

Evaluation of Anti-inflammatory Activity of Seeds of *Melia azedarach* (Linn.) in Albino Wistar Rats

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ABSTRACT

Objective: The objective of present investigation is to evaluate the anti-inflammatory activity of seeds of *Melia azedarach* (Linn.) in albino wistar rats.

Materials and Methods: A descriptive study was undertaken to perform the qualitative analysis and pharmacological screening. The seeds were extract with hexane by soxhalation method. For evaluating the anti-inflammatory activity, carrageenan induced paw oedema and formalin induced inflammation test were used with the hexane extract of seeds of *Melia azedarach* Linn. (Meliaceae) in wistar rats.

Results: The percent reduction in carrageenan induced paw edema was 52.77 and 91.66% at doses of 100 mg/kg and 200 mg/kg body weight of the extract respectively. Where as in formalin induced paw edema it was found to reduce the inflammation 15.21 % and 20.86% at doses of 100 mg/kg and 200 mg/kg. respectively.

Conclusion: From the study it is clear that Bakayan seed extract significant anti inflammatory activity in rodents which is comparable with that of reference drug Indomithacin. The anti-inflammatory activity is probably due to the presence of palmitic acid and linoleic acid.

KEYWORDS: Carrageenan, Formalin, Hexane extract.

INTRODUCTION

Inflammation is a local response of living mammalian tissues to the injury. It is a body defense reaction in order to eliminate or limit the spread of injurious agents. There are various components to an inflammatory reaction that can contribute to the associated symptoms and tissue injury. Oedema formation, leukocyte infiltration and granuloma formation represent such components of inflammation^[1]. Oedema formation in the paw is the result of a synergism between various inflammatory mediators that increase vascular permeability and/or the mediators that increase blood flow^[2]. Several experimental models of paw oedema have been described. Carrageenan-induced paw oedema is widely used for determining the acute phase of inflammation. Histamine, 5-hydroxytryptamine and bradykinin are the first detectable mediators in the early phase of carrageenan-induced inflammation^[3] whereas prostaglandins are detectable in the late phase of inflammation^[4]. Drugs which are in use presently for the management of pain and inflammatory conditions are either narcotics e.g. opioids or non-narcotics e.g. salicylates and corticosteroids e.g. hydrocortisone. All of these drugs possess well known side and toxic effects. Moreover, synthetic drugs are very expensive to develop and whose cost of development ranges from 0.5 to 5 million dollars. On the contrary many medicines of plant origin had been used since long time without any adverse effects. Exploring the healing power of plants is an ancient concept. For centuries people have been trying to alleviate and treat disease with different plant extracts and formulations^[5]. It is therefore essential that efforts should be made to introduce new medicinal plants to develop cheaper drugs. Plants represent still a large untapped source of structurally novel compounds that might serve as lead for the development of novel drugs^[6]. Screening of the plants for their biological activity is done on the basis of either their chemotaxonomic investigation or ethnobotanical knowledge for a particular disease. Identification of a particular compound against a specific disease is a challenging long process. Importance of the plant lies in their biologically active principles. There are two types of plant chemicals, primary metabolites such as sugars, proteins, amino acids, chlorophylls etc. The other category of chemicals is called secondary metabolites, which includes alkaloids, terpenoids, saponins and phenolic compounds. These chemicals exert a significant physiological effect on the mammalian system^[7].

Melia azedarach Linn. (Meliaceae) also known as Bakayan is a small attractive deciduous tree often grown for shade or ornament on roadsides, parks and other open situations. The seeds are traditionally used for antioxidant, immunomodulatory, antiulcer and antitumor property. The phytoconstituents present in the seeds, are likely to be beneficial in anti-inflammatory process. Therefore, it was prepared to investigate the anti-inflammatory potential of *Melia azedarach* Linn [8].

Melia azedarach L., a member of the Meliaceae family commonly named “paraíso,” is a tree that grows easily in Argentina, where it is widespread in both rural and urban areas. *M. azedarach* L. also grows in the southern United States and is known as the Chinaberry tree, Indian lilac, or white cedar. It is used for medicinal [9], Ornamental and timber purposes. The effectiveness of extracts from fruits and leaves of *M azedarach* L. has been previously demonstrated against insects [10]. Unfortunately, *M azedarach* L. fruits are popularly believed to be toxic, but toxicity assays of the fruit extract carried out on mammals have shown no adverse effects either orally administered to rat [11] or in dermal and ocular irritation tests. For these reasons, we decided to study the effect of *M azedarach* L. fruit extract and oil on lice and nits. The fruits were chosen because they are a renewable resource from the tree with high yields of extract.

MATERIALS AND METHODS

Plant materials

The seeds of *Melia azedarach* Linn. were collected from Aushatang Ayurvedic College, Lokmanya Nagar and Saifee Nagar Indore. Authentication of plant on basis of pharmacognostic study and organoleptic characteristics was done by **Dr. P.G. Diwakar** Joint Director Botanical survey of India, Koregaon Park, Pune. A voucher specimen number (BSI/WRC/Tech./2010/537) has deposited in museum of Dept. of Botany, Botanical survey of India.

Preparation of the seed extracts

The seeds of *Melia azedarach* Linn. were collected from Aushatang Ayurvedic College, Lokmanya Nagar and Saifee Nagar Indore. The seeds were then pulverized to powder using the traditional wooden mortar and pestle. Oil from 500g of each seed powder was extracted in 300 ml of hexane by Soxhlet apparatus at room temperature. The solvent was removed by rotary

evaporation at temperature below 45 °C. The hexane-extracted oils *Melia azedarach* were tested for repellency against the sand flies both in the laboratory and the field ^[12].

Preliminary phytochemical screening of extract

Preliminary phytochemical analysis was carried out to check and identify the active constituents of the hexane extract of *Melia azedarach* seeds such as saponins, flavonoids, terpenoids, amino acids, proteins, alkaloids, and carbohydrates by using foam formation test, Dragendroff and Mayer test, lead acetate test, Millons test, Biuret test and Fehling's test, respectively.

Acute toxicity study

The acute oral toxicity study was carried out as per the 425 guideline set by Organization for Economic Co-operation and Developments (OECD). The hexane extract was administered at the dose level of 2000 mg/kg. One tenth of the median lethal dose (LD₅₀) was taken as an effective dose. The LD₅₀ was greater than 2000 mg/kg for the hexane extract of the seed.

Experimental animals

Albino Wistar rats (150-200 g) of either sex born and reared in the animal house of College of Pharmacy, IPS Academy, Indore, M.P. from a stock originally purchased from Sudhakar Rao Naik Institute of Pharmacy, Pusad, M.H. were used for the study. Animals were placed randomly in polypropylene cages (five/cage) with husk as bedding. Standard laboratory condition of temperature 25± 2 °C, relative humidity 55± 5% and 12h/12h light/dark cycle were maintained throughout the experiment. Animals were kept for 1 week to acclimatize to laboratory conditions before starting the experiment; they were given free access to water and standard rat feed during experimentation.

Experimental Methods

Carrageenan induced paw edema

Four groups of 5 rats each were allotted to different treatments. Group I (control) was treated with distilled water (10 ml/kg, p.o.), used as vehicle. Group II was treated orally with 10 mg/kg of Indomethacin used as reference. Group III and IV were treated orally with *Melia azedarach*

Linn. seed extract 100 & 200 mg/kg. One hour after, edema was induced by injection of carrageenan (0.1 ml, 1% w/v in saline) into the subplantar tissue of the right hind paw. The linear paw circumference was measured using the vernier caliper method. Measurements were made immediately before injection of the phlogistic agent and at 30 min, 3 h, 6 h, 24 h, 48 h and 72 h until the peak of the effect was observed ^[13] .

Formalin induced paw edema

Male Wistar rats (150-190 g) were divided into four groups of five animals each inflammation was produced by subaponeurotic injection of 0.1 ml of 2% formalin in normal saline solution in the right foot, on the first and third day of the test. The groups were made as described in previous model. However, the treatment was give for 10 days. The rat paw circumference was measured daily for 10 days by vernier caliper method. The percent inhibition of the mean increase in the paw edema of each group was calculated on the tenth day and compared with the control ^[14] .

Statistical analysis

All values are expressed as mean±SD. The values obtained for the above parameters in case of the extract were compared with standard drug and control group by using repeated measure one-way Analysis of Variance (ANOVA) followed by Tukey's test. $P < 0.05$ was considered as significant. Graph pad prism (version 5.01) software was used for statistical analysis.

RESULTS

Preliminary phytochemical screening of extract

Preliminary phytochemical screening of seed extract revealed presence of alkaloids, flavonoids, carbohydrates, proteins and amino acids, phenolic compounds and steroids.

Acute Toxicity Studies

No mortality was observed after treatment with the highest tested dose (2000 mg/kg *p.o.*) of the hexane extract. The extract was found to be safe up to the dose of 2 g/kg *p.o.*

Anti-inflammatory activity

Carrageenan induced paw edema

The extract at the test doses 100 and 200 mg/kg body weight reduced the oedema induced by carrageenan by 52.77 and 91.66% respectively at 72 h, whereas the standard drug showed 97.22% of inhibition as compared to the control group which is represented in table I.

Table- 1 Effect of hexane extract of *Melia azedarach* on carrageenan induced paw edema in rats

Groups	Paw edema (mm)					
	0.5 h	3 h	6 h	24 h	48 h	72 h
Control	0.22±0.04	0.39±0.17	0.46±0.17	0.58±0.29	0.38±0.16	0.36±0.18
Indomethacin	0.14±0.02**	0.08±0.03**	0.06±0.02***	0.04±0.01**	0.03±0.01***	0.01±0***
HEMA 100 mg/kg	0.19±0.01	0.28±0.02	0.22±0.01**	0.19±0.01*	0.17±0.01*	0.17±0.01*
HEMA 200 mg/kg	0.15±0.02*	0.11±0.02**	0.08±0.02***	0.06±0.01*	0.04±0.01***	0.03±0*

Values are expressed as mean ± S.D. (n=5). *p < 0.05, **p < 0.01, ***p < 0.001 when compared with vehicle-control by one way analysis followed by tukey's test.

Formalin induced paw edema

Hexane extract of seeds of *Melia azedarach* also effective in formalin induced paw edema. Normal saline solution was used as vehicle in control and indomethacin was used as standard drug. Hexane extract of seeds of *Melia azedarach* dose dependently reduce inflammation in

each animal and statistical significance was observed at doses of 100 and 200 mg/kg respectively which is represented in table II.

Table- 2 Effect of hexane extract of *Melia azedarach* (Linn.) on formalin induced paw edema in rat measure on 10th days

Groups	Edema (mm)	Inhibition (%)
Control	5.16±.26	-
Indomethacin 10 mg/kg	3.73±.1***	27.64%
HEMA 100 mg/kg	4.38±.21**	15.21%
HEMA 200 mg/kg	4.08±.04***	20.86%

Values are expressed as mean ± S.D. (n=5). *p < 0.05, **p <0.01, ***p <0.001 when compared with vehicle-control by one way analysis followed by tukey's test.

DISCUSSION

Inflammation is defined as the local response of living mammalian tissue to injury due to any agent. It is a body defense reaction in order to eliminate or limit the spread of injurious agent as well as to remove the consequent necrosed cells and tissue.

Even though a range of drugs are available for the treatment of inflammation many of these donot fulfill all the requirements and have side effects. Recently, there has been much intrest in naturals medicine derived from the traditional knowledge of plant pharmacological property. Large number of medicinal plant has been shown to possess anti-inflammatory activity. Our results revealed that administration of hexane extract inhibited the oedema starting from the first hour and during all phases of inflammation, which is probably inhibition of different aspects and chemical mediators of inflammation.

In spite of tremendous development in the field of synthetic drugs during recent era, they are found to have some or other side effects, whereas plants still hold their own unique place, by the way of having no side effects. Therefore, a systematic approach should be made to find out the efficacy of plants against inflammation so as to exploit them as herbal anti inflammatory agents. The enzyme, phospholipase A2, is known to be responsible for the formation of mediators of inflammation such as prostaglandins and leukotrienes which by attracting polymerphonuclear

leucocytes to the site of inflammation would lead to tissue damage probably by the release of free radicals. Phospholipase A2 converts phospholipids in the cell membrane into arachidonic acid, which is highly reactive and is rapidly metabolized by cyclooxygenase (prostaglandin synthesis) to prostaglandins, which are major components that induce pain and inflammation^[15]. It is well known that carrageenan induced paw edema is characterized by biphasic event with involvement of different inflammatory mediators. In the first phase (during the first 2 h after carrageenan injection), chemical mediators such as histamine and serotonin play role, while in second phase (3 – 4 h after carrageenan injection) kinin and prostaglandins are involved^[16]. *Melia azedarach* L. (*Meliaceae*), also known as Chinaberry or Persian lilac tree, is a deciduous tree that is native to India and has long been recognized for its medicinal and insecticidal properties^[17].

CONCLUSION

Preliminary phytochemical screening of seed extract revealed presence of alkaloids, flavonoids, carbohydrates, proteins and amino acids, phenolic compounds and steroids. Anti-inflammatory activity was evaluated by carrageenan induced paw edema and formalin induced paw edema in rats. The percent reduction in inflammation was 52.77 and 91.66% at doses of 100 mg/kg and 200 mg/kg body weight of the extract respectively. The extract was also found to reduce the inflammation 15.21 % and 20.86% at doses of 100 mg/kg and 200 mg/kg.

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