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ANTI-INFLAMMATORY ACTIVITY OF THE AERIAL PARTS EXTACTS OF *CAESALPINIA MIMOSOIDES* LAMK

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ABSTRACT: *Caesalpinia mimosoides* Lamk. is a prickly climbing shrub belongs to *Leguminosae* family. various parts of this plant have been used in the treatment of ulcer, inflammation, wound healing and arthritis. The present study aimed at the evaluation of anti inflammatory property of different extracts of aerial parts by *in vitro* and *in vivo* methods. *In vitro* methods were estimated by protein denaturation and proteinase inhibition method. An *in vivo* method was estimated on the carragenan paw edema. Acute toxicity studies were performed as per OECD-423 guidelines. Toxicity signs and symptoms were not observed. Both methods showed significant anti-inflammatory activity of the different extracts tested.

INTRODUCTION: Inflammation is a sequential process induced by several biological stimuli such as ischemia, thermal or physical injury, infectious agents and antigen- antibody interactions leads to release of allergic mediators, which causes injury [1]. It is the defense mechanism of the body processed to get rid of the injurious stimuli and induce the tissue healing process.

The commercially available anti inflammatory drugs have severe side effects such as water and salt retention, cancer, renal damage and gastro intestinal disturbances. The side effects of existing synthetic anti inflammatory drugs led to search for a new potential natural drug with lesser or no side effects [2].

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Caesalpinia mimosoides Lam is a member of Leguminosae family. A prickly climbing shrub, with branches bearing small prickles is mainly distributed in the south of China and grows in countries like India, Myanmar, as well as in northern and north-eastern parts of Thailand [3]. Young sprouts and leaves are edible and sour and are traditionally used as a carminative and a remedy for dizziness [4]. The methanolic extract of *C. mimosoides* shoot tips was reported to exhibit antioxidant activity. Moreover, the aqueous and the ethanol extracts contained gallic acid, the antioxidative compound. This plant showed moderate antioxidant activity, and high tannin and total phenolics contents, which led us to examine it further for other biological activity [4]. The present study was designed to investigate the anti inflammatory activity of aerial part extract of *Caesalpinia mimosoides* by *in vitro* and *in vivo* model.

MATERIALS AND METHODS:

Plant Materials: Aerial parts of *Caesalpinia mimosoides*, was collected from Kottayam district,

Kerala during the month of March 2013 and was identified and authenticated by the botanist, Mr. Rogimon P. Thomas, Department of Botany, C.M.S. College, Kottayam, Kerala. A voucher specimen (No. 265) was preserved at C.M.S. College, Kottayam.

Preparation of Extract:
Shade dried and powdered aerial parts (100g) of *Caesalpinia mimosoides* was soaked in rectified spirit in a round bottom flask. After soaking it for one day, it was refluxed with ethanol 95% (2 Litre) for 3 hours and the clear solution was decanted off. The extraction was repeated thrice. The combined extract was concentrated to a semisolid consistency. Thus total ethanolic extract was obtained. The fractionation of the ethanolic extract was carried out using solvents in the increasing order of polarity i.e. petroleum ether(PEE), chloroform(CHE) & ethyl acetate(EAE). Each fraction was concentrated, weighed and stored for further studies.

Animals:
Adult Wister *albino* rats (150g -200 g) of either sex were used for the *in vivo* evaluation. They were housed under standard laboratory conditions and were fed with standard animal feed and water *ad libitum*. The experimental protocol was approved by institutional animal ethical committee.

Acute toxicity test:
Acute toxicity study was performed as per OECD guidelines 423. (Acute toxicity class method-The IAEC approval No. is 024/MPH/UCP/CVR/13). 3 female Wistar albino rats were used for the study. The extract was administered orally at the dose of 2000 mg/kg and observed at half an hour intervals for 4 hr, then after 24 hr. There were no mortality and no signs of toxicity.

***In vitro* anti-inflammatory activity:**
Inhibition of Protein Denaturation:

1. The Test solution (0.5ml) consist of 0.45ml of Bovine serum albumin (5%W/V aqueous solution) and 0 .05ml of test solution.
2. Test control solution (0.5ml) consist of 0.45ml of bovine serum albumin (5%W/V aqueous solution) and 0 .05ml of distilled water.
3. Product control (0.5ml) consists of 0.45ml of distilled water and 0.05 ml of test solution.
4. Standard solution (0.5ml) consists of 0.45ml of Bovine serum albumin (5%w/v aqueous solution) and 0.05ml 0f Diclofenac sodium.

All the above solutions were adjusted to pH 6.3 using 1N HCl. The samples were incubated at 37°c for 20minutes and the temperature was increased to keep the samples at 57°c for 3minutes. After cooling, add 2.5 ml of phosphate buffer to the above solutions. The absorbance was measured using UV-Visible spectrophotometer at 660nm [5, 6]. The experiments were performed in triplicate. The r++++++++++esults were tabulated.

$$\% \text{ inhibition} = 100 - \frac{(\text{O.D of test solution} - \text{O.D of product control}) \times 100}{\text{O.D of test control}}$$

Proteinase inhibitory activity:
The reaction mixture (2ml) was containing 0.06mg trypsin, 1ml of 25mM Tris HCl buffer (pH7.4) and 1ml test sample of different concentrations of different solvents. The reaction mixture was incubated at 37ºC for 5min and then 1ml of 0.8% (w/v) casein was added. The mixture was incubated for an additional 20min, 2ml of 70% perchloric acid was added to terminate the reaction. Cloudy suspension was centrifuged and the absorbance of the supernatant was read at 280nm against buffer as blank [5]. The experiment was performed in triplicate. The percentage of inhibition of proteinase inhibitory activity was calculated. The experiments were performed in triplicate. The results were tabulated.

$$\text{Percentage Inhibition} = \frac{(\text{Absorbance of control} - \text{absorbance of test}) \times 100}{\text{Absorbance of control}}$$

***In vivo* anti-inflammatory activity:**
Carrageenan induced rat paw edema model:
The animals were divided into four groups of six animals each, so a total of 24 animals were used and divided according to the following manner:

Group 1: Positive control rats (Inflammatory control)

Group 2: Inflammation induced rats, given Diclofenac sodium (75mg/kg - orally)

Group 3: Inflammation induced rats, given ethanolic extract (200mg/kg- orally)

Group 4: Inflammation induced rats, given ethanolic extract (400mg/kg-orally)

Paw oedema was induced on each rat by injecting 1 mL of carrageenan on physiological saline to the right hind paw. The ethanolic extract at different concentrations (200 and 400µg/ml) were administered orally 30 minutes prior to carrageenan administration. Paw volumes were measured immediately after injection and then at 60, 120, 180 minutes by mercury displacement method using plethysmograph. The percentage inhibition of paw volume in extract treated groups was compared with control [7].

Percentage of edema inhibition = [(Vc - Vt) / Vc] ×100

Vc- Volume of edema in control group
Vt- volume of edema in treated group

Statistical analysis:
Statistical analysis was done using one way analysis of variance followed by Dunnets test. *p* values greater than 0.05 were considered as significant.

RESULTS:

Acute toxicity studies:
The extracts of *C.mimosoides* did not show any sign of toxicity up to 2000 mg/kg body weight and hence it was considered to be safe.

***In vitro* Anti-inflammatory activity:**
The production of auto antigen in certain arthritic disease may be due to denaturation of protein, membrane lysis and proteinase action. Different concentrations (50,100,200,250µg/ml) of *C. mimosoides* showed significant anti-inflammatory activity against protein denaturation and proteinase enzyme. The ethyl acetate extract of the *Caesalpinia mimosoides* showed higher inhibition when compared with the other extracts.

***In vivo* anti-inflammatory activity:**
The anti inflammatory activity of total ethanolic extract was studied on carrageenan induced rat paw oedema model in wistar albino rats by using plethysmograph. The ethanolic extracts of *Caesalpinia mimosoides* at the doses of 200 and 400 mg/kg significantly reduced the carrageenan induced edema. The maximum percentage inhibition of extract and Diclofenac sodium were noticed at the 3rd hr of carrageenan administration. Extract and Diclofenac sodium showed significant inhibition of 51.39% and 73.74% at doses of 400mg/kg and 75mg/kg respectively. The measurement of paw volume and the percentage inhibition of inflammation were calculated and tabulated in **Table 3**.

TABLE 1: INHIBITION OF PROTEIN DENATURATION

Concentration (µg/ml)	Extracts	IC_{50} values (µg/ml)
50	Diclofenac	70.75
100	PEE	235.8
200	CHE	215.23
250	EAE	75.04

TABLE 2: PROTEINASE INHIBITORY ACTIVITY

Concentration (µg/ml)	Extracts	IC_{50} values (µg/ml)
50	Diclofenac	68.72
100	PEE	245.36
200	CHE	217.6
250	EAE	95.21

TABLE: 3 EFFECT OF *C.MIMOSOIDES* ROOT BARK EXTRACTS ON CARRAGEENAN INDUCED RAT PAW EDEMA

Treatment	Mean paw edema volume in M±SEM				Percentage (%) inhibition			
	0hr	1hr	2hr	3hr	0hr	1hr	2hr	3hr
Control	2.250± 0.07638	2.750± 0.0885	2.90± 0.2017	2.983± 0.3167	-	-	-	-
Standard (Diclofenac sodium 75mg/ml)	2.00± 0.06325	1.817± 0.1302**	1.00± 0.1751***	0.7833± 0.0872***	11.11%	33.92%	65.51%	73.61%
Low dose (200mg/kg)	2.200± 0.06325	2.314± 0.1424	2.153± 0.3585	1.688± 0.3301*	2.22%	15.85%	25.75%	43.41%
High dose (400mg/kg)	2.119± 0.06146	2.133± 0.2741	1.850± 0.3603*	1.450± 0.3118**	5.91%	22.43%	36.21%	51.39%

Values are Mean+SEM, n=6 ANOVA followed by multiple comparison Dunnet test. *P<0.05, **P<0.01, ***P<0.001 was considered as significant when compared to positive control.

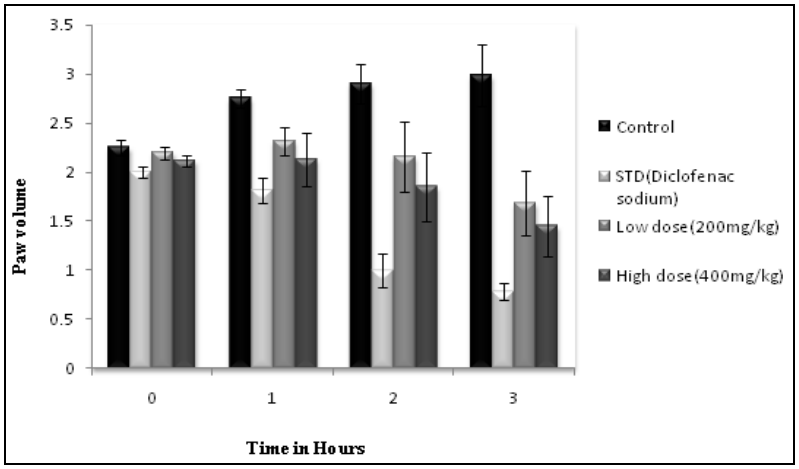

FIG.1: ANTI-INFLAMMATORY ACTIVITY BY CARRAGEENAN INDUCED PAW EDEMA MODEL

DISSCUSSION: Denaturation of proteins is a well documented cause of inflammation. As a part of the investigation on the mechanism of the anti-inflammatory activity, ability of extract to inhibit protein denaturation was studied. It was effective in inhibiting heat induced albumin denaturation at different concentrations as shown in Table 1. The IC_{50} value of the standard diclofenac sodium was found to be 70.75µg/ml and it was comparable with the IC_{50} value of EAE (IC_{50} 75.04µg/ml). The ethyl acetate extract of the *Caesalpinia mimosoides* showed higher inhibition of protein denaturation when compared with the other extracts.

Neutrophils are known to be rich source of serine proteinase and are localized at lysosomes. It was previously reported that leukocytes proteinase play an important role in the development of tissue damage during inflammatory reactions and significant level of protection was provided by proteinase inhibitors. So by inhibiting the proteinase enzyme, the tissue damage as well as release of inflammatory mediators can be blocked. In the proteinase inhibitory activity screening the ethyl acetate fraction showed higher inhibitory activity when compared with other extracts (Table 2). The ethyl acetate fraction showed an IC_{50} value of 95.21µg/ml which was comparable with the IC_{50} value of the standard diclofenac (68.72µg/ml) for the inhibition.

The local acute inflammation model used in this study induced a biphasic oedema consisting on an early phase followed by a more sustained late phase. The early phase of carrageenan oedema was related to the production of immediate inflammation mediators such as histamine, serotonin and bradykinin in the inflamed tissue. The late phase was related to neutrophil infiltration and production of reactive species such as hydrogen peroxide, superoxide radical, peroxinitrite and pro-inflammatory prostanoids. The second phase is sensitive to most clinically effective anti-inflammatory agents. The anti-inflammatory activity exerted by TEE of *Caesalpinia mimosoides* suggests that the effect may be due to the inhibition of prostaglandin, kinin, bradykinin and lysozyme synthesis.

The total ethanolic extract of *Caesalpinia mimosoides* possesses varying degree of anti inflammatory activity when tested at two different doses (**Table 3**). The total etanolic extract of *Caesalpinia mimosoides* at the dose of 400 mg/kg showed high significant anti inflammatory activity at the 3rd hour of administration, where it caused 51.39% inhibition, as compared to that of 75 mg/kg of standard diclofenac (73.61%).

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