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Phytopharmacological evaluation and anti-asthmatic activity of *Ficus religiosa* leaves

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ABSTRACT

Objective: To discuss phytopharmacological potential and anti-asthmatic activity of *Ficus religiosa* (*F. religiosa*) (L.). **Methods:** Fresh leaves of *F. religiosa* were obtained from Vastrapur Lake, Ahmedabad, and dried to obtain powder. Histamine and acetylcholine were used to guinea pigs to establish bronchospasm model. In *in vivo* study, the aqueous extract of *F. religiosa* leaves (AEFR) at doses of 150 and 300 mg/kg was administered to guinea pigs, and the broncho-protective activity of AEFR was compared with aminophylline at 25 mg/kg. While in *in vitro* study, and 10 g/mL, 20 g/mL, 30 g/mL of AEFR was administered to guinea pigs, respectively, and mast cell stabilizing activity of AEFR was compared with ketotifen at 10 g/mL. **Results:** In the *in-vivo* model, pre-treatment with aminophylline (25 mg/kg, *ip.*) could significantly delay the onset of histamine induced pre-convulsive dyspnea, compared with vehicle control. Administration of AEFR (150 and 300 mg/kg, *ip.*) also produced significant effect on latency to develop histamine & acetylcholine induced pre-convulsive dyspnea. In the mast cell stabilizing model, AEFR at 10, 20 and 30 μ g/mL could significantly increase the number of intact cells. **Conclusions:** It can be concluded that AEFR is effective on histamine & acetylcholine induced bronchospasm in guinea pigs. In addition, AEFR can potentiate the number of intact cells in the mast cell stabilizing model.

1. Introduction

Ficus religiosa (*F. religiosa*)L. (Moraceae), popularly called peepal a sacred (bodhi) tree has got mythological, religious and medicinal importance in Indian culture. It can be used for the treatment of asthma, cough and other respiratory disorders[1–3]. *Ficus* Linn being the largest genus of the family Moraceae comprises about 755 fig tree species worldwide[4]. *F. religiosa* is a Bo tree, which sheltered the Buddha as he divined the “Truths.” And that is why it is religiosal[5].

F. religiosa (L.) is a large perennial tree, glabrous when young, found throughout the plains of India upto 170 m altitude in the Himalayas. It is largely planted as an avenue and roadside tree especially near temples[6].

The potential therapeutic benefits of leaf juice and fruits of *F. religiosa* have been documented in folk medicine[3]. 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 gonorrhoea, bleeding, paralysis, diabetes, diarrhoea, bone fracture, as antiseptic, astringent and antidote[3]. The leaves have been reported to have the hypoglycemic, anti-convulsant, wound healing, anti-ulcer, anti-oxidant and immunomodulatory activity[7–12]. Various constituents from leaves have been reported including carbohydrate, protein, lipid, lupeol campesterol, stigmaterol, and tannic acid[13].

No substantial scientific evidence is available to support ethnomedicinal use of leaves from *F. religiosa*[14,15]. Therefore, the present study is designed to investigate the effect of leaves using *in vivo* histamine induced bronchospasm in guinea pigs and measuring its anti-allergic activity by mast cell stabilizing model of rats.

2. Materials and methods

2.1. Drugs and chemicals

Histamine hydrochloride, acetylcholine chloride and compound 48/80, were procured from S.D. Fine Chem. Ltd., Mumbai, Central Drug House, New Delhi and M.P. Biomedicals, France, respectively. Distilled water was used

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for the preparation of extracts. HPLC grade of methanol and all other chemicals of analytical grade were from Rankem Chemicals (Pvt.) Ltd. India.

2.2. Preparation of the extract

Leaves of *F. religiosa* were collected from Ahmedabad, Gujarat, India in October 2010 and the sample was identified and authenticated by Dr. Punjani BL, Ethnobotanist, P.G. Centre in Botany, Smt. S.M. Panchal Science College, Talod, Gujarat, India. Voucher specimen (IPS/PCOG/MPH10–11/510) has been preserved at our university for future reference. The aqueous extract of the leaves was prepared from 2 kg of leaves. It yielded 10.74% (w/w) of dry extract. The extract was dried under rotary vacuum evaporator to obtain solvent free extract. The extract was subjected to qualitative phytochemical tests^[16].

2.3. Animals

The experimental protocol (IPS/PCOG/MPH10–11/2015) was approved by the Institutional Animal Ethical Committee. Age matched adult guinea pigs (350–500 g) were acclimatized in animal house with normal cycles of day and night, under standard conditions at (25±2)°C and 55%–65% relative humidity.

2.4. In vivo studies of broncho-protective activity in guinea pigs against various mediators

Guinea pigs of either sex were selected and randomly divided into six groups. Drugs were administered intraperitoneally (*ip.*) by dissolving the water extract in normal saline. The single dose treatment was given 45 minutes before challenged with 2% w/v histamine hydrochloride and 4%w/v acetylcholine chloride aerosol. The onset time of respiratory distress (pre-convulsion dyspnea) was noted^[17]. The onset time of pre-convulsion dyspnea during challenge with these agents was also recorded. Guinea pigs with onset time of preconvulsion dyspnea longer than 120 s were considered insensitive and discarded. Group I served as vehicle control, and treated by normal saline; Group II by AEFRL at 150 mg/kg; Group III by AEFRL at 300 mg/kg; and Group IV by aminophylline at 25 mg/kg.

2.5. Mast cell stabilizing activity on rat mesentery

Adult male albino CF1 rats were killed with ether. Pieces of mesentery with connecting lobes of fat and blood vessels were rapidly dissected out and placed in Ringer–Locke solution. From each rat 10–15 pieces were obtained. The pieces were then placed in solutions of 48/80 in Ringer–Locke for (30±1) min. The pieces of mesentery were removed to a 4% formaldehyde solution containing 0.1% toluidine blue for 20 to 30 min. And then they were transferred through acetone and xylene and mounted on slides. Usually two pieces of mesentery was used in each concentration of the drug. From these pieces five microscope fields were selected at random under 100× magnification from widely separated areas of mesentery^[18].

2.6. Acute toxicity studies

F. religiosa is one of the oldest known human foods having

a very high safety profile^[19]. It has been reported that the hydroalcoholic extract of the leaves up to dose (2 000 mg/kg) did not produce any mortality^[20].

2.7. Statistics

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

3. Results

3.1. Phytochemical constituents

The preliminary phytochemical screening has revealed the presence of carbohydrates, amino acids, alkaloids, gums, phenolics, tannins and flavonoids in aqueous extract of *F. religiosa* leaves.

3.2. Effect of the aqueous extract on histamine and acetylcholine induced bronchospasm in guinea pigs

Pre-treatment with aminophylline (25 mg/kg, *ip.*) has significantly delayed the onset of pre-convulsive dyspnea induced by aerosol exposure of histamine compared with vehicle control ($P<0.05$). The administration of AEFRL at 150 and 300 mg/kg also significantly delay the onset time in dose-dependent way ($P<0.05$) (Table 1).

Similarly when the guinea pigs were exposed to the aerosol of acetylcholine, significant activity of AEFRL can be seen ($P<0.05$). The increase in latency was noticed upon exposure to acetylcholine aerosol as compared to vehicle control (Table 1).

3.3 Mast cell stabilizing activity on rat mesentery

The control group showed (86.07±0.45)% degranulation of mast cell, while groups treated with AEFRL (10–30 μ g/mL) and ketotifen (10 μ g/mL) significantly ($P<0.05$) protected degranulation of mast cells. The protection percentage was 52.52%, 79.29% and 88.37% in AEFRL treated groups, respectively, and 76.70% in ketotifen treated group. The protective effect of AEFRL at 20 μ g/mL was comparable with that of ketotifen (Table 2).

4. Discussion

Animal model of acute bronchospasm by guinea pig is established by exposing to histamine aerosol. And the anti-asthmatic potential of compounds can be tested by measuring the onset time for the development of pre-convulsion dyspnea^[21,22]. In present study, a single administration of the AEFRL significantly delayed the development of histamine-induced pre-convulsion dyspnea as compared to vehicle control animals, suggesting that the aqueous extracts at the doses employed in the present study are effective on bronchospasm induced by histamine. On the basis of results in this study it may be concluded that *F. religiosa* leaves are effective on bronchospasm induced by histamine or acetylcholine in guinea pigs.

The degranulation of mast cell occurs in response to the

Table 1The effect of AEFRL on histamine and acetylcholine induced bronchospasm in guinea pigs (mean \pm SEM).

Treatment group	Dose(mg/kg)	Pre-convulsion time(s)	
		Histamine	Acetylcholine
Vehicle control	–	68.0 \pm 3.5	85.0 \pm 3.5
Aqueous extract	150	125.0 \pm 9.6*	235.0 \pm 9.6 *
Aqueous extract	300	167.0 \pm 5.7*	273.0 \pm 5.7 *
Aminophylline	25	84.0 \pm 11.2*	202.0 \pm 11.2*

*:P<0.05 vs. vehicle control.

Table 2The effect of AEFRL on compound 48/80 induced mast cell granulation in rats (mean \pm SEM).

Treatment group	Concentration(μ g/mL)	% Degranulation (Ruptured cells)
Positive control	–	10.08 \pm 0.05
Negative control (Compound 48/80)	–	86.07 \pm 0.45
Aqueous extract	10	40.87 \pm 0.22 *
Aqueous extract	20	17.82 \pm 0.09 *
Aqueous extract	30	10.00 \pm 0.07*
Ketotifen	10	20.05 \pm 0.13*

*: P<0.05 vs. negative control group.

immunological stimuli in which antigen antibody reactions are predominant. AEFRL at 10–30 μ g/mL) significantly protects compound 48/80 induced degranulation of mast cell in a dose dependent manner. The protective effect of AEFRL at 20 μ g/mL is comparable with that of ketotifen.

In conclusion AEFRL is effective in treatment of asthma. Hence, it can be suggested that leaves of *F. religiosa* could be used for the treatment bronchial asthma, as it is currently being used in folklore system of the medicine.

Conflict of interest statement

We declare that we have no conflict of interest.

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