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ANTI-INFLAMATORY ACTIVITY OF *ACACIA NILOTICA* PODS EXTRACTS IN EXPERIMENTAL RATS

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ABSTRACT

Inflammation is body's response to disturbed homeostasis occurs mainly due to infection, injury or trauma resulting in systemic and local effects. The Roman writer Celsus in1st Century AD named the four Cardinal Signs of inflammation as Rubor (redness), Tumor (swelling), Calor (heat) and Dolor (pain). *Acacia nilotica* has long been used in folk medicine in treatment of diarrhoea, snake bite, malaria, smallpox, fever, scabies; ulcer, and stomach disorders. [Prajapti *et al* 2003]. Although a lot of work has been done on the pharmacological activities and phytoconstituents isolation of seed and leaves of *A. nilotica* but no work has been done on anti-inflammatory activity of pods extract of *A. nilotica.* Therefore, the aim of the present work is "Evaluation of anti-inflammatory activity of pods extracts of *Acacia nilotica*." The work was initiated with authentication of plant *Acacia nilotica*. Morphological, Acute toxicity study aims at establishing the therapeutic index. Extracts were found safe up to 2000 mg/kg. *In-vitro* and in-*vivo* anti-inflammatory activity of ethyl acetate, ethanolic extract of *Acacia nilotica* was evaluated by using hyaluronidase inhibition assay and the carrageenan induced paw edema models.

INTRODUCTION

Inflammation associated with many diseases. The drug which are available presently in market itself cause ulcer, hence currently search for new anti-inflammatory agents that have few side effect is undertaken by many researchers. Many medicines of plant origin had been used since ages without any adverse effects. It is therefore essential that efforts should be made to introduce new medicinal plants to develop more effective and cheaper drugs. Plants

represent a large natural source of useful compounds that might serve as lead for the development of novel drugs.

The use of medicinal plants has been an important alternative as therapeutic source of treatment of various diseases and disorders. Remarkably, still 80% of the world population relies on traditional medicines. World Health Organization encourages the conventional medicines as they are inexpensive, easily and abundantly available, and also with least adverse effects. There are many medicinal herbs and spices, which find place in day-to-day uses, many of these, are used as herbal remedies.

The interest regenerated in the complementary system of medicine, is because of side and adverse effects of allopathic medicine, particularly in the treatment of chronic diseases, like hypertension, diabetes and asthma patients' needs to take medicines lifelong. Certainly, intake modern medicine for an extended period of time bound to cause complications in overall health. Hence, there is a growing claim for alternative systems of medicine which uses medicines from natural sources.

Acacia nilotica has long been used in folk medicine in treatment of diarrhoea, snake bite, malaria, smallpox, fever, scabies; ulcer, and stomach disorders. [Prajapti *et al* 2003]. Although a lot of work has been done on the pharmacological activities and phytoconstituents isolation of seed and leaves of *A. nilotica* but no work has been done on anti-inflammatory activity of pods extract of *A. nilotica.* Therefore, the aim of the present work is "Evaluation of anti-inflammatory activity of pods extracts of *Acacia nilotica*."

Animal used

Wistar *rats* of either sex weighing 150 to 200 gm were used in the present study. The experimental animals were maintained under standard laboratory conditions in an animal house of Nanded Pharmacy College, which is approved by the committee for the purpose of control and supervision on experiments on animals (CPCSEA) Protocol. Animals were kept under 12 h light/dark cycles and controlled temperature (24 ± 2°C) and fed with commercial pellet diet and water *ad libitum*. All animals were acclimatized to the laboratory environment for at least one week before the commencement of experiment. The experimental protocol for the study was followed according to the norms of Institutional Animal Ethics Committee.

Safe dose calculations

Determination of acute oral toxicity is usually an initial screening step in the assessment and the evaluation of the toxic characteristics of all compounds. For present study of calculation of safe dose of Nerium indicum Mill. Stem extracts was done by referring standard references. Many researchers carried out acute oral toxicity study of selected plant material & its extracts. Such method will enable a judgment with respect to classifying the test substance to one of a series of toxicity classes defined by fixed LD_{50} cut-off values. Researchers evaluated for acute toxicity of plant as per OECD guideline No. 423. (Acute oral toxicity-class method) were considered for calculating experimental safe dose.

Anti-inflammatory activity

In-vitro method

Name of the analysis method: Hyaluronidase inhibition assay method

Solvent Used **:** DMSO

No. of Samples **:** 02

Standard drug used **:** Indomethacin

Concentrations screened **:** 10, 50 and 100 µg

Principle

The Hyaluronidase is the intercellular substance which maintains intercellular cementing substance and thereby maintains membrane permeability and hence play important role in inflammation like pathological condition and hence inhibition of hyaluronidase can be well correlated with anti-inflammatory activity.

Both extracts of *Acacia nilotica* at different concentrations10, 50, 100µg in solvent DMSO produced significant anti-inflammatory activity. The ethyl acetate extract at 100µ concentration showed 38.22% inhibition while ethanol extract at 100µ concentration showed 41.24% inhibition and the standard drug indomethacin has produced a percentage inhibition ofn92.47%.

METHODOLOGY

Medium containing 3-5U hyaluronidase in 100µl of 20mM sodium phosphate buffer (pH 7.0) with 77mM sodium chloride & 0.01% BSA (bovine serum albumin)was preincubated with different concentrations (10, 50, 100 µg/ml) of the test compound for 15 min at 37°C.

100µl of hyaluronic acid was added to the incubation mixture and incubated for a further 45 min at 37 °C. & after standing at room temperature for 10 min, the absorbance of the reaction mixture was measured at 600 nm. The absorbance in the absence of enzyme was used as the reference value for maximum inhibition. The inhibitory activity of test compound was calculated as the percentage ratio of the absorbance in the presence of test compound vs. absorbance in the absence of enzyme. Compound was tested in a range of 10µg -100µg in the reaction mixture.

Indomethacin (Indo) was used as reference standard.

***In-vivo* method**

In-vivo anti-inflammatory study of Acacia nilotica Linn was carried out using carragenan induced paw edema.

IAEC Approval

Male albino wistar rats weighing 200-300 g were used in the present study. The experimental animals were maintained under standard laboratory conditions in an animal house approved by the committee for the purpose of control and supervision on experiments on animals(CPCSEA) under 12 h light/dark cycle and controlled temperature (24 ±2°C) and fed with commercial pellet diet and water *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethics Committee, Nanded Pharmacy College, Nanded, Maharashtra, India.

Protocol Approval No. **R-2: XII dated 28/02/2015**

Animals used: Albino Rats Wistar Strain

Weight: 250±5 g

Route of administration: P.O.

Housing Condition: Animals were housed in a group of six in separate cages under controlled conditions of temperature (22± 2°C). All animals were given standard diet and water regularly.

Carragenan induced rat paw edema

Principle

Among the many methods used for screening of anti-inflammatory drugs, one of the most commonly employed techniques is based upon the ability of such agents to inhibit the edema

produced in the hind paw of the rat after injection of a phlogistic agent. Many phlogistic agents (irritants) have been used, such as brewer's yeast, formaldehyde, dextran, egg albumin, kaolin, sulfated polysaccharides like carragenan. The effect can be measured in several ways. Usually, the volume of the injected paw is measured before and after application of the irritant and the paw volume of the treated animals is compared to the controls. Many methods have been described how to measure the paw volume by simple and less accurate and by more sophisticated electronically devised methods. (Vogel, 2007)

Experimental design

The following groups were considered for the study, each group containing six animals.

Table: Animal grouping for *in-vivo* anti-inflammatory screening.

Group No.	Group	Treatment and dose/day
I	Control	1 ml
II	Ethyl acetate extract(EaE)	100 mg/kg
III	Ethyl acetate extract(EaE)	200 mg/kg
IV	Ethanolic extract(EE)	100 mg/kg
V	Ethanolic extract(EE)	200 mg/kg
VI	Indomethacin	20 mg/kg

Procedure

Albino rats of either sex weighing between 200-300 g were selected and divided into 6 groups of six animals each. All these animals were fasted for 12 hrs before the beginning of the experiment and water was given *ad libitum*. In animals of all groups acute inflammation was produced by sub plantar injection of 20 µl of freshly prepared 1% suspension of carragenan in normal saline in right hind paw of rat. The paw thickness was measured using plethysmometer before and at an interval of 0, 30, 60, 120, 180, 240 min and 24 hrs after carragenan challenge in each group. Animals were pretreated with drug/extracts one hour before carragenan injection as depicted in Table. The increase in paw volume were measured, inhibition were calculated by comparing with control group.

$$\textbf{\% Inhibition} = \frac{(V_t - V_o)_{Control} - (V_t - V_o)_{test}}{(V_t - V_o)_{Control}} \times 100$$

Where, V_t =Volume of paw edema at time t.

V_o = Volume of paw edema before administration of test sample (Predose)

Statistical Analysis

Presentation of results was done in tabular form. All results expressed as Mean ±Standard Error. The results were expressed as mean ± S.E.M. Data was analyzed by one-way ANOVA test.

RESULTS

Anti-inflammatory activity

***In-vitro* antiinflammatory activity by Hyaluronidase Inhibition Assay**

Table 6.6.: *In-vitro* antiinflammatory activity of plant extracts by Hyaluronidase inhibition assay.

Sample	OD @600 nm	% inhibition
EEAN(10 µg)	0.255	28.67
EEAN(50 µg)	0.27	30.34
EEAN(100 µg)	0.34	38.20
EaEAN(10 µg)	0.221	24.83
EaEAN 50 µg)	0.23	25.84
EaEAN(100 µg)	0.367	41.24
Indomethacin(10 µg)	0.271	30.45
Indomethacin(50 µg)	0.456	51.24
Indomethacin(100 µg)	0.823	92.47

EEAN: Ethanolic extract of *Acacia nilotica*, EaEAN: Ethyl acetate extract of *Acacia nilotica*

The Ethyl acetate extract of *Acacia nilotica* at 10,50 and 100µ concentration exhibit 28.65%, 30.34% and 38.20% percent inhibition whereas the ethanolic extract of *Acacia nilotica* at 10,50 and 100 µg concentration exhibit 24.83%, 25.8% and 41.24% percent inhibition respectively in comparison with that of standard drug indomethacin at 10,50 and 100 µg concentration.

EEAN: Ethanolic extract of *Acacia nilotica*, EaEAN: Ethyl acetate extract of *Acacia nilotica.*

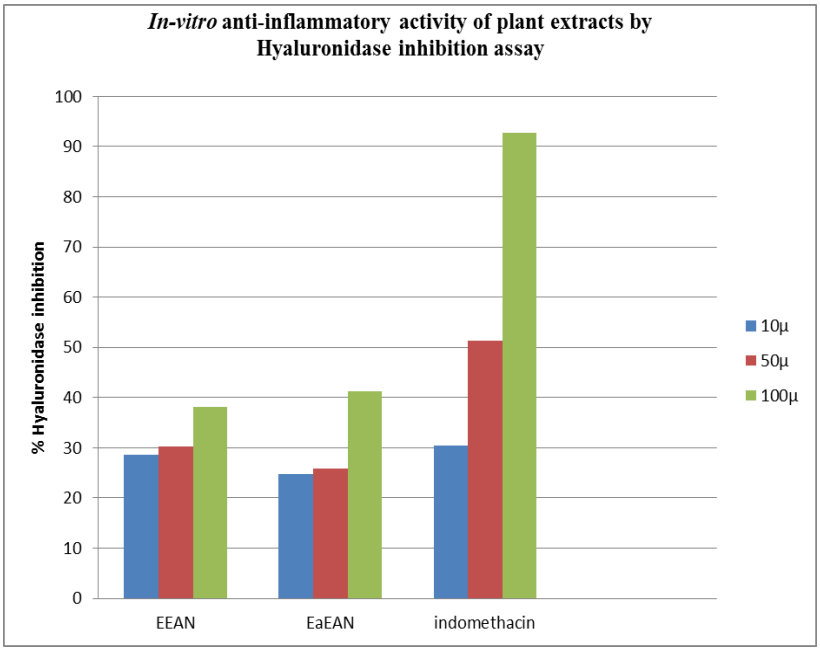

Figure: The *In-vitro* anti-inflammatory activity of *Acacia nilotica* Linn pods.

***In-vivo* Carragenan induced paw edema**

Table: *In-vivo* Anti-inflammatory activity of Ethanolic extract of *Acacia nilotica* (100 mg/kg) against Carragenan induced rat paw edema.

Sr. No	Time(min)	Paw edema volume(ml)			% inhibition	
		EEAN 100 mg/kg	control	Standard (indomethacin)	EEAN 100 mg/kg	Standard Indomethacin
1	Predose	1.326±0.154	0.931±0.118	1.243±0.154	NA	NA
2	30 min	1.588±0.146	1.918±0.112	1.741±0.149	73.46%	79.86%
3	60 min	1.503±0.144	2.008±0.116	1.636±0.152	80.18%	80.16%
4	120 min	1.443±0.144	2.131±0.114	1.533±0.154	90.27%	92.12%
5	180 min	1.405±0.144	2.301±0.100	1.436±0.157	94.27%	95.12%
6	240 min	1.365±0.152	2.403±0.096	1.378±0.157	96.99%	97.81%
7	24 hr	1.388±0.154	1.965±0.117	1.348±0.156	88.54%	88.88%

n=6; **EEAN: Ethanolic extract of *Acacia nilotica***

From above table it shows that EEAN has significant anti-inflammatory activity in comparison with control and in relation with indomethacin, it revealed comparable activity in a given dose of EEAN (100mg/kg) at 240 min shows 96.99% percent inhibition, while Indomethacin (20mg/kg) at 240 min shows 97.81% percent inhibition.

Table: *In-vivo* Anti-inflammatory activity of Ethanolic extract of *Acacia nilotica* (200 mg/kg) against Carragenan induced rat paw edema.

Sr. No	Time(min)	Paw edema volume(ml)			% inhibition	
		EEAN 200 mg/kg	Control	Standard (indomethacin	EEAN 200 mg/kg	Standard Indomethacin
1	Predose	1.211±0.114	0.931±0.118	1.243±0.154	NA	NA
2	30 min	1.488±0.106	1.918±0.112	1.741±0.149	68.35%	79.86%
3	60 min	1.403±0.105	2.008±0.116	1.636±0.152	85.55%	80.16%
4	120 min	1.343.104	2.131±0.114	1.533±0.154	89.75%	92.12%
5	180 min	1.303±0.113	2.301±0.100	1.436±0.157	94.91%	95.12%
6	240 min	1.265±0.109	2.403±0.096	1.378±0.157	96.29%	97.81%
7	24 hr	1.288±0.114	1.965±0.117	1.348±0.156	88.32%	89.84%

n=6; **EEAN: Ethanolic extract of *Acacia nilotica***

From above table it shows that EEAN has significant anti-inflammatory activity in comparison with control and in relation with indomethacin, it revealed comparable activity in a given dose of EEAN (200mg/kg) at 240 min shows 96.29% percent inhibition, while Indomethacin (20mg/kg) at 240 min shows 97.88% percent inhibition.

Table: *In-vivo* Anti-inflammatory activity of Ethyl acetate extract of *Acacia nilotica* (100 mg/kg) against Carragenan induced rat paw edema.

Sr. No	Time(min)	Paw edema volume(ml)			% inhibition	
		EaEAN 100 mg/kg	control	Standard (indomethacin	EaEAN 100 mg/kg	Standard Indomethacin
1	Predose	1.153±0.171	0.931±0.118	1.243±0.154	NA	NA
2	30 min	1.67±0.142	1.918±0.112	1.741±0.149	47.21%	79.86%
3	60 min	1.723±0.146	2.008±0.116	1.636±0.152	47.06%	80.16%
4	120 min	1.63±0.151	2.131±0.114	1.533±0.154	60.23%	92.12%
5	180 min	1.48±0.149	2.301±0.100	1.436±0.157	76.13%	95.12%
6	240 min	1.43±0.144	2.403±0.096	1.378±0.157	81.17%	97.81%
7	24 hr	1.303±0.178	1.965±0.117	1.348±0.156	85.48%	89.84%

n=6; **EaEAN: ethyl acetate extract of *Acacia nilotica***

From above table it shows that EaEAN has insignificant anti-inflammatory activity in comparison with control and in relation with indomethacin, it revealed comparable activity in a given dose of EaEAN(100mg/kg) at 240 min shows 54.17% percent inhibition, while Indomethacin(20mg/kg) at 240 min shows 97.88% percent inhibition.

Table: *In-vivo* Anti-inflammatory activity of Ethyl acetate extract of *Acacia nilotica* (200 mg/kg) against Carragenan induced rat paw edema.

Sr. No	Time(min)	Paw edema volume(ml)			% inhibition	
		EaEAN 200 mg/kg	Control	Standard (indomethacin	EaEAN 200 mg/kg	Standard Indomethacin
1	Predose	1.306±0.119	0.931±0.118	1.243±0.154	NA	NA
2	30 min	1.583±0.123	1.918±0.112	1.741±0.149	71.94%	79.86%
3	60 min	1.478±0.120	2.008±0.116	1.636±0.152	80.05%	80.16%
4	120 min	1.425±0.119	2.131±0.114	1.533±0.154	90.13%	92.12%
5	180 min	1.36±0.115	2.301±0.100	1.436±0.157	94.08%	95.12%
6	240 min	1.34±0.120	2.403±0.096	1.378±0.157	96.75%	97.81%
7	24 hr	1.36±0.119	1.965±0.117	1.348±0.156	86.23%	89.84%

n=6; **EaEAN: ethyl acetate extract of *Acacia nilotica***

From above table it shows that EaEAN has insignificant anti-inflammatory activity in comparison with control and in relation with indomethacin, it revealed comparable activity in a given dose 0f EaEAN(200mg/kg) at 240 min shows 97.75% percent inhibition, while Indomethacin(20mg/kg) at 240 min shows 97.81% percent inhibition.

DISCUSSION

The present study is an attempt for providing traditional claims about the plant *Acacia nilotica* mentioned in Ayurvedic tests and evaluation for its anti-inflammatory activity. Acute toxicity study aims at establishing the therapeutic index. Extracts were found safe up to 2000 mg/kg. The use of medicinal plants has been an important alternative as therapeutic source of treatment of various diseases and disorders. Its rising acceptance in the medical community has been due to the fact that several plants with biological activities have been scientifically investigated and their efficacy and safety have been verified (Vane *et al.* 1995).

The continuous research in the field of synthetic drugs in recent years is accompanied by numerous unwanted side effects, such as NSAIDs shows gastric ulcer & glucocorticoids shows adrenal suppression, as major side effects. Whereas plants have their unique place with least side effects, hence present work, *In-vitro* and in-*vivo* anti-inflammatory activity of ethyl acetate, ethanolic extract of *Acacia nilotica* was evaluated by using hyaluronidase inhibition assay and the carrageenan induced paw edema models.

Both extracts of *Acacia nilotica* at different concentrations 10, 50, 100µg in solvent DMSO produced significant anti-inflammatory activity. The ethyl acetate extract at 100µg concentration showed 38.22% inhibition while ethanol extract at 100µg concentration showed

41.24% inhibition and the standard drug indomethacin has produced a percentage inhibition of 92.47%. From such results it might be concluded that the *Acacia nilotica* pods may have promising anti-inflammatory activity.

The carrageenan induced paw edema model has been commonly used as an experimental model for acute inflammation and is believed to be biphasic event. The initial phase occurs between 0 and 1.5 h after the injection of the phlogistic agent, has been accredited to the action of inflammatory mediators such as histamine, 5-HT, etc. Second phase (1.5-2.5 h) is mediated by bradykinin on vascular permeability (Yonathan *et al* 2006).

In this study, abatement of edema appeared after 30 min & this effect was continued from 180 min up to 24 hrs. Hence this is suggestive of ethanolic extract of *Acacia nilotica* possibly acts by inhibiting the synthesis, release and action of histamine, 5-HT, Bradykinin & prostaglandin too.

Saponins, glycosides, phenolic compounds and flavonoids are reported to possess anti-inflammatory properties. The ethyl acetate extract 100 mg/kg, 200 mg/kg), ethanolic extract (100mg/kg, 200 mg/kg) exhibited anti-inflammatory activity. Both extracts have produced significant anti-inflammatory activity. The maximum percentage reduction in paw edema observed with the ethanol extract of *Acacia nilotica* and the standard drug indomethacin has produced a percentage reduction of 97.81%.

CONCLUSION

Acacia nilotica pods were used for studying pharmacognostical, phytochemical and pharmacological evaluations. The *in-vitro* study has showed that ethyl acetate and ethanolic extract of *Acacia nilotica* does possess significant anti-inflammatory activity with 10µ, 50µ and 100µ concentrations and with high concentration of ethanolic extract (100µ) it shows 41.24% inhibition. This *In-vivo* study has showed that ethyl acetate and ethanolic extract of *Acacia nilotica* does possess significant anti-inflammatory activities with 100 mg/kg and 200 mg /kg. The results, thus, might support the traditional use of this plant in inflammatory process. The future scope of the study includes isolation and fractions of extract of *A. nilotica* and further in detail screening of the active principle (s) in order to come up with the active compound (s) responsible for the anti-inflammatory properties of the plant. Moreover, other studies should be performed to confirm the exact mechanism (s) and anti-inflammatory activity of the plant in chronic inflammatory models.

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