A COMPARATIVE STUDY OF ANTI-INFLAMMATORY ACTIVITY OF ETHANOLIC AND AQUEOUS EXTRACTS OF ACACIA NILOTICA LINN

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ABSTRACT

Objective: *An ethanolic and aqueous extracts of stems of Acacia Nilotica Linn was investigated for its anti-inflammatory activity. The results of the investigation were compared.*

Materials & methods: *Carrageenan induced rat paw edema method was followed for the investigation of anti-inflammatory activity. 200mg/kg and 400mg/kg of ethanolic and aqueous extracts of Acacia Nilotica were used for the study. Ibuprofen was used as a standard drug for the investigation.*

Results & Discussion: *The study showed significant anti-inflammatory activity for the stems of Acacia Nilotica Linn.*

Conclusion: *Comparatively, ethanolic extract of Acacia Nilotica showed better anti-inflammatory activity than an aqueous extract.*

Key words: Stem, Carrageenan, Ibuprofen, Anti-inflammatory, Ethanolic, Aqueous.

Introduction

In acute inflammation the tissue becomes red, swollen, tender or painful, there is local heat and the patient may be febrile. At a cellular level the capillaries become more permeable and fluid and other elements from the blood leak into the tissue spaces. Phagocytic cells including leucocytes migrate into the area and rupture of cell lysosomes releases lytic enzymes into the tissues. Inflammation may also be chronic, as in rheumatoid arthritis [1]. Anti-inflammatory drugs by acting on and modifying the response of the innate immune system to a

challenge are useful in many settings to damp down as over exuberant or pathologically prolonged inflammatory response. Immunomodulatory agents, which act on components of the adaptive immune response, are important for the treatment of complex autoimmune disease and in preventing allograft rejection. The majority of NSAIDs are anti-inflammatory because they inhibit COX in the periphery. COX inhibition in most chronic inflammatory conditions, while useful for symptomatic relief, does not modify the cause of disease [2].

Acacia Nilotica is commonly known as gum Arabic tree, Egyptian thorn, karuvela maram etc., which is belongs to the family *Fabaceae*. It's a medium to large tree that can reach a height of 10 m, with an average of 4-7 m in height. The bark is blackish grey or dark brown in mature trees and deeply grooved, with longitudinal fissures. The young branches are smooth and grey to brown in color. It is native from Egypt, across the Maghreb and Sahel, south to Mozambique and KwaZulu-Natal, South Africa and east through Arabian Peninsula to Pakistan, India and Burma. The bark exudes an edible gum and is used medicinally. The decoction of the bark can be used as cough remedy. Other parts of the tree were used to treat eye diseases or as a tranquillizer and as an aphrodisiac. A root extract was used in the treatment of tuberculosis, impotence, diarrhea, haemorrhages, toothache, dysentery and gonorrhea [3,4].

The plant parts were scientifically explored for its antimicrobial [5], antibacterial, antifungal [6], antiviral [7], antibiotic [8], antimalarial [9], anti-diarrhea [10], antioxidant [11], antihypertensive [12], anti-mutagenic [13] and anthelmintic activity [14]. However, an extended literature survey revealed that, the stems of *Acacia Nilotica Linn* was not scientifically proved for its anti-inflammatory activity. Hence, the present study was undertaken to explore its anti-inflammatory activity.

Materials and Methods

Collection of Plant Material

Stems of *Acacia Niotica Linn* was collected from road side of Badvel, YSR Dist, Andhra Pradesh, India and the same was authentified by Dr.K.Madhava Chetty, Assistant Professor, S.V.University, Tirupati. Voucher specimen was deposited at department of Pharmacognosy for further reference.

Preparation of extracts

The collected plant material was cleaned thoroughly and dried under shade. The dried material was powdered using a mixer grinder and used for the extraction. The powdered material

was evenly packed in the Soxhlet apparatus and then extracted with various solvents from non-polar to polar successively. After each extraction, the extracts were filtered through Whattmann filter paper to remove any impurities if present. Then they were concentrated by the process of distillation and transferred into a beaker. The remaining solvent was evaporated on a water bath, cooled and placed in a desiccator to remove any excessive moisture. Then the extracts were packed in air tight containers for their further use in the phytochemical screening and experimental studies.

Detection of phytochemical constituents

Chemical constituents present in any plants are generally classified into two main categories i.e., organic constituents and inorganic constituents. Organic constituents include Carbohydrates, steroids, glycosides, alkaloids, flavonoids, saponins, proteins, tannins and phenolic compounds etc. Various chemical tests were performed on the extracts of the plants to identify the presence of these chemical constituent. n-hexane, chloroform, ethyl acetate, ethanol and water extracts of stems of *Acacia Nilotica* were subjected to preliminary phytochemical screening for the detection of the presence or absence of various phytoconstituents such as alkaloids, carbohydrates, glycosides, flavonoids, saponins, tannins/phenols, triterpenoids, phytosterols, proteins and amino acids [15,16].

Procurement of Animals

Swiss albino mice (20-30gm) and Wistar albino rats (200-250gm) of either sex and of appropriate same age used in the present study were procured from Sri Venkateshwara Enterprises, Bangalore, Karnataka, India. The animals were fed with standard pellet diet (Hindustan Lever Ltd, Bangalore) and water ad libitum. The animals were kept under alternate cycle of 12 hours in darkness and light. The animals were acclimatized to the laboratory condition for one week before starting the experiment.

Acute toxicity studies

A key stage in ensuring the safety of drugs is to conduct toxicity tests in appropriate animal models. Toxicity studies involve a test in which single dose of the drug used in each animal for the determination of LD_{50} or median lethal dose, i.e., the dose required to kill 50% animals used in the studies. It is considered sufficiently adequate LD_{50} with confidence limits is established on one common laboratory species, in general, mice by the standard method. In the present work, acute toxicity studies were performed for the Ethanolic and Aqueous extracts of stems *Acacia Nilotica* [EEAN and AEAN] using different doses according to OECD guidelines [17]. For the

pharmacological studies, the amount of dose administered was adjusted on the basis of observation during the toxicity studies.

Anti-inflammatory activity [18-20]

The inflammatory reaction is readily produced in rats in the form of paw edema with the help of irritants. Substances such as carrageenan, formalin, bradykinin, histamine, 5-hydroxytryptamine, mustard or egg whites when injected in the dorsum of the foot of rats they produce acute paw edema within few minutes of the injection. Carrageenan-induced paw edema is the most commonly used model in experimental pharmacology. Hence the same was selected to screen the anti-inflammatory effect of EEAN & AEAN in rats.

Carrageenan is a sulphated polysaccharide obtained from sea weed (*Rhodophyceae*) and by causing the release of histamine, 5-HT, prostaglandins and bradykinin it produces inflammation and edema. Six groups of rats (6 in each group) were administered with the following dosage schedule.

Group I: Control group animals received 2ml/kg of 1% NaCMC.

Group II: Test group animals received EEAN 200mg/kg in 1% NaCMC p.o.

Group III: Test group animals received EEAN 400mg/kg in 1% NaCMC p.o.

Group IV: Test group animals received AEAN 200mg/kg in 1% NaCMC p.o.

Group V: Test group animals received AEAN 400mg/kg in 1% NaCMC p.o.

Group VI: Standard drug group animals received Ibuprofen 5mg/kg.

One hour after the administration of the above scheduled doses, edema was induced to the animals by subcutaneous injection of carrageenan solution (0.1ml of 1% carrageenan in normal saline) on the plantar surface of the hind paw of rats. For measuring the paw volume, the paw of the animals were marked with ink at the level of lateral malleolus and immersed in the mercury column of a Plethysmometer. The paw volume was measured immediately (0 hour) after the carrageenan injection and then at 3, 6, 12 and 24th hour. The percentage inhibition of inflammation was calculated by using the following formula,

Percentage inhibition of inflammation = $(A - B)/A \times 100$

Where A and B denotes mean increase in paw volume of control and drug treated animals respectively.

Statistical Analysis

Statistical analysis was performed with One Way ANOVA followed by Dunnett's test using Graph Pad Instat 3 software.

Results and Discussion

The results of the study showed the presence of alkaloids, glycosides, flavonoids, tannins/phenols and saponins in ethanolic extract and alkaloids, glycosides, carbohydrates, tannins/phenols, saponins and amino acids in aqueous extract of *Acacia Nilotica.*

Acute toxicity study of EEAN & AEAN showed that these extracts are safe to use in animals even at a dose of 2000mg/kg orally. From the results obtained, two doses (200mg/kg & 400 mg/kg) were selected for the further investigation of plant extracts for its medicinal properties.

Mean paw volume (ml) and percentage inhibition of inflammation on Carrageenan induced rat paw edema by the administration of EEAN & AEAN are shown in table-1 and the bar diagram were given in fig.1. The percentage inhibition is given in fig.2. Rats showed increased paw volume at 3rd and 6th hour of the experiment. Standard drug showed reduction in paw volume with the percentage inhibition of 72.58% at 24th hr. Groups EEAN and AEAN at the dose of 400mg/kg showed percentage inhibition of 63.71% and 56.45% respectively. The results of which are comparable with that of standard.

Table-1

Anti-inflammatory effect of EEAN & AEAN on Carrageenan induced rat paw edema

Groups		Mean paw volume (ml)					% inhibition at 24 hr
		0 hr	3 hr	6 hr	12 hr	24 hr	
I	Control	0.82 ± 0.12	1.42 ± 0.13	1.47 ± 0.14	1.36 ± 0.14	1.24 ± 0.12	---------
II	EEAN 200mg	0.85 ± 0.09	1.18 ± 0.11	1.12 ± 0.07	0.98 ± 0.09*	0.87 ± 0.12*	29.84%
III	EEAN 400mg	0.79 ± 0.08	1.02 ± 0.07*	1.05 ± 0.08*	0.82 ± 0.12**	0.45 ± 0.04***	63.71%
IV	AEAN 200mg	0.81 ± 0.18	1.22 ± 0.08	1.24 ± 0.06	1.09 ± 0.06	0.94 ± 0.04*	24.19%
V	AEAN 400mg	0.80 ± 0.04	1.06 ± 0.06*	1.08 ± 0.08*	0.92 ± 0.11*	0.54 ± 0.09**	56.45%
VI	Standard	0.81 ± 0.15	1.02 ± 0.11*	0.97 ± 0.12**	0.71 ± 0.12**	0.34 ± 0.08***	72.58%

Each value represents the mean ± SEM, n=6. * p<0.05, ** p<0.01 and *** p<0.01. Groups II to VI are compared with group I.

Fig.1

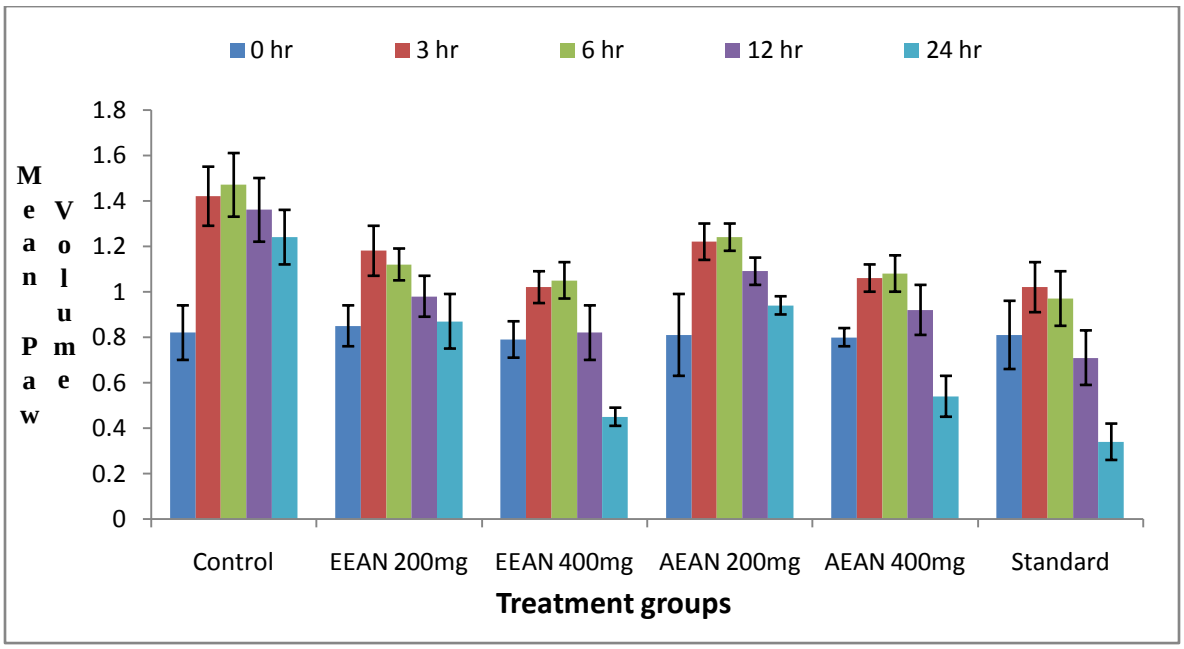
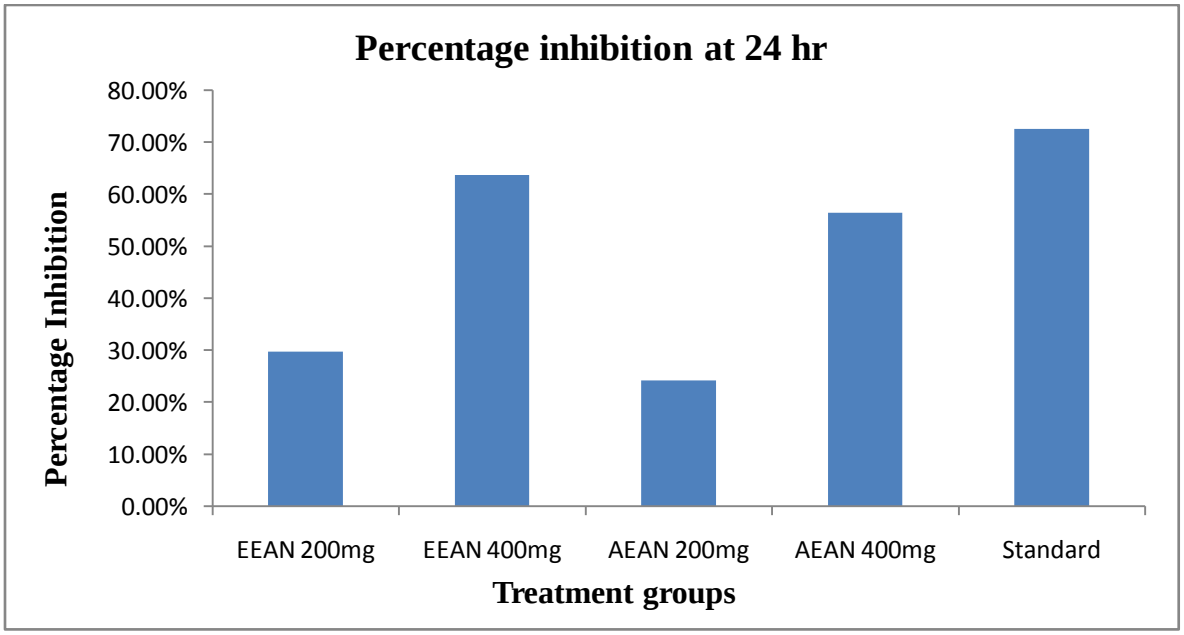


Fig.2



Summary and Conclusion

In the current target-rich and lead-poor scenario, ethnopharmacology and drug discovery using natural products remain important issues as many modern drugs have their origin in ethnopharmacology [21]. Presently, there is a positive trend in favor of traditional and integrative health sciences in both research and practice. The common approaches to drug discovery includes the use of chemical biology, serendipity, chemical synthesis, combinatorial chemistry and genomics along with innovative approaches involving ethnopharmacology, reverse pharmacology, holistic, system biology and personalized medicine.

The mainstream in pharmaceutical research is moving away from single molecule or single target approach to combinations and multiple target approaches. "Safety" remains the most important starting point and the efficacy becomes a matter of validation in this approach. Traditional knowledge, Modern Medicine and Modern science with systems orientation constitute a golden triangle that will converge to form an innovative discovery engine for newer, safer, economical and effective therapies. Drugs developed between 1981 and 2002 showed that products of natural-derived drugs comprised 28% of all new chemical entities launched into market and 24% of these new chemical entities were natural mimic compounds constituting 52% of all new chemical entities, advises that natural products are imperative sources for new drugs and are also good lead compounds suitable for further modification during drug development [22,23].

Inhibition of Carrageenan inflammation in rats is one of the most suitable test procedures to screen anti-inflammatory agents as reported by many investigators [24]. The sub plantar injection of carrageenan developed edema of high intensity and persisted for 3 hr after injection. The development of carrageenan induced edema is bi-phasic where release of histamine, serotonin and kinins are seen in first phase whereas, release of prostaglandins is observed in the second phase [25,26]. The inhibitory action of the tested extracts on carrageenan induced paw edema in rats may be mediated through either of the mediators alone or in combination. Results suggest that ethanolic extract of *Acacia Nilotica* at its higher doses showed more significant percentage inhibition of paw edema, which is closer to the inhibition by a standard drug. Comparison of results of ethanolic extract of Acacia Nilotica with an aqueous extract of Acacia Nilotica suggests that the ethanolic extract possess more significant anti-inflammatory activity than an aqueous extract.

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