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INVIVO ANTI-INFLAMMATORY ACTIVITY STUDIES ON *SIMAROUBA GLAUCA* LEAVES

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ABSTRACT

The aim of the study is to carry out the in vivo anti-inflammatory activity on leaves of *Simarouba glauca*. The *in-vivo* anti- inflammatory activity of the ethanolic extract *Simarouba glauca* has been done by on croton oil induced ear edema method. Topical application of croton oil promoted an increase in the thickness of the ear. Upon application of the ethanolic extract (1%, 3% and 5%), the result was observed as 23.53%, 45.92% and 59.79% and standard 72.64%. The maximum inhibition of croton oil-induced ear edema was observed as 59.79% when compared with standard. The ethanolic extract possess better activity which was comparable to the standard drug (Dexamethasone cream).

KEYWORDS: anti-inflammatory activity, dexamethasone cream, medicinal plant.

INTRODUCTION

Inflammation is a complex process, which is frequently associated with pain and involves occurrences such as: the increase of vascular permeability, increase of protein denaturation and membrane alteration. When cells in the body are damaged by microbes, physical agents or chemical agents, the injury is in the form stress. Inflammation of tissue is due to response to stress. It is a defensive response that is characterized by redness, pain, heat and swelling and loss of function in the injured area. Loss of function occurs depends on the site and extent of injury. Since inflammation is one of the body's nonspecific internal systems of defense, the response of a tissue to an accidental cut is similar to the response that results from other types of tissue damage, caused by burns due to heat, radiation, bacterial or viral invasion.[1]

When tissue cells become injured they release kinins, prostroglandins and histamine. These work collectively to cause increased vasodilation (widening of blood capillaries) and permeability of the capillaries. This leads to increased blood flow to the injured site. These substances also act as chemical messengers that attract some of the body's natural defense cells a mechanism known as chemotaxis. Inflammation can be classified as either acute or chronic. Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes (especially granulocytes) from the blood into the injured tissues. A cascade of biochemical events propagates and matures the inflammatory response, involving the local vascular system, the immune system, and various cells within the injured tissue. Prolonged inflammation, known as chronic inflammation, leads to a progressive shift in the type of cells present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process.

The mechanisms of inflammation involve a series of events in which the metabolism of arachidonic acid plays an important role. It can be metabolized by the Cyclooxygenase (COX) pathway to prostaglandins and thromboxane A2, or by the 5-lipoxygenase (5-LOX) pathway to hydroperoxy-eicosatetraenoic acids (HPETE's) and leukotrienes (LT's), which are important biologically active mediators in a variety of inflammatory events. Upon appropriate stimulation of neutrophils, arachidonic acid is cleaved from membrane phospholipids and can be converted to leukotrienes and prostaglandins through 5-LOX or COX pathways respectively. Inhibition of 5-LOX and COX-leads to decreased production of LTs and PGs, such a drug would have the potential to provide anti-inflammatory and analgesic effects with a reduction in the GI side-effects. Furthermore, inflammatory processes also involve reactive oxygen species started by leukocyte activation. Therefore, screening of antioxidant properties may provide important information about the potential activity of a drug on inflammatory processes.[2] *Simarouba glauca* also known as paradise tree has a long history of herbal medicine in many countries and belongs to the family *Simaroubaceae*. The *Simaroubaceae* family includes 32 genera and more than 170 species of trees and brushes of pan tropical distribution.[3] The bark and leaf extract of *Simarouba* is well known for its different types of pharmacological properties such as haemostatic, anthelmenthic, antiparasitic, antidysentric, antipyretic and anticancerous. The bark is used to cure fever, malaria, stomach and bowel disorders, haemorrhages and ameobiasis. Leaf, fruit pulp and seeds are possessing medicinal properties such as analgesic, antimicrobial, antiviral,

astringent, stomachic tonic and vermifuge. The crushed seeds are used as antigo against snake bites.

obtained. The extract was subjected to preliminary phytochemical screening and pharmacological activities.

Assessment of *in vivo* anti-inflammatory activity

Ear edema induced by croton oil

The edema is expressed as increase in ear thickness due to inflammation. The ear thickness is measured before and after induction of the inflammatory reaction using variation in the.

MATERIALS AND METHODS

Collection of plant material

The leaf of *Simarouba glauca* was collected from the local area of mid land of idukki in kearala state. The plant was identified, confirmed and authenticated by Dr. Gurukar Mathew Botanist and Head of the Department of Botany, Bharathi College, Bharathinagara. The leaves were collected and shade dried under room temperature. The dried material was pulverized separately into coarse powder by mechanical grinder. The coarse powder was subjected to hot continuous extraction with ethanol in a Soxhlet extractor.

Extraction

Continuous hot extraction process- 1kg of dry coarse powder of the leaf of *Simarouba glauca* was extracted with ethanol by hot continuous using Soxhlet apparatus. The extraction was continued until the solvent in the thimble become clear. After complete extraction, the extract was filtered and solvent was distilled off. The extract was concentrated for drying in desiccators over anhydrous calcium chloride. A dark green residue was weight of the ear.croton oil was used for the study.

The 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, IIV and V served as test using concentrations 1%, 3% and 5% respectively. Group 6 received dexamethasone cream (0.1%) used as standard.

For tests in rat the irritant is composed as follows (v/v): 1 part Croton oil, 10 parts ethanol, 20 parts pyridine, 69 parts ethyl ether. 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 were dissolved in this solution. For tests in rats either sexwere used. Six animals were used for controls and each test group. The test compounds are dissolved in a concentration of 10 mg/ml for rats in the irritant solution. On both sides of the right ear 0.02 ml was applied. Control group receive only the irritant solvent. The left ear remain untreated. The irritant was applied under either anesthesia. After four hours of the application the animals are sacrificed under anesthesia. Both ears are removed and weighed immediately. The weight difference between the treated and normal control ear is recorded indicating the degree of inflammatory edema. The result compare with standard.[4]

% ear oedema= [Wt.of control ear –Wt.ofcontrol test ear] / Wt.of control earx100

Statistical analysis

The results were expressed as the mean ± S.E.M. The Statistical analysis was determined by one way ANOVA followed by dunnet test.

RESULTS

Preliminary phytochemical screening

The qualitative analysis of extracts of *Simarouba glauca* were carried out and extracts showed the presence of various chemical constituents such as carbohydrates, saponins, terpenoids, glycosides, phenolics, flavonoids, and tannins. The results are shown in Table 1.

Table No:-1 Preliminary phytochemical screening.

SI. No	Tests	Crude ethanol Extract of *Simaroubaglauca*
1	Alkaloids	-
2	Carbohydrates	+
3	Saponins	+
4	Terpenoids	+
5	Flavanoids	+
6	Proteins	-
7	Glycosides	+

(+) indicates present (–) indicates absent.

Table No. 2:-*In vivo* anti inflammatory activity of *Simarouba glauca by* croton oil induced method.

Normal control	Croton oil control	Test			Standard
		1%	3%	5%	
0.21	0.81	0.69	0.49	0.29	0.22
0.22	0.79	0.56	0.46	0.32	0.24
0.19	0.78	0.58	0.39	0.30	0.22
0.18	0.80	0.62	0.37	0.34	0.20
0.19	0.79	0.59	0.45	0.45	0.21
0.21	078	0.57	0.39	0.39	0.20

The table shows ear weight(gm).

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 Group VI received dextramethasone cream (0.1).

The *in-vivo* anti- inflammatory activity of the ethanolic extract *Simarouba glauca* has been done by on croton oil induced method. Topical application of croton oil was promoted an increase in the thickness of the ear. Upon application of the ethanolic extract of *Simarouba glauca* various concentration in rat the ear edema reduced effectively.

Table. No. 3:-Anti inflammatory potential of plant extract in experimental animals.

Treatment	Dose	Ear weight (mean ±SEM)	% inhibition of ear edema
Croton oil control	0.02ml	0.786±0.0071	-
Normal control	-	0.2±0.0063	-
Test	1%	0.601±0.0195	23.53%
	3%	0.425±0.0196	45.92%
	5%	0.316±0.0117	59.79%
Standard	0.1%	0.215±0.0061	72.64%

As shown in table, topically applied ethanolic extract from 1%, 3% and 5% and result was observed as 23.53%, 45.92% and 59.79% and standard 72.64%. The maximum inhibition of croton oil-induced ear edema was observed as 59.79% when compared with standard.

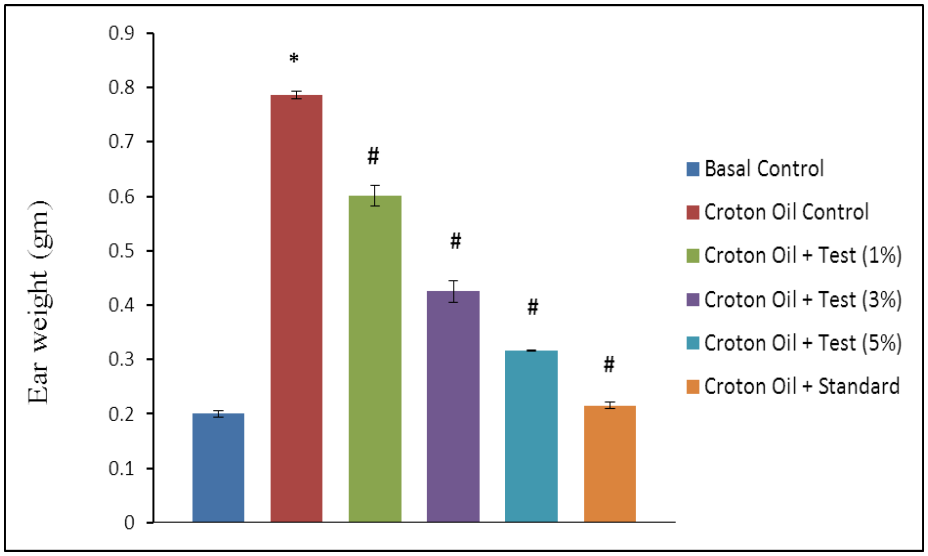

Fig No. 1:-*In vivo* anti inflammatory potential of *Simarouba glauca* by using croton oil induced ear edema in experimental animals

Note :All the values are the mean ± SEM of 6 animals in each group.
*, P < 0.01 compared with the Basal control group

#, P < 0.01 compared with the Croton Oil control group

DISCUSSION

Inflammation is part of biological response of body tissue to harmful stimuli, such as pathogens, damaged cells or irritants. Inflammation caused by release of chemicals from tissues and migrating cells. Most strongly implicated are the prostaglandins (PGs), leukotriens(LTs), histamine, bradykinin and platelet activating factor(PAF) and interleukin-1.

Arachidonic acid (AA) is metabolized into various mediators that induce the formation of edema, such as PGE_2, LTC_4 and LTD_4. The plasma membrane epidermal cells produce AA, which is oxidized to form prostaglandins, leukotrienes, and thromboxanes, responsible for inflammation. As part of the immune response elicited by antigens such as phospholipase A_2 (PLA_2). Thus, it is possible to identify, in this model compounds that inhibit AA metabolism into prostaglandins (PG) and leukotrienes. The nonsteroidal anti-inflammatory drugs (NSAID) inhibit the COX pathway thereby impeding the synthesis of PG.[5]

Invivo anti inflammatory activity was done for the extracts of *Simarouba glauca* by using croton oil induced method. The ethanolic extract fabricates significant activity at 5%

(59.75%) at by inhibition of edema and its effect was compared with the standard drug dexamethasone cream.

CONCLUSION

Finally, it is concluded that the ethanolic extract of *Simarouba glauca* possess significant anti-inflammatory activity. This may be due to presence of carbohydrates, flavonoids, glycosides, saponin, tannins and diterpenoids and triterpenoids, thus it proves the traditional information of the plant is scientifically validated and confirmed by anti inflammatory activitiy.

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