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# Bronchospasm potentiating effect of methanolic extract of *Ficus religiosa* fruits in guinea pigs

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ABSTRACT

*Ethnopharmacological relevance:* The sacred tree Peepal (*Ficus religiosa* family: Moraceae) has numerous therapeutic utility in folk medicine.
*Aim of the study:* It has been reported to be used in ethno medical treatment of asthma and also in epilepsy due to its high serotonin content, which has been implicated in pathophysiology of asthma, this led us to carry out the present study.
*Materials and methods:* The *in vivo* studies of histamine induced bronchospasm in guinea pigs and *in vitro* isolated guinea pig tracheal chain and ileum preparation.
*Results:* Pre-treatment of guinea pigs with ketotifen (1 mg/kg, p.o.) has significantly delayed the onset of histamine aerosol induced pre-convulsive dyspnea, compared with vehicle control ($281.8^a \pm 11.7$ vs. $112.2 \pm 9.8$). The administration of methanolic extract (125, 250 and 500 mg/kg, p.o.) did not produced any significant effect on latency to develop histamine induced pre-convulsive dyspnea. On the other hand, methanolic extract of the fruits at the doses employed (i.e., 0.5, 1 and 2 mg/ml) has significantly potentiate the $EC_{50}$ doses of both histamine and acetylcholine in isolated guinea pig tracheal chain and ileum preparation. In addition, HPLC analysis of the methanolic extract showed the presence of high amounts of serotonin (2.89%, w/w).
*Conclusions:* On the basis of data, it may be concluded that *Ficus religiosa* fruits have been found to be ineffective against histamine induced bronchospasm in guinea pigs. In addition, methanolic extract of the fruits have shown to potentiate the bronchoconstriction induced by both histamine and acetylcholine on guinea pig tracheal chain preparation.



## 1. Introduction

*Ficus religiosa* L. (Moraceae), popularly called peepal a sacred (bodhi) tree has got mythological, religious and medicinal importance in Indian culture for the treatment of asthma, cough and other respiratory disorders (Kala et al., 2006; Prasad et al., 2006; Ripu and Rainer, 2006). Ficus Linn being the largest genus of the family Moraceae comprises about 755 fig tree species worldwide (Van Noort et al., 2007). The potential therapeutic benefits of leaf juice and fruits of *Ficus religiosa* has been documented in folk medicine (Ripu and Rainer, 2006). The leaf juice has been used for the treatment of asthma, cough, sexual disorders, diarrhoea, haematuria, ear-ache and toothache, migraine, eye troubles, gastric problems and scabies; leaf decoction as an analgesic for toothache; stem bark in gonorrhea, bleeding, paralysis, diabetes, diarrhoea, bone fracture, as antiseptic, astringent and antidote (Ripu and Rainer, 2006) and the fruits have been used for the treatment of asthma, other respiratory disorders and scabies (Bhattarai, 1993).

The *Ficus religiosa* fruits claimed to have potential anticonvulsant activity (Vyawahare et al., 2007). Antiepileptic activity of methanolic extract of the fruits has been documented due to the high amount of serotonin content (Bliebtrau, 1968; Singh and Goel, 2009). On other hand, an increased level of free serotonin has shown to be positively correlated with severity of bronchial asthma and negatively regulated pulmonary function (Lechin et al., 1994, 1996). Thus, it has been suggested that an increasing concentration of free serotonin in plasma may be detrimental to pulmonary function, particularly in asthmatic patients (Cazzola et al., 1995; Cazzola and Matera, 2000). No substantial scientific evidence is available to support that fruits of *Ficus religiosa* may be indeed useful for their ethanomedicinal uses. Therefore, the present study is designed to investigate the effect of fruits using *in vivo* histamine induced bronchospasm in guinea pigs and *in vitro* guinea pig tracheal chain preparation.

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## 2. Materials and methods

### 2.1. Plant material

The fruits were collected from Chandigarh in the month of November–December and were authenticated by Prof. (Dr.) Promila Pathak, Associate Editor, The Orchid Society of India, Dept. of Botany, Punjab University, Chandigarh. The fruits were shade dried, coarsely powdered and stored in an airtight container. Specimen is preserved in herbarium vide Specimen No. 17362 in Dept. of Botany, Punjab University, Chandigarh.

### 2.2. Preparation of the extract

The coarsely powdered dried fruits (750 g) were first defatted with petroleum ether (40–60 °C) and then dried marc was extracted with 90% methanol in soxhlet apparatus for 12 h. The extract was concentrated and dried to constant weight (34.50 g) in vacuum evaporator. Dried marc was re-extracted three times with distilled water at room temperature for 24 h and the aqueous extract was dried to constant weight using rotary evaporator. Both extracts were stored in desiccators till further use.

### 2.3. Drugs and chemicals

Histamine and serotonin procured from Acrose, Belgium. Methanol and petroleum ether were of HPLC grade and all other standard chemicals used in this study were of analytical grade, purchased from Rankem Chemicals (Pvt.) Ltd. India.

### 2.4. Animals

The experimental protocol used in the present study was approved by the Institutional Animal Ethical Committee. Age matched adult guinea pigs (600–800 g) were acclimatized in animal house with normal cycles of day and night under standard conditions at an ambient temperature 25 ± 2 °C and 55–65% relative humidity.

### 2.5. In vivo studies on histamine induced bronchospasm (asphytic reaction) in guinea pigs

Guinea pigs of either sex were selected and randomly divided into six groups. The drugs were administered orally by suspending in 0.5% sodium carboxyl methyl cellulose in water. The single dose treatment was given one and half hour before challenged with 0.25% histamine hydrochloride aerosol. The time to onset of respiratory distress (pre-convulsion dyspnea) was noted (Sheth et al., 1972). The time to onset of pre-convulsion dyspnea during challenge with these agents was recorded; the guinea pigs with pre-convulsion dyspnea more than 120 s were considered insensitive and discarded. The following experimental protocol was employed: Group I: vehicle control (0.5% CMC, p.o.) treated; Group II: ketotifen (1 mg/kg, p.o.) treated; Group III: fruits powder of *Ficus religiosa* (1000 mg/kg, p.o) treated; Group IV: the methanol extract of the fruits (125 mg/kg, p.o.) treated; Group V: methanol extract of the fruits (250 mg/kg, p.o.) treated; Group VI: methanol extract of the fruits (500 mg/kg, p.o.) treated.

### 2.6. In vitro studies on isolated guinea pig tracheal preparation

Male guinea pigs (200–250 g) were starved overnight, with free access to water. Animals were sacrificed by exsanguinations and tracheae were immediately removed. The trachea was dissected and cut transversely into 2-mm segments between the segments of cartilage so as to give a number of rings of tracheal muscle. The tracheal rings were tied together with cotton thread to form a chain, which was placed in an organ bath containing Krebs bicarbonate buffer at 37 °C, continuously aerated with 95% $O_2$ and 5% $CO_2$ (Sheth et al., 1972). The tissue was stabilized for 45 min and then isotonic contractions were recorded by exposing to graded doses of histamine. Firstly, $EC_{50}$ and $E_{max}$ values of histamine in individual isolated preparations were established. Subsequently, contractile response of both $EC_{50}$ and $E_{max}$ dose of histamine using a separate tissue preparation was recorded in the presence of varied concentrations (0.5, 1 and 2 mg/ml) of methanol extract of fruits. The responses were expressed as the percentage of the maximum response induced by histamine.

### 2.7. In vitro studies on isolated guinea pig ileum preparation

The ileum was dissected and tied with cotton thread which was placed in organ bath containing Tyrode buffer kept at 37 °C. The tissue was stabilized for 45 min and then isotonic contractions were recorded by exposing to graded doses of histamine and acetylcholine to establish an $EC_{50}$ and $E_{max}$ values in individual preparations. Subsequently, contractile response of both $EC_{50}$ and $E_{max}$ doses of histamine and acetylcholine were again recorded in the presence of different doses (0.5, 1 and 2 mg/ml) of methanolic extract of the fruits. The responses were expressed as the percentage of the maximum response induced by histamine and acetylcholine, respectively.

### 2.8. Acute toxicity studies

*Ficus religiosa* is one of the oldest known human foods having a very high safety profile (Lansky et al., 2008). It has been reported that the intra methanolic extract of the fruits up to dose (1000 mg/kg, i.p.) did not produce any neurotoxic effects and mortality (Singh and Goel, 2009).

### 2.9. Phytochemical screening

The fruits and mathanolic extracts were subjected to phytochemical screening for the following classes of compounds such as alkaloids, steroids, carbohydrates, phenolic compounds, flavonoids, proteins and saponins (Harborne, 1998).

### 2.10. HPLC-UV detection of serotonin in methanolic extract of the fruits (Han et al., 2006)

The standard solution of serotonin was prepared by dissolving 10 mg of pure serotonin in 10 ml of methanol (1 mg/ml). Sample solution of the fruit's methanolic (1 mg/ml) extract was prepared by centrifugation at 4500 × *g* for 10 min and the supernatant was used for further investigations. Both standard and sample solutions were filtered through a 0.45 μm membrane filter. Prior to HPLC analysis, the optimized UV spectra of pure serotonin was determined at wavelength scanning between 200 and 400 nm using a standard UV system. The fruit extract solution was analyzed using HPLC system with a Photodiode detector. Separation was performed on a Thermohypersil C-18 column (150 mm × 4.6 mm × 3 μm) using 25 mM phosphate buffer (pH 2.5): acetonitrile at 95:5 (v/v) at a flow rate of 1 ml/min. The injection volume was 20 μl and the peaks were identified by comparison with the retention times of the standard solution.

### 2.11. Statistics

All values were expressed as mean ± SEM. The data were analyzed by one-way ANOVA followed by Turkey multiple range test. The $p < 0.05$ was considered to be statistically significant.