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Short Report

Evaluation of Bronchodilator and Anti-Anaphylactic Activity of *Myrica sapida*

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ABSTRACT

Background: Asthma is a chronic inflammatory disorder of the airways. The available treatment options have major limitations owing to low efficacy, associated adverse events and compliance issues. Therefore, the health burden of bronchial asthma is increasing globally at an alarming rate, providing a strong impetus for the development of new therapeutics. *Myrica sapida* is known traditionally in Ayurveda to possess anti-asthmatic activity. Hence, the present investigation was undertaken to evaluate the bronchodilator and anti-anaphylactic activity of the stem bark of *Myrica sapida*. **Methods:** Experimental models studied were acetylcholine induced bronchospasm in guinea pigs, egg albumin induced anaphylaxis in guinea pigs, *in vitro* studies on tracheal strip of egg albumin sensitized guinea pigs. **Results:** Treatment with ethanolic extract of *M. sapida*, 75 mg/kg, orally resulted in significant protection against acetylcholine aerosol induced bronchospasm and allergen induced anaphylaxis in guinea pigs. Ethanolic extract of *M. sapida* (75 mg/kg, p.o.) prevented the potentiation of responses and also produced a decrease in pD_2 value of histamine and acetylcholine in guinea pig tracheal strip. **Conclusion:** These results suggest that *M. sapida* possesses bronchodilator activity, has potent inhibitory effect on immediate hyper-sensitivity reactions and decreases bronchial hyper-responsiveness. *Iran. Biomed. J. 12 (3): 191-196, 2008*

Keywords: Anti-asthmatic activity, *Myrica sapida*, Bronchial hyper-responsiveness, Potentiation

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INTRODUCTION

Asthma is a chronic inflammatory disorder of the airways. It involves in complex interactions between many cells and inflammatory mediators that results in inflammation, obstruction (partially or completely reversible after treatment or resolves spontaneously), increased airway responsiveness (i.e. hyper-responsiveness) and episodic asthma symptoms [1]. Research indicates that airway hyper-responsiveness is important in the pathogenesis of asthma and that the level of airway hyper-responsiveness usually correlates with the clinical severity of asthma [2].

The available treatment options have major limitations owing to low efficacy, associated adverse events and compliance issues [3]. As a result, there is high prevalence of usage of complementary and alternative medicines for treatment of this disease. Ayurveda, an ancient system of Indian medicine, has recommended several drugs from indigenous plant sources for the treatment of bronchial asthma and allergic disorders [4]. *Myrica sapida* (commonly known as Kaiphal, Myricacea Family) is a small to moderate-sized tree varying from 3 to 15 m from place to place in sub-tropical Himalayas from the Ravi River eastward to Khasi, Jaintia, Naga and Lushai hills of India at altitudes of 900-2100 m. The plant is known to possess varied medicinal properties. The bark of this medicinal plant has been reported to be used in the treatment of asthma, fever, dyspnoea, throat and lung affections, chronic bronchitis, typhoid, dysentery, diuresis, cough, stomatitis, chronic gonorrhea, headache, piles, gleet, liver complaints, sores, uterine stimulant, excessive burning sensation, appetizer, toothache and ulcers

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[5]. The oil from the bark is used in earache. The oil obtained from the flowers is tonic and useful in earache, diarrhoea, inflammation and paralysis. A paste of the seeds is externally used as rubefacient in cholera. It is also used as bitter, astringent, carminative and tonic [6]. In the present study, the effect of *M. sapida* was studied on various *in vivo* and *in vitro* methods for evaluation of bronchodilator and anti-anaphylactic activity.

MATERIALS AND METHODS

Plant material. Stem bark of *M. sapida* was obtained from commercial supplier of Ahmedabad (India). The plant was identified and authenticated by Dr. Minoo Parabia, Department of Bioscience, Veer Narmad South Gujarat University, Surat, Gujarat (India). A voucher specimen of plant was deposited in the Herbarium of the Department of the above mentioned.

Preparation of ethanolic and methanolic extract. The stem bark reduced to coarse powder was macerated with ethanol for 48 h, filtered and filtrate was evaporated under reduced pressure to obtain dry extract. The extract was stored in cool and dry place and used for pharmacological evaluation. Same procedure was followed for preparation of methanolic extract.

Animals. Hartley strain guinea pigs (350-500 g) of either sex, housed in standard conditions of temperature (22 ± 2°C), relative humidity (55 ± 5%) and light (12 h light/dark cycles), were used and fed with green vegetables. The Institutional Animal Ethics Committee approved the experimental protocol (project no. 6012 dated 19/12/06).

Acetylcholine induced bronchospasm in guinea pigs. Experimental animals were divided into 2 groups, each containing six animals [7]. The animals of group I and group II placed in an aerosol chamber were exposed to 0.4% acetylcholine bromide aerosol under constant pressure 1 kg/cm^2 on day 0 without any treatment and time for preconvulsive dyspnoea (PCD) was noted. The end point for PCD was determined from the time of aerosol exposure to the onset of dyspnoea leading to the appearance of convulsions. As soon as PCD commenced, the animals were removed from chamber and placed in fresh air to recover. This time for PCD was taken as day 0 value. After 15 days of wash out period, the animals of group I and group II were administered with ethanolic extract orally (p.o., 75 mg/kg) and methanolic extract (120 mg/kg, p.o.) of *M. sapida*, suspended in 0.5% carboxy methyl cellulose (CMC) respectively. Two hours later, the animals were exposed to 0.4% acetylcholine bromide aerosol and time for PCD was noted. The % increase in time of PCD was calculated using the following formula [8].

$$\text{Percentage increase in time of PCD} = \left(1 - \frac{T_1}{T_2}\right) \times 100$$

Where: T_1= time for PCD onset on day 0, T_2 = time for PCD onset on day 15

Egg albumin induced anaphylaxis in guinea pigs. Guinea pigs (n = 20) were sensitized by two intraperitoneal injections of 0.5 ml and 10% w/v solution of egg albumin at a 48-h interval. After sensitization, the animals were divided into two groups of 10 animals each. Animals of group I received 0.5% CMC and served as control group. Animals of group II received ethanolic extract of *M. sapida* (75 mg/kg, p.o., once daily) suspended in 0.5% CMC for 14 days. On day 14, two hours after treatment, the animals were challenged with 0.5 ml of 2% w/v solution of egg albumin into the saphenous vein. Guinea pigs were observed for the onset of symptoms such as dyspnoea and cyanosis, duration of persistence of symptoms (min.) and mortality. The severity score with respect to symptoms was recorded using the method of Gupta *et al*. [9]: increased respiratory rate (2), dyspnoea for 10 min. (4), dyspnoea and cyanosis for 10 min. (8), and collapse (10). Guinea pigs remaining alive after the antigen challenge were counted to record the percentage of mortality due to anaphylactic shock by using the following equation [10]:

$$\text{Mortality rate (\%)} = \frac{\text{Number of guinea pigs collapsed} \times 100}{\text{Total number of experimental animals}}$$

In vitro studies on isolated tracheal preparations. The animals were divided into three groups of six animals each i.e. group-I (control), group-II (sensitized), group-III (sensitized + treatment). The animals of group I received 0.5% CMC for 14 days. The animals of group II and group III were sensitized with egg albumin (1 ml, 10% w/v, i.p.) on the first day. The animals of group III were administered with ethanolic extract of *M. sapida* (75 mg/kg, p.o., once daily for 14 days) suspended in 0.5% CMC. Two hours after the last dose of drug

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Table 1. Effect of *M. sapida* on acetylcholine bromide aerosol induced bronchospasm in guinea pigs.

GROUP	Pre convulsion time (min)		Percent protection	
	Ethanolic extract (75 mg/kg, p.o.)	Methanolic extract (120 mg/kg, p.o.)	Ethanolic extract (75 mg/kg, p.o.)	Methanolic extract (120 mg/kg, p.o.)
Before treatment	2.645 ± 0.3414	2.160 ± 0.5102	-	-
After treatment	8.030 ± 2.0560*	5.677 ± 1.5330*	67.06%	61.95%

Statistical analysis by paired *t*-test; values are mean ± SEM; n = 6 in each group; significantly difference between before and after treatment *P<0.05.

administration (on 14th day), group II and group III animals were challenged with egg albumin (0.5 ml, 2% w/v) through saphenous vein. After 3 hours of the challenge of egg albumin or just prior to thedeath of animals, which ever was earlier, the animals were sacrificed for isolation of trachea [11]. Guinea pigs were stunned by a sharp blow on the head and sacrificed by cutting neck blood vessels. The trachea and ileum were rapidly dissected free of surrounding tissues and placed in Petri dish containing oxygenated Krebs-Henseleit solution (NaCl, 114.0 mM; $CaCl_2$, 2.5 mM; KCl, 4.7 mM; glucose, 11.7 mM; $NaHCO_3$, 25 mM; $MgCl_2$, 1.2 mM; KH_2PO4, 1.2 mM). Tracheal strips were prepared by cutting the trachea spirally. Tracheal strip was suspended in organ tube containing Krebs-Henseleit solution at 37 ± 1°C under a uniform tension of 1.5 g, continuously bubbled with 95% O_2 and 5% CO_2. After an initial equilibration period of 90 min., the responses of the trachea and ileum to histamine and acetylcholine were recorded on a student physiograph (Bio Devices, India) using isotonic transducer.

Calculation of pD_2 value. The dose response curve was constructed by plotting the percentage of maximal response produced by each dose against the log molar concentrations of the drug. The 50 percent point of the curve was projected on the concentration axis and the value readout was EC50 (median effective dose or concentration producing 50 percent of maximal response). The potency was then expressed as pD2 value (mean negative log of molar concentration producing 50 percent of maximal response) calculated by using the formula [12]:

$$pD_2 = - \log (EC50)$$

where EC50 = molar concentration of drug (Histamine/Acetylcholine) producing 50 percent of maximal response.

Statistical analysis. The results of various studies were expressed as mean ± SEM and analyzed statistically using paired '*t*'-test or one way ANOVA to find out the level of significance. Data were considered statistically significant at P<0.05.

RESULTS

Effect of acetylcholine induced bronchospasm in guinea pigs. Ethanolic extract (75 mg/kg, p.o.) and methanolic extract (120 mg/kg, p.o.) of *M. sapida* significantly prolonged the latent period of PCD (P<0.05) as compared to the control, following exposure to acetylcholine bromide aerosol (Table 1). The activity of ethanolic extract was found to be better than methanolic extract.

Egg albumin induced anaphylaxis in guinea pigs. As shown in Table 2, an intravenous challenge of egg albumin resulted in a fatal anaphylactic shock in 70% of the animals in control group characterized by symptoms of dyspnoea, asphyxia and collapse. Pretreatment with ethanolic extract of *M. sapida* (75 mg/kg, p.o.) significantly protected the sensitized guinea pigs against anaphylactic shock as the onset of symptoms were delayed, less severe and none of the animals collapsed (Table 2).

Table 2. Effect of *M. sapida* on egg albumin induced anaphylaxis in rats.

Group	Pre convulsion time (min)			Percent protection
	Onset (min)	Duration (min)	Severity (score)	Mortality (%)
Group I (Control)	1.246 ± 0.056	23.66 ± 0.345	9.4 ± 0.306	70%
Group II (*M. Sapida*, 75 Mg/Kg, P.O., For 14 Days)	2.914 ± 0.088*	7.054 ± 0.131*	3.0 ± 0.615*	0%

Statistical analysis for symptoms by paired *t*-test; values are mean ± SEM; n = 10 in each group; significantly different from control group *P<0.001.

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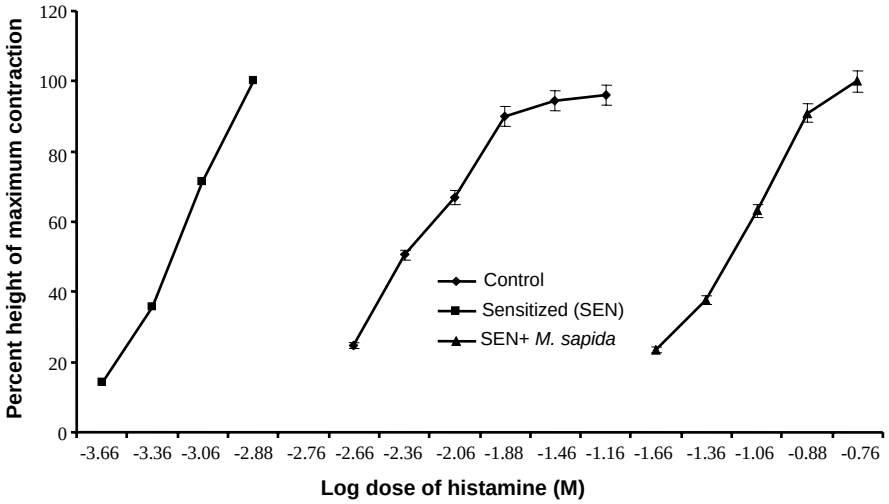

Fig. 1. Effect of ethanolic extract of *M. sapida* on histamine induced contractions in tracheal strip preparation of guinea pigs. Each point represents mean ± SEM of 6 experiments. NS, non-significant difference between sensitized, control and treated group.

The in vitro studies on isolated smooth muscle preparations. pD_2 value of histamine and acetyl choline in sensitized group (3.21 ± 0.011 and 3.36 ± 0.021) were found to be non-significantly greater compared to control group (2.36 ± 0.15 and 3.21 ± 0.028) (Figs. 1 and 2). Cumulative log concentration response curve of histamine and acetylcholine in animals pretreated with ethanolic extract of *M. sapida* (75 mg/kg, p.o., for 14 days) showed a clear rightward shift compared to control and sensitized group. pD_2 value of histamine and acetyl choline in animals pretreated with ethanolic extract of *M. sapida* (75 mg/kg, p.o., for 14 days) (1.21 ± 0.035 and 2.31 ± 0.095) was found to be non-significantly lower compared to sensitized control group (Figs. 1 and 2).

DISCUSSION

Asthma, a chronic relapsing inflammatory disease, is characterized by hyper-reactive airways, leading to reversible bronchoconstriction. The inflammation causes an associated increase in

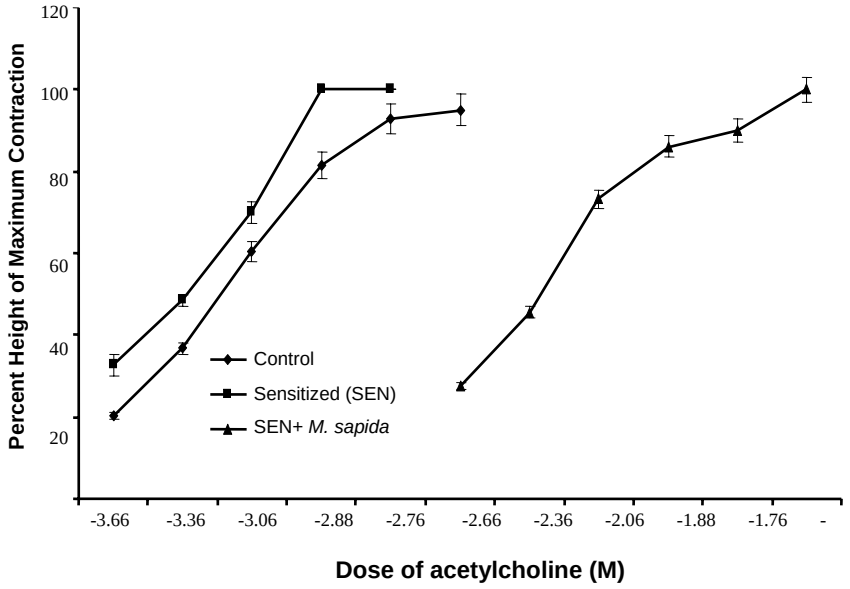

Fig. 2. Effect of ethanolic extract of *M. sapida* on acetylcholine induced contractions in tracheal strip preparation of guinea pigs. Each point represents mean ± SEM of 6 experiments. NS, non-significant difference between sensitized, control and treated group.

airway responsiveness to various stimuli. Broncho-constriction, cough, mucus production and airway hyper-sensitivity to bronchoconstriction mediators are the main clinical manifestations of asthma and these features correlate well with the severity of the disease. Single mediator approach to asthma therapy is difficult as the disease process involved in asthma is complex [13].

Parasympathetic nerves are characterized by their release of neurotransmitter acetylcholine. The vagus nerve sends motor fibers from the brain to smooth muscle cells in the bronchial walls and the stimulation of the vagus nerve releases acetylcholine, which binds to specific "cholinergic" receptors on smooth muscle cells within the bronchial walls and thus constricts the airways. Thus, it was concluded that cholinergic stimulation causes bronchoconstriction through airway smooth muscle contraction [14]. The present findings show that ethanolic and methanolic extract of *M. sapida* prolonged the time of PCD in guinea pigs following acetylcholine aerosol induced bronchospasm. In context to this, it may be concluded that protective effect of ethanolic and methanolic extract of *M. sapida* against acetylcholine induced bronchospasm may be due to its anti-cholinergic activity. Moreover, the bronchodilating activity following treatment with *M. sapida* was found to be greater for ethanolic extract compared to methanolic extract.

The presence of acetylcholine muscarinic receptors and histamine H_1 sensitive excitatory receptors in airway smooth muscle of man and animals has been reported [14-17]. Histamine and acetylcholine induced contraction in sensitized animals pretreated with ethanolic extract of *M. sapida* were less as compared to those in sensitized and non-sensitized guinea pig trachea. The potency of a drug may be expressed as EC50 or pD_2 and lower the EC50/higher the pD_2 value, higher the potency of drug [12]. In context to this, an increase in pD_2 value of histamine and acetylcholine in sensitized animals indicate their increased potency which can be attributed to hyper-responsiveness induced by egg albumin. However, decreased pD_2 value of histamine and acetylcholine in sensitized animals pretreated with ethanolic extract of *M. sapida*, suggests that the potency of histamine and acetylcholine decreases on pretreatment with ethanolic extract. This finding fits well with the current observation that pretreatment with *M. sapida* decreased pD_2 value indicating the prevention of hyper-responsiveness to histamine and acetylcholine resulting in protective effects in bronchial asthma.

The present findings reveal protection against egg albumin induced anaphylactic shock characterized by decrease in intensity and delay in the development of symptoms of dyspnoea, asphyxia and collapse. In line with this notion, anti-anaphylactic effect of *M. sapida* may be due to inhibition of phenomenon of sensitization or non-availability of antibodies on the mast cell surface. All these findings reveal the bronchodilator and anti-anaphylactic activity of *M. sapida* indicating its beneficial use in asthma.

Moreover, phytochemical screening of *M. sapida* indicates the presence of glycosides like myricetin-3 rhamnoside, quercetin. Glycosides isolated from various plant sources are proposed to have anti-asthmatic activity through several mechanisms which include prevention of allergen induced bronchial obstruction [18], inhibition of histamine release from mast cells and polymorphonuclear leucocytes [19, 20], spasmolytic activity by relaxation of smooth muscle (tracheal muscle) [21], anti-anaphylactic activity [22], antiallergic activity [23,24] and bronchodilator activity [25] by protecting against histamine induced bronchospasm. Thus, it can be concluded that glycosides may contribute to the bronchodilator and anti-anaphylactic activity of *Myrica sapida* in the experimental animals.

In conclusion, the results of this study demonstrate that the possible mechanism of action appears to be not only prevention of hyper-responsiveness in smooth muscle but also inhibition of immediate hyper-sensitive reaction by an allergen, stabilization of mast cell membrane, or non-availability of antibodies on the mast cell surface.

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