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Original Research Article

## Evaluation of anti-asthmatic and anti-cholinergic activity of ethanolic extract of *Tectona grandis* Linn. bark

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### ABSTRACT

**Background:** Asthma is a chronic disease that affects approximately 300 million people worldwide. *Tectona grandis* Linn. bark, also known as Teak (English), is traditionally used to treat asthma. However, the scientific data on anti-asthmatic and anti-cholinergic of this plant has got little attention. An attempt has been based on ethanolic extract of bark of *Tectona grandis* Linn. shown a tremendous effect on asthma when comparative study was done with normal and treated group.

**Methods:** The anti-asthmatic activity of a 95% ethanol and 5% distilled water extract of dried and fresh *Tectona grandis* Linn. bark, was evaluated against histamine and acetylcholine-induced preconvulsive dyspnea (PCD) in guinea pigs fasted for 24 h were exposed to an atomized fine mist of 2% histamine dihydrochloride and acetylcholine aerosol (dissolved in normal saline) using nebulizer at a pressure of 300 mmHg in the histamine chamber (24×14×24 cm, made of perplex glass). They were divided in groups Mepyramin (8 mg/kg) intraperitoneally, atropine aerosol and *Tectona grandis* bark formulation (2.5, 5, 10 gm/kg) were administered orally 30 min prior to exposure. Animals, which did not develop typical asthma within 6 minutes, were taken as protected.

**Results:** Ethanolic extract of *Tectona grandis* Linn. bark at 5 and 10 gm/kg significantly reduce bronchoconstriction as compared to control group along with significant mast cell stabilization activity.

**Conclusions:** In conclusion, the present study shows that the ethanolic bark extract of *Tectona grandis* Linn. has potential antiasthmatic and anticholinergic action in histamine and acetylcholine broncocontraction in guinea pigs.

**Keywords:** Anti-asthmatic, Anti-cholinergic, *Tectona grandis* Linn. bark extract

### INTRODUCTION

Asthma is the inflammation of the airways which results in increased contractability of the bronchial smooth muscles.<sup>1,2</sup> This narrowing of the airway which result wheezing. In asthma there is liberation of endogenous and intrinsic mediators like histamine, leukotrienes (LTs), bradykinin, prostaglandins (PGs), nitric oxide, platelet activating factors (PAF), chemokines and endothelin from mast cells during the allergic reactions which result inflammation of the air passages in the lungs. Worldwide

incidence of bronchial asthma is 7-10%.<sup>3</sup> Adrenergic drug is main modality of treatment.<sup>4</sup> Triggered factors for inflammation of bronchus are various infections, dust, cold air, exercise, emotion, perfumes, chemical, environmental tobacco smoke and histamine.<sup>5</sup> symptomatic relief is main goal of the treatment. In, India Ayurveda mentioned numerous herbs for therapeutic use in asthma. *Tectona grandis* Linn. (Verbenaceae) is one of them important plants. It is a large deciduous tree, distributed all over India. It is commonly known as Sagwan (Hindi), Saka (Sanskrit) and Teak tree (English).<sup>6</sup> Its bark is acrid and used in the treatment of bronchitis,

hyperacidity, constipation, diabetes, anthelmintic, leprosy and skin diseases. Methanol extract has shown antibacterial activity.<sup>6-8</sup> According to literature Tectoionols A and B (apocarotenoids), naphthaquinone neanthraquinone glycoside, tectol, tectograndiol, lapachol, steroidal moieties and tannin are major components present in *Tectona grandis* which might be responsible for various medicinal use of herb. Although, there is no scientific proof of the efficacy of plant extracts for antiasthmatic activity with the use of histamine with guinea pig and also anticholinergic property also not known.

The aim of present study was to evaluate antiasthmatic effect of *Tectona grandis* antihistaminics with exploration of anticholinergic property.

## METHODS

### Collection of plant materials

The bark of *Tectona grandis* Linn. were collected from the outfield of Sewagram District, Wardha Maharashtra February-March 2007. The collected plant bark extract were deposited and shade dried in the Department of Pharmacology, Mahatma Gandhi Institute of Medical Sciences Sewagram Wardha.

### Drugs and chemicals

Histamine dihydrochloride, acetylcholine chloride, atropine sulfate, mepyramine melete, and toluidine blue were purchased from CAL Laboratories, supplied by Kasliwal Brothers, Indore. Histamine solution was freshly prepared in normal saline (NaCl, 8.5 gm/l). All the other chemicals were of analytical grade.

### Extraction

Bark of *Tectona grandis* were washed with distilled water to remove dirt and soil, and shade dried. The dried materials were powdered by mixture and passed through a 10-mesh sieve.<sup>6,9</sup> Then 500 gm of plant bark powder was macerated with 95% of ethanol and 5% distilled water, after 24 hours of maceration, extraction done with 95% ethanol with 5% distilled water in percolator after percolation for 48 hours. Extract was dried in fan so that solvents evaporated and get crude ethanol extract.

### Experimental animals

Antihistaminic and anticholinergic studies were conducted on guinea pigs (350-500 gm) of either sex. For mast cell stabilization paradigm, adult Wistar albino rats weighing 140-160 gm of either sex were used. All the experimental animals were fed on commercial pellet diet (Poshak Ahar Pvt. Ltd. India). They were group housed under standard conditions of temperature (22±20°C), relative humidity (60±5%) and 12:12 light/dark cycle was maintain. They were divided in groups of six animals

each. Group 1 was the saline fed group served as control Group 2 was treated with a standard drug. Group 3 was bark extract 2.5 gm/kg and Group 4 was bark extract 5 gm/kg. Group 5 was 10 gm/kg of bark extract. Before experimentation, the animals were kept on fast for 24 hours but water was given *ab de libitum*. During experiments, animals were also observed for any alteration in their general behavior. All the experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC).

### Preparation of plant extract

*Tectona grandis* Linn. bark extract formulation was suspended in 1% SCMC in distilled water and administered orally. The control animals were given an equivalent volume of SCMC vehicle. The standard group received Mepyramine spray and atropine sulphate I.P.<sup>10</sup>

### Pharmacological screening (antiasthmatic paradigms)

#### Histamine-induced bronchospasm in guinea pigs

The guinea pigs fasted for 24 hours were exposed to a 2% histamine dihydrochloride aerosol (dissolved in normal saline) using nebulizer with pressure of 300 mmHg in the histamine chamber (24×14×24 cm, made of perplex glass). Guinea pigs which were exposed to histamine aerosol showed progressive signs of difficulty in breathing than followed by convulsions, asphyxia and death. The time required for development of convulsion is called pre-convulsion time (PCD).<sup>11-13</sup> Preconvulsion time can be judge accurately by experience and observation. When animal reached to preconvulsion time, animal were removed from chamber and placed in fresh air to recover. In this experiment the criterion used was time for onset of dyspnea and percent of protection from convulsant was calculated by the percentage protection offered by the standard and test drug against asphyxia was calculated, using the formula: Percentage protection= [(T2-T1)/T2]×100. Three days prior to the experiment screening was done and the animal who develop asthma within 3 minute with histamine were selected for the experiment, along with it we also given habituation practice to retain them in histamine chamber. Animals were divided in groups of six animals each. Mepyramine (8 mg/kg) administrated intraperitoneally and *Tectona grandis* Linn. bark extract formulation (2.5, 5, 10 gm/kg) were administered orally 30 minutes prior to exposure. Animals, which did not develop typical asthma within 6 minutes were taken as protected.

#### Acetylcholine- induced bronchospasm in guinea pigs

Procedure was similar as describe above to the histamine aerosol study except that animals were exposed to aerosol of 0.5% acetylcholine in another set of animals (n=6).<sup>14-16</sup> Atropine sulphate (2 mg/kg) was used as a standard drug. Degranulation of mast cell carried out by compound 48/80 it was the same method described by Kaley et al

with little modification.<sup>2</sup> Male albino rats were sacrificed by ketamine anesthesia. The albino rats were immediately injected with 15 ml of prewarmed (37°C) buffered salt solution (NaCl 137 mM; KCl 2.7 mM; MgCl 2; 1 mM; CaCl 20.5 mM; NaH<sub>2</sub>PO 40.4 mM; Glucose 5.6 mM; HEPES 10 mM) into the peritoneal cavity, and massage was given gently for 90 seconds. Peritoneum was exposed by giving midline incision. Aspiration of pale fluid by using blunted plastic Pasteur pipette and collected in plastic centrifuge. It was centrifuged at 1000 rpm for 5 minutes, supernatant discarded to reveal a pale cell pellet. The cell pellets were re-suspended in fresh buffer and re-centrifuged. The centrifuged fluid was incubated with the test compounds or disodium cromoglycate, before challenge with compound 48/80. The incubated fluid spread over glass slides and the mast cells were stained with 1% toluidine blue and counterstained with 0.1% light green. After drying slides in air. Mast cells counted from randomly selected high power objective fields (450X). The effect of *Tectona grandis* on mast cells was studied by incubating the mast cells for 10 minutes with the above formulation in a concentration of 2.5, 5 and 10 gm/ml along with standard drug prednisone (10 mg/kg). After incubation it was exposed to degranulator, compound 48:80 (10 µg/ml) for 10 minutes, again mast cell were spread over glass slide. The percent degranulation of the mast cells was calculated. Compared degranulation with control and standard drug with *Tectona grandis* bark extract.

All data of the experiment was statistically analyzed by Dunnett's t-test and two way ANOVA.

#### Acute toxicity test (determination of LD50)<sup>13,17</sup>

For the acute toxicity (LD50) done by method describe by Kabore Adama. Albino mice (20-25 gm) of either sex were used. Doses of *Tectona grandis* Linn. bark extract 1, 2, 4, 8, 16gm/kg were administered orally, one dose for each group. The treated animals were monitored for 24 hours for mortality and general behaviour. On above dose there was no occurrence of any adverse effects in animals. So, from the result of the above step, three different doses of 2.5, 5 and 10 gm/kg were chosen and administered orally respectively to three groups of one mouse per group. The treated animals were again monitored for 24 hours.

## RESULTS

The results of the present study revealed mast cell stabilization, antihistaminic and anti-cholinergic actions of *Tectona grandis* Linn. bark extract. In the mast cell stabilization paradigm, weight of granuloma was 220mg. while in the plant extract group it was 120, 96mg in dose of 5 and 10gm/kg which is significant (p<0.001). Compound 48/80 (10 µg/ml) produced about 76% degranulation of mast cells. Pretreatment with prednisolone (10 mg/kg) and the herbal formulation (5 and 10 gm/kg) significantly reduced (p<0.01)

degranulation of mast cells as compared to compound 48/80-treaed control group (Table 3) which give the protection of 54%, 89%, 97% protection. These results suggest that herbal formulation protects mast cells from compound 48/80-evoked degranulation.

**Table 1: Effect of *Tectona grandis* Linn. formulation on histamine-aerosol in guinea pigs.**

Treatment	Dose	Preconvulsion time (seconds)	Protection
Control	Saline	120.2±3	--
Mepyramine	8 mg/kg i.p.	560±3.4	78
<i>Tectona grandis</i> dose I	2.5 gm/kg orally	280±2.2	58*
<i>Tectona grandis</i> dose II	5 gm/kg orally	320±1.5	63*
<i>Tectona grandis</i> dose III	10 gm/kg orally	430±2.5	72**
<i>Tectona grandis</i> + mepyramine	10 gm/kg (orally) + 8 mg/kg (i.p.)	630 ±2.5	81**

\*p value<0.05; \*\*p value<0.001.

In the histamine aerosol study, the control animals showed convulsion during the first 3 minutes of the experiment was selected for experiment. Prior treatment of herbal formulation (2.5, 5, 10 gm/kg) protected the animals proconvulsant time was increase 280, 320, 430 seconds. In control group proconvulsant time was 120 second (Table 1). It shows that *Tectona grandis* bark extract has significant effect in bronchial asthma (p<0.01). The development of asphyxia produced by histamine aerosol confirming that it has antihistaminic activity. The *Tectona grandis* bark extract has highly significant effect when it is used with standard drug the proconvulsant time was 630 second. Histamine had major role in asthma.<sup>2</sup> The close resemblance of pulmonary responses to histamine challenge in both guinea pigs and humans, as well as the anaphylactic sensitization made this species the model of choice. In the present study, guinea pigs were used because of the extreme sensitivity of their airways to the primary mediators of bronchoconstriction, including histamine and leukotrienes, and their ability to be sensitized to foreign proteins.

Acetylcholine-aerosol provoked bronchoconstriction in all animals of control group proconvulsant time was 110 seconds, while it was significantly increases to 280, 320, 370 seconds in herbal formulation (2.5, 5 and 10 gm/kg) (p<0.01) from acetylcholine-induced bronchoconstriction. Atropine sulphate (a standard antimuscarinic drug, 2 mg/kg, i.p.) significantly prolonged pre-convulsion time which is 480 seconds (p<0.01) and protected animals from asphyxia (Table 2).

**Table 2: Effect of *Tectona grandis* Linn. extract on acetylcholine induced bronchospasm in guinea pigs.**

Treatment	Dose	Preconvulsion time (seconds)	Protection
Control	Saline	110 ± 3	--
Atropine sulphate	2 mg/kg (i.p.)	480 ± 3.4	77
<i>Tectona grandis</i> dose I	2.5 gm/kg orally	280± 2.2	61*
<i>Tectona grandis</i> dose II	5 gm/kg orally	320± 1.5	65*
<i>Tectona grandis</i> dose III	10 gm/kg orally	370± 2.5	70**
<i>Tectona grandis</i> + atropine sulphate	10 gm/kg orally+ 2 mg/kg i.p.	550 ± 2.5	80**

\*p value<0.05; \*\*p value<0.001.

But when standard drug used with plant bark extract, the preconvulsant time markedly increases, it was 550 seconds. This shows when plant extract combine with standard drug has synergistic action.

**Table 3: Effect of *Tectona grandis* Linn. extracts on mast cell.**

Treatment	Dose i.p.	Weight of granuloma (mg)	Protection
Control	Saline	220 ± 3	-
Prednisolone	10 mg/kg	90 ± 3.4	90
<i>Tectona grandis</i> dose I	2.5 gm/kg	160 ± 2.2	47
<i>Tectona grandis</i> dose II	5 gm/kg	120± 1.5	54*
<i>Tectona grandis</i> dose III	10 gm/kg	96 ± 2.5	89**
<i>Tectona grandis</i> + Prednisolone	10 gm/kg and 10 mg/kg	60 ± 2.5	97***

\*p value<0.05; \*\*p value<0.001; \*\*\*p value<0.0001.

## DISCUSSION

In bronchial asthma, there is injury to mast cells and basophiles by antigenic stimulation and causes release of histamine. Histamine which is liberated by mast cell destruction causing smooth muscle contraction, increased vascular permeability and mucus formation. Histamine provoke bronchoconstriction, it causes the bronchial hyperactivity. Mast cells with mediator is main factor for initiation of early phase of allergic reaction and also responsible for initiation of chronic allergic reaction.<sup>18</sup> For the study of agonist and antagonist on bronchus and

bronchial tree the contraction of tracheal chain in vitro is use. Guinea pig is very sensitive to histamine and it is ideal model for testing H1 antihistaminics drugs in histamine chamber. Guinea pig tracheal chain preparation is suitable for screening of the antihistaminic activity.<sup>18</sup>

In present study *Tectona grandis* Linn. bark extract also shows increase the preconvulsant time as compared to control in histamine induced bronchoconstriction. This shows that *Tectona grandis* has antihistaminics property.

In acetylcholine induced bronchoconstriction *Tectona grandis* also increase in preconvulsant time significantly. As the muscarinic receptors (M2) present on bronchus.<sup>19</sup> This shows that *Tectona grandis* has anticholinergic activity also.

Result study on *Tectona grandis* Linn. suggest that, there was appreciable decrease in severity of symptoms of asthma and also simultaneously improvement in lung function parameters. Along with it *Tectona grandis* Linn. has significant mast cell stabilizing activity.

## CONCLUSION

In conclusion, the results of present investigation suggest that, *Tectona grandis* Linn. bark extract formulation have significant bronchodilator activity against histamine and acetylcholine. However, further studies are suggested to establish molecular mechanism and also to isolate and characterize the active principles responsible for the action. Further study is ongoing to characterize the active principles of the extract which are responsible for antiasthmatic activity.

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