doses of 100 mg/kg and 200 mg/kg, one hour before ip injection of acetic acid (300 mg/kg of 3% solution). The positive control group received acetyl salicylic acid (100 mg/kg, po, Astra-IDL Ldt. India). The numbers of abdominal writhing were measured for 15 min after 5 min of injection of acetic acid. Results were expressed as percentage inhibition of abdominal contractions with respect to control [6,7].

Anti-inflammatory activity

Anti-inflammatory activity was evaluated using carrageenan-induced rat paw edema method. Rats were divided into four groups of six animals each. The first group served as the control and received vehicle only (1% C M C), second and third group was treated with HEMAR (100 mg/kg, 200mg/kg respectively) and the fourth group was administered with standard drug, Ibuprofen (200 mg/kg, orally). A mark was made left hind paws just below the tibiotarsal junction so that every time the paw could be dipped in the mercury column of plethysmometer up to the mark to ensure constant paw volume. After forty-five minutes of the treatment, an inflammatory edema was induced in the left hind paw by injection of 0.1 ml of Carrageenan (1% w/v) in the plantar tissue of all the animals. The increase in the paw volume was measured in control, standard and sample treated groups after 3 h of Carrageenan injection [8].

The degree of edema formation was assayed by the percentage increase in paw treated with standard drug and those treated with extract. These were compared with the increased paw volume of control animals. Thus, percentage inhibition of paw volume in treated animals i. e. edema rate (E) % = (Vt/Vc) x100, which was used for calculating the percentage inhibition rate % = 1-(Vt./Vc) x100, where Vt and Vc are the mean relative changes in the paw volume of the test and control respectively [9].

Results and Discussion

Pain and inflammation is associated with many pathophysiology of various clinical conditions like arthritis, cancer and vascular diseases. The abdominal contraction response induced by glacial acetic acid is a sensitive procedure to establish peripherally acting analgesics. This response is thought to involve local peritoneal receptors. Intraperitoneal administration of acetic acid causes an increase in peritoneal fluids of PGE2 and PGF2∝ involving in part, peritoneal receptors and produces algesia by inducing

capillary permeability and liberating the noxious endogenous substances including serotonin, histamine, prostaglandin, bradykinin and substance p that sensitize pain nerve endings.

In present investigation, at the doses of 100 and 200 mg/kg body weight, extract shows significantly ($p<0.01$) inhibition of the frequency of acetic acid induced abdominal contraction in rat. Percent inhibition of writhing was 82.23 % and 88.94 % respectively. (Table 1).

Table 1: Effect of *Melia azedarach* L. root extract on acetic acid induced writhing in rats

Group	Treatment	Dose mg/kg	Writhing	% inhibition
I	Control (vehicle, 1%CMC)	-	25.33 ±1.046	-
II	HEMAR	100,po	4.5±0.4282**	82.23
III	HEMAR	200,po	2.833± 0.4014**	88.94
IV	Aspirin	100,po	2.167±0.167**	91.44

Values are mean ±SEM, n=6, ** $p<0.001$, compared with control group,

It has been suggested that acetic acid stimulates the vanniloid receptor (V R I) and bradykinin B2 receptor in the pathway comprising sensory afferent C-fibres [10]. Therefore, the observed activity is because of the interfering the synthesis or release of those endogenous substances or desensitization of the nerve fibers involved in pain transmission pathway.

Inflammation induced by carrageenan involves three distinct phases of the release of mediators including histamine and serotonin in the first phase (0-2 h), kinin in the second phase (3 h) and prostaglandin in the third phase (>4 h) [11].

After 3 h HEMAR, in the doses of 100 mg/kg, non-significantly reduced the paw volume but at 200 mg/kg dose, it showed significant ($p<0.01$) reduction in the paw volume. % inhibition was 26.45 % Ibuprofen treated group also showed significant ($p<0.01$) reduction in the paw volume after 3h, % inhibition was 50.39 %, so the extract shows anti-inflammatory activity, comparable with standard drug (Table 2).

Table 2: Effect of *Melia azedarach* L. root extract on carrageenan induced paw edema in rats

Group	Treat-ment	Dose mg/kg	Paw volume (ml) at 3h	% edema rate	% inhibition of edema
I	Control (vehicle, 1%CMC)	-	1.399±0.026	-	-
II	HEMAR	100, po	1.188±0.126	84.92	15.08
III	HEMAR	200, po	1.029±0.055**	73.55	26.45
IV	Ibuprofen	200, po	0.694±0.035**	49.61	50.39

Values are mean ±SEM, n=6, ** $p<0.01$, compared with control group

The ability of extract to suppress abdominal writh and inhibition of the phases of carrageenan, confirm the analgesic and anti-inflammatory properties of the extract which are probably mediated via inhibition of prostaglandin synthesis as well as peripheral inhibitory mechanism, which may be of potential benefit for the management of pain and inflammatory disorders. The ethanolic root extract of this plant mainly contain terpenoids, sendanolactone, kulactone and beta sitosterol [12].

In phytochemical study, we found the presence of tannins and saponins in the extract. The analgesic and antiinflammatory activity of roots of *Melia azedarach* may be attributed to the precence of these constituients.

Conclusion

In present investigation the extract showed anti-inflammatory activity comparable with standard drug ibuprofen. Extract also showed significant analgesic activity, comparable with standard drug aspirin.

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