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IN-VIVO ANTI-INFLAMMATORY ACTIVITY ON TENDER LAVES OF *ARTOCARPUS HIRSUTUS LAM*

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ABSTRACT

Artocarpus hirsutus (wild jack) endogenous to Kerala has wide medicinal values which are well documented in the third volume of Hortus Malabaricus, the oldest comprehensive printed book on the natural plant wealth of Asia. The decoction of roots and bark are supposed to cure diarrhea. The leaves when used with white camphor and root of curcuma are believed to treat venereal bubones and chronic hemorrhage respectively. The juice from the cooked unripe fruits are believed to induce appetite and also when applied to the anus relieve the pains of hemorrhage. The ethanol extract of *Artocarpus hirsutus lam* was screened for its anti-inflammatory activity *in vivo*. Ear edema was induced by topical application of 0.02ml croton oil was done. After formation of edema, the test drug prepared with irritant solution in different concentrations (1%, 3% and 5%) topically applied in the ear. The tests compared with standard are dexamethasone. From the results, it may be concluded that tender leaves of *Artocarpus hirsutus lam* possess significant anti-inflammatory activity. The activity may be due to the presence of flavanoids, saponin and coumarin glycosides, and terpenoids.

KEYWORDS

Artocarpus hirsutus lam, Ear edema, Flavanoids and Terpenoids.

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INTRODUCTON

Inflammation is a normal, protective response to tissue injury caused by physical trauma, noxious chemicals, or microbiologic agents. Inflammation is the body's effort to inactivate or destroy invading organisms, remove irritants, and set the stage for tissue repair. When healing is complete, the inflammatory process usually subsides. However, inappropriate activation of our immune system can result in inflammation leading to rheumatoid arthritis (RA). Normally, our immune system can differentiate between self and nonself. In RA, white

blood cells (WBC) view the synovium (tissue that nourishes cartilage and bone) as nonself and initiate an inflammatory attack. WBC activation leads to activation of T lymphocytes (the cell-mediated part of our immune system), which will recruit and activate monocytes and macrophages. These will secrete proinflammatory cytokines, including tumor necrosis factor (TNF)- α and interleukin (IL)-1 into the synovial cavity. These cytokines will then cause 1) increased cellular infiltration into the endothelium due to release of histamines, kinins, and vasodilator prostaglandins; 2) increased production of C-reactive protein by hepatocytes (a marker for inflammation); 3) increased production and release of proteolytic enzymes (collagenases and metalloproteinases) by chondrocytes (cells that maintain cartilage), leading to degradation of cartilage and joint space narrowing; 4) increased osteoclast activity (osteoclasts regulate bone breakdown), resulting in focal bone erosions and bone demineralization around joints; and 5) systemic manifestations in which organs such as the heart, lungs, and liver are adversely affected. In addition to T-lymphocyte activation, B lymphocytes are also involved and will produce rheumatoid factor (inflammatory marker) and other auto antibodies with the purpose of maintaining inflammation. These defensive reactions will cause progressive tissue injury, resulting in joint damage and erosions, functional disability, and significant pain and reduction in quality of life¹. The plant *Artocarpus hirsutus Lam* is Moraceae family. The uses of unripe fruits are sour, sweet, an aphrodisiac, an astringent, and thermogenic. An infusion of the bark is applied to treat small cracks and pimples on the skin. Dry leaves are used for treatment of buboes and hydrocele². This aim of the study is to investigate *In vivo* anti-inflammatory activity of extract of *A. hirsutus lam*. *Artocarpus hirsutus lam* were widely used in ethno medicine for the treatment of inflammatory and related disorders, their anti-inflammatory properties have not yet been pharmacologically evaluated. Phytoconstituents such as steroids, terpenoids, flavanoids, phenols and tannins are explored as anti-inflammatory agents from various medicinal plants³. These are presented in this plant. Hence, the present study was undertaken to evaluate anti-inflammatory activity of

ethanol leaf extract by croton oil induced ear edema method.

MATERIAL AND METHODS

Plant material

Collected tender leaves of *Artocarpus hirsutus lam* collected and shade dried and it was converted into moderately coarse powdered and extracted with ethyl alcohol for 27 hours by soxhlet apparatus. Dried extract was used for the Phytochemical screening, and *in vitro* anti-arthritis activity.

Drugs and Chemicals

All organic solvents and other reagents were procured from SD Fine chemicals Ltd. Mumbai and were of analytical grade. Croton oil was obtained from Aline exporters

Preparation of extract

The powdered plant material (300g) was defatted by extracting with ethanol in soxhlet extractor. The drug was macerated with distilled water for 24 hours and then filtered. The marc obtained was again macerated with distilled water and filtered. The filtrates were combined and evaporated to dryness. The dried extracts were kept in dessicator. The percentage yield of extract was 7.8w/w.

Preliminary phytochemical screening

The Phytochemical analysis was done. Major chemical constituents such as flavanoids, terpenes, coumarin and saponin glycosides are presented.

ASSESSMENT OF *IN VIVO* ANTI-INFLAMMATORY ACTIVITY

Extracts use

The ethanol extract of *Artocarpus hirsutus lam* was prepared by using ethanol and freshly prepared irritant solution was applied topically to ear of the experimental animals.

Croton-oil induced ear edema in rats

For tests in rats the following mixture is prepared (v/v): 4 parts Croton oil, 10 parts ethanol, 20 parts pyridine, 66 parts ethyl ether. The standards and the test compounds are dissolved in this solution. For tests in rat's male Sprague-Dawley rats with a weight of 150 to 200 g are used. Rats were divided into 6 groups of 6 animals each, Group I served as normal control. Group II served as croton oil control. Group III, IV and V served as test using concentrations 1%, 3%, and 5% respectively. The

tests, ethanol extract of *Artocarpus hirsutus* Lam are to be dissolved in the irritant solution at different concentration (1%, 3% and 5%) were applied topically to the ear of rats. Group VI received dexamethasone cream (0.1%), used as standard. Croton oil controls receive only the irritant solvent. The left ear remains normal control. The irritant is applied under ether anesthesia. Four hours after application the animals were sacrificed under anesthesia. Both ears are removed and weighed immediately and the weight difference between the treated and normal control ear is recorded indicating the degree of inflammatory edema. Drug effects were calculated as percent inhibition of edema using the equation^{4,5},

$$\frac{(\text{wt. of normal control ear} - \text{wt. of croton oil control ear}) - (\text{wt. of normal control ear} - \text{wt. of test ear})}{\text{wt. of normal control ear} - \text{wt. of croton oil control ear}}$$

Statistical analysis

Data are expressed as mean \pm SEM. Statistical analysis was performed using ANOVA followed by Dunnett's test. P values less than 0.05 ($P < 0.05$) were considered significant. The data obtained were analyzed using the Instat software program.

RESULTS

Croton oil induced ear edema in rats

The results of this study demonstrated that the plant extract produces a reduction in ear edema induced by croton oil. The variations in the weight of the ear taken as study parameter after and before formation of edema by four hours. The control group produced maximum edema in ear was 60.5mg. The ear of standard (Dexamethasone) group was weighed as 25.1mg. Plant extract with irritant solution prepared in the concentration of 1%, 3% and 5% which was applied topically over the ear edema formed. The test group (1, 3 and 5%) was found to be 51.3, 46.3 and 42.1mg and the group of normal control rats was found to be 21.3mg.

DISCUSSION

Anti-inflammatory activity is one of the traditional uses of this plant. This plant extract with irritant solution, which is applied topically over the ear edema formed was observed for four hours from the time of application. The effects of plant extracts in a model of cutaneous inflammation in rats (ear edema

induced by croton oil) shown significant results. Croton oil is a phlogistic agent extracted from *croton tiglium* L., *Euphorbiaceae*, and it has an irritant and vesicular effect on the skin. Croton oil contains phorbol esters which produces an acute inflammatory reaction characterized by vasodilatation, polymorpho nuclear leukocyte infiltration to the tissue and edema formation. These changes are triggered by protein kinase C activation, which promotes an increase in the activity of phospholipase A₂. 12-O-tetradecanoylphorbol-13-acetate and other phorbol esters are the primary irritants in croton oil. It has been reported that cyclooxygenase inhibitors and 5-lipoxygenase inhibitors are highly effective against inflammation caused by 12-O-tetradecanoylphorbol-13-acetate. The events of inflammation in this model occur in the first two hours, followed by increased thickness of the ear as result of cell leakage, which reaches a peak in the sixth hour and then tends to decrease⁶. The results shown that the extract was able to inhibit the inflammatory responses induced by croton oil. It suggests that the extract could inhibit the vasodilatation, leukocytes infiltration and cytokines (IL 1 β , TNF α).

The results of this study demonstrated that plant extract produces a reduction in ear edema induced by croton oil. The percentage inhibition of the edema of standard(Dexamethasone) was 90.20% ($P < 0.01$) and the test 1%, 3% and 5% was 23.27%, 36.06%, 46.72% ($P < 0.01$) respectively. Anti-inflammatory effect of the plant extract was studied scientifically and validated by this research work.

Table No.1: In- vivo anti-inflammatory activity potential of *A. hirsutus lam* in experimental animals by croton oil induced ear edema method

S.No	Treatment	Dose	Ear wt. Mean± SEM	Wt. Difference (Normal control-treated) Mean± SEM	% inhibition of ear edema
1	Normal control	-	0.213±0.8433	-	-
2	Croton oil control	0.02ml/ear	0.605±0.0042*	0.391±0.0083	0.00
3	Croton oil + test	1%	0.5133±0.003#	0.30±0.0093	23.27
		3%	0.463±0.0021#	0.25±0.0089	36.06
		5%	0.4217±0.004#	0.208±0.0116	46.72
4	Croton oil + standard	0.1%	0.2516±0.011#	0.0383±0.0060	90.204

Note: All the values are the mean ± SEM of 6 animals in each group

*, P <0.01 Compared with the normal control group

#, P <0.01 Compared with the croton oil control group

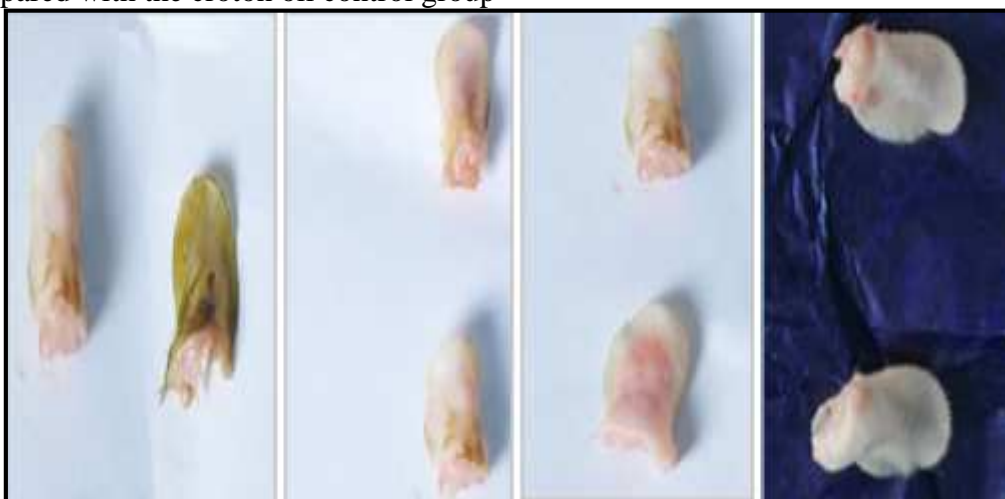


Figure No.1: Image for ears of test (1%, 3% and 5%) and standard

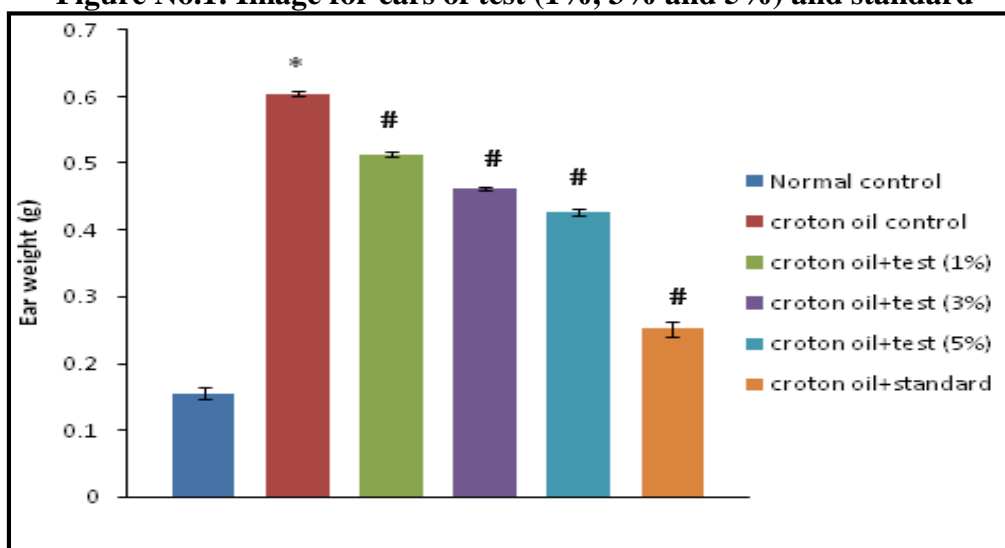


Figure No.2: Inhibitory effect of the ethanol extract of *Artocarpus hirsutus lam* on ear edema induced by croton oil

Note: All the values are the mean ± SEM of 6 animals in each group

*, P <0.01 Compared with the normal control group

#, P <0.01 Compared with the croton oil control group

CONCLUSION

In the present study, the qualitative phytochemical analysis showed the presence of flavonoids and other active constituents in *Artocarpus hirsutus lam*. From the results, it may be concluded that tender leaves of *Artocarpus hirsutus lam* possess significant anti-inflammatory activity. The *in vivo* anti-inflammatory effect of extract was evaluated against croton oil induced ear edema and it was inhibited by the extract. Anti-inflammatory activity involves mechanisms that are able to reduce the formation of edema and the number of leukocytes that migrate to inflammatory foci. Our future aim is to isolate the chemical constituents responsible for those activities. Hence it could be beneficial for further work as active anti-inflammatory agent.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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