Effect of Various Extracts of *Tectona grandis* Linn. Bark on Bronchitis

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Summary

Bronchial asthma is a chronic inflammatory disease, characterized by both bronchoconstriction and airway inflammation which leads to bronchial hyper-responsiveness to various stimuli, in which many cell types play a role, more important being mast cells, eosinophils and T-lymphocytes. Various extracts of *Tectona grandis* Linn. bark were screened for antiasthmatic activity by using different *in-vivo* animal models like mast cell degranulation and capillary permeability. The results of these studies indicated that ethyl acetate extract of *Tectona grandis* Linn. bark showed significant (p< 0.001) antiasthmatic activity. The anti-asthmatic activity of ethyl acetate extract can be attributed to mast cell stabilizing and adaptogenic activity suggestive of its potential in management of asthma.

Keywords: *Tectona grandis*, Mast cell degranulation, Capillary Permeability.

Introduction

Bronchial asthma is a complex disease of lung characterized by reversible airways obstruction, airway inflammation, excessive mucus production and airway hyper responsiveness. Due to rapid industrialization and urbanization, its prevalence is predicted to increase more rapidly in the coming years. Although limited data is available on the asthma prevalence in India, according to the “Global Burden of Asthma Report”, the increase is likely to be dramatic, particularly in India. A wide variation ranging from 4-19% is reported in the prevalence of asthma in school-going children from different parts of India. The prevalence of current-wheezing in children in Delhi is 16.7% and the cumulative prevalence is 20.8%. Another study conducted in Bangalore has reported the prevalence as high as 29.5%. The pathological condition results from a complex interaction between genetic and environmental factors¹⁴. For managing asthma attacks, symptomatic relief is the foremost requirement. In India various traditional systems mentioned numerous herbs for therapeutic use in asthma. *Tectona grandis* Linn. (Verbenaceae) is one of the important plants mentioned in Ayurveda for asthma.
It is a large deciduous tree, distributed all over Indian. It is commonly known as sagwan (Hindi), saka (Sanskrit) and teak tree (English). Various chemical constituents are present in this plant like steroids, glycosides, flavonoids, tannins and terpenoids etc. Tectoionols A & B (apocarotenoids), naphthaquinone-anthraquinone glycoside, tectol, tectograndiol, lapachol, steroidal moieties and tannin are major components reported which might be responsible for various medicinal use of herb. The bark is astringent, acrid, sweet and useful in the treatment of asthma. Flowers are acrid, bitter and useful in the treatment of bronchitis, biliousness and urinary discharges. The wood is acrid, sedative, anthelmintic, expectorant and useful in the treatment of gravid uterus, piles, leucoderma, dysentery, headache and burning pain over liver region.

Although, there is no scientific proof of the efficacy of plant extracts for antiasthmatic activity, the aim of this study was to evaluate antiasthmatic effect of Tectona grandis.

Materials and Methods

1. Plant Material Collection and Extraction
Bark of T. grandis were collected from Ahmednagar district of Maharashtra in Sep 2008 and authenticated by Mr. P.G. Diwakar, Botanical Survey of India, Pune, India. The voucher specimen was deposited bearing no. DGTEG1. The collected material was cleaned and air dried at 35-40°C and pulverized in electric grinder. The powder was subjected to successive solvent extraction in soxhlet extractor using petroleum ether (60-80°C), ethyl acetate and ethanol as solvent and the marc left was macerated with water. All the extracts were vacuum dried to yield petroleum ether extract (PEE) (2.81% w/w), ethyl acetate extract (EAE) (6.48% w/w), ethanol extract (ETE) (5.19% w/w) and aqueous extract (AQE) (8.86% w/w), respectively. The extracts were stored in a refrigerator for further use.

2. Chemical and Reagents Used
All the reagents used were of analytical grade obtained from Sigma Chemical Co., St. Louis, USA and Fine Chemicals Ltd., Mumbai, India.

3. Animals
Swiss albino mice of either sex weighing 20-25g were housed under standard laboratory conditions. The animals were fed with standard pellet diet (Amrut laboratory animal feed, Sangali, India) and had free access to water. All the experiments were approved and conducted as per the guidelines of Institutional animal ethical committee (448/01/C/CPCSEA).

4. Acute Toxicity Studies
The acute toxicity study for all extracts of T. grandis Linn. bark were performed using Swiss albino mice as per OECD guideline. All the extract were administered orally with increasing dose and was found safe up to dose of 2000 mg/kg for all extract.

5. Anti-asthmatic Activity Evaluation
A. Mast Cell Degranulation
Mice were divided into six groups (n=6). Control group (group I) received vehicle (5% Tween 80 in normal saline) and other groups PEE, EAE, ETE and AQE received single dose (as 100 mg/kg, p.o., each) (Group III, Group IV, Group V and Group VI respectively). Disodium chromoglycate (200µg/kg, i.p.) (Group II) was used as positive control. A three day drug treatment schedule was followed. On the fourth day, each animal was injected with 4 ml/kg, 0.9% saline solution, in to peritoneal cavity.
By gentle massage, peritoneal fluid was collected after five min. and transferred into siliconised test tube containing 7-10 ml RPMI 1640 buffer medium (pH 7.2-7.4). This solution was then centrifuged at 400-500 rpm. Pellets of mast cell were washed with same buffer medium twice by centrifugation, discarding supernatant. These cells were challenged with clonidine (50µg), incubated at 37°C in a water bath for 10 min followed by staining with 1% toludine blue and observed under microscope (45 X). Total 100 cells were counted from different visual area. Percent protections against degranulations were calculated.9,10.

B. Capillary Permeability

Mice were divided into six groups (n=6). The mice in the control group were sensitized with bovine albumin and Freund’s adjuvant 0.05 ml given i.p. Three weeks later the animals were challenged with the same dose of bovine albumin. At the same time Evan’s blue injected i.v. in a dose of 200 mg/kg. The mice were sacrificed 30 min. later 5 ml of saline was injected i.p. and the abdominal wall was gently massaged for a minute. The abdomen was then incised and peritoneal fluid was collected and filtered after passage through glass wool. It was centrifuged at 3000 rpm for 15 min. The group II received Standard drug (Disodium chromoglycate). The group III, IV, V and VI received petroleum ether, ethyl acetate, ethanol and aqueous extracts of Tectona grandis respectively in a dose of 100 mg/kg, i.p. 24 h and 2 h before the challenge. The transmittance of the dye depends on capillary permeability was determined by measuring the optical density on a spectrophotometer11,12.

Results and Discussion

The present study deals with screening of antiasthmatic activity of various extracts of bark of Tectona grandis Linn. Mast cells are widely distributed in the connective tissue with a preferential localization adjacent to small blood vessels. The allergic process has an important inflammatory component in which mast cell activation and degranulation are the first phenomena observed. During this process, mast cells release several inflammatory mediators like histamine, 5-HT, PAF and a variety of cytokines which can elicit many events associated with allergic inflammation such as edema formation. The mast cells contain basophil granules literally loaded with active substances which causes vascular and other tissue reactions similar to those characteristic of inflammatory processes which contributes to the late asthmatic reaction of congestion and mucus hypersecretion. When these cells arrive, they degranulate and perpetuate underlying airway inflammation13,14.

Clonidine, α2 adrenoreceptor agonist, releases histamine from mast cells identified histamine containing mast cells, which is inhibited by histamine H1 receptor antagonists but not by H2 receptor antagonist10,15.

In the present study, the groups of animals pretreated with ethyl acetate extract (100mg/kg p.o.) of Tectona grandis Linn. resulted in significant reduction in degranulation of mast cells and offered significant protection when challenged with clonidine indicating mast cell stabilizing activity (Graph 1).
Graph 1: Effect of various extracts of bark of *Tectona grandis* on Clonidine induced Mast cell degranulations

All the values are expressed as mean ± SEM of a sample size of n=6; ** P<0.001, compare with control treated group, One way ANNOVA followed by Dunnett’s test.

In present study, the ethyl acetate extract of *T. grandis* significantly decreased the capillary permeability in mice at the dose of 100 mg/kg p.o. as is evident from its effect on optical density of the dye. The effect of ethyl acetate extract on capillary permeability shows that the same mechanism may be involved as that of anti-allergic action (Graph 2).

**Graph 2. Effect of Various Extracts of *Tectona grandis* on Capillary Permeability**

All the values are expressed as mean ± SEM of a sample size of n=6; ** P<0.001, * P<0.05 Control compare with standard and test groups, one way ANNOVA followed by Dunnett’s test.
Conclusion

Thus, it can be concluded from the results obtained in the present investigation that ethyl acetate extract of *Tectona grandis* Linn. possess significant (P<0.001) antiasthmatic activity compare to all other extract at a dose of 100mg/kg body weight. The anti-asthmatic activity of ethyl acetate extract of bark of *Tectona grandis* Linn. can be attributed to mast cell stabilizing and adaptogenic activity, suggestive of its potential in treatment and prophylaxis of asthma.Future prospective involves identification of lead molecule responsible for anti asthmatic activity of *T. grandis* Linn. bark extract.

References

8. OECD guideline