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Pharmacological basis for the medicinal use of *Morus alba* in gut and airways disorders

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Abstract

Crude extract of *Morus alba* at 100 mg/kg exhibited protective effect against castor oil-induced diarrhea in mice. In isolated rabbit jejunum, *M. alba* (0.3-10 mg/mL) inhibited the spontaneous contractions and caused glibenclamide-sensitive inhibition of low K^+ (20 mM)-induced contractions, with mild effect on high K^+ (80 mM). Similarly, cromakalim caused inhibition of low K^+, but not of high K^+, while verapamil did not differentiate in its inhibitory effect on two concentrations of K^+. *M. alba* (3.0-30 mg/kg) caused suppression of carbachol (100 µg/kg)-induced increase in inspiratory pressure of anaesthetized rats. In guinea-pig trachea, *M. alba* completely inhibited low K^+ contractions, with partial effect on high K^+. *M. alba* (0.3-1.0 mg/mL) caused leftward shift of isoprenaline-induced inhibitory concentration response curves, like papaverine. These results indicate that *M. alba* possesses a combination of K_{ATP} channel opening, weak Ca^{++}-antagonist and phosphodiesterase inhibitory mechanisms, which explain its medicinal use in hyperactive gut and airways disorders.

Introduction

Morus alba Linn. (Family:Moraceae), commonly known as "Mulberry" and locally, "Shahtut" is found in Khyber Pakhtunkhwa, Punjab and Baluchistan provinces of Pakistan and other parts of the world. It is a medium sized tree, 3-6 m high with whitish or dark purple fleshy fruits, 1.5-3 cm long and edible (Baquar, 1989). *M. alba* is used in folk medicine to treat diarrhea, asthma, cough, dyspepsia, eye problems, intestinal ulcers, headaches, hemoptysis, hepatopathy, hoarseness, lumbago, melancholia, scabies, smallpox and splenopathy (Baquar, 1989; Brown, 1995; Shinwari et al., 2003; Deshpande et al., 2008) as well as considered useful as anthelmintic, antibacterial, aphrodisiac, antirheumatic, diuretic, expectorant, antihypertensive, laxative and sedative agent (Nadkarni, 1976; Chiej 1984; Duke and Ayensu, 1985; Bown, 1995; Usmanghani et al., 1997; Wiart, 2002).

Phytochemical studies on the plant revealed the presence of numerous constituents that include sugar, pectin, citrates, malates, phytosterol, sulfur, essential oils, quercetin, inokosterone, ecdysterone, phytosterol, rutin, morocetin, isoquercitrin, umbelliferone, scopolin, hexenal, trigonelline, choline, adenine, aspartic acid, chlorogenic acid, phytosterol, deoxynojirimycin, moracetin, gamma amino butyric acid, sitosterol, fiber, calcium, vitamin A, vitamin B1, vitamin B2, vitamin C, sodium, potassium, carotenes, amino acids, prenylflavones, glycoside, astragal, scopolin, skimmin, roseoside II and benzyl D-glucopyranoside (Nadkarni, 1976; Doi et al., 2001).

M. alba is reported to possess antidiabetic (Chen et al., 1995), anti-inflammatory (Hong et al., 2002; Choi and Hwang, 2005), hepatoprotective (Oh et al., 1999), anti-

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melanocytic (Baurin et al., 2002; Lee et al., 2002), anti-cancer (Kim et al., 2000), antihyperlipidimic (Doi et al., 2001) properties. In present research, we provide evidence that *M. alba* exhibits antidiarrheal, anti-spasmodic and bronchodilatory activities, mediated through K^+ channel activation, weak Ca^{++} channel blockade (CCB) and phosphodiesterase (PDE) inhibitory pathways, which explains the medicinal use of *M. alba* in hyperactive gut and airways disorders, such as diarrhea and asthma.

Material and Methods

Plant material and extraction

The fresh fruit of *M. alba* was collected from the trees in April 2008. The plant material was cleaned and approximately 1.7 kg of the material was soaked in aqueous-methanol (70%) at room temperature (25 ± 2.0° C) for 24 hours with occasional shaking. It was passed through a clean muslin cloth and then through filter paper. This procedure of soaking and filtration was repeated twice more (Williamson et al., 1998). All the filtrates were combined and evaporated to dryness on a rotary evaporator under reduced pressure (-760 mmHg) at 35-40^{0}C to a semisolid residue of light brown color with sweetish aroma, the crude extract of *M. alba* , yielding approx. 14.6%. *M. alba* was soluble in normal saline (0.9%) and distilled water for use in the *in vivo* and *in vitro* experiments.

Animals

Rabbits (1-1.2 kg), guinea-pigs (500-550 g), Sprague-Dawley rats (200-250 g) and Balb-C mice (20-25 g) of local breed and either sex were used for this study housed at the Animal House of the Aga Khan University, maintained at 23-25°C and were given a standard diet and tap water. Rabbits starved for 24 hours were sacrificed by blow on back of head. Experiments performed complied with rulings of Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, 1996).

Chemicals

Acetylcholine chloride (ACh), carbachol (CCh), isoprenaline, loperamide, glibenclamide, papaverine, potassium chloride and verapamil were obtained from Sigma Chemicals Co, St Louis, MO, USA and cromakalim from Tocris, Ellisviille, MO, USA. Pentothal sodium (thiopental) and castor oil were respectively obtained from Abbot Laboratories and KCL Pharma, Karachi, Pakistan. Chemicals used for making physiological salt solutions were: Calcium chloride, glucose, magnesium chloride, magnesium sulfate, potassium dihydrogen phosphate, sodium bicarbonate, sodium dihydrogenphosphate (Merck, Darmstadt, Germany and sodium chloride from BDH Laboratory supplies, Poole, England. The chemicals used in phytochemical analysis include: Acetic anhydride, aluminum chloride, ammonium hydroxide, ferric chloride (Sigma Chemical Co, USA), benzene, chloroform, hydrochloric acid and petroleum ether (BDH Laboratory Supplies, England). All chemicals used were of analytical grade.

Phytochemical screening

Preliminary investigation of the plant extract for the presence of various phytochemical classes, such as saponins, coumarins, sterols, terpenes, flavonoids, anthraquinones and tannins was done according to reported methods (Edeoga et al., 2005). Plant material was noted as positive for flavonoids when it gave yellow color with aluminum chloride reagent and for tannins, when green or black color was produced with aqueous ferric chloride. For the detection of sterols and terpenes, plant material was treated with petroleum ether and subsequently extracted with chloroform. The appearance of green to pink (for sterols) and pink to purple colors (for terpenes) was then noted after treatment of chloroform layer with acetic anhydride and concentrated hydrochloric acid in succession. Presence of saponins was based on the appearance of froth upon vigorous shaking of diluted samples. The observation of yellow florescence under the uv-torch of filter paper previously exposed to the vapors from boiling plant material confirmed coumarins. Lastly, for detecting anthraquinones, the extract was dissolved in 1% HCl, then in benzene; the extract showed pink-violet to reddish color with ammonium hydroxide.

Castor oil-induced diarrhea

Mice were fasted for 24 hours before the experiment. Animals were housed in individual cages and divided in five groups, each containing 10 mice. The first group received saline (10 mL/kg, p.o.) and served as a negative control. The dose of the test extract (100 mg/kg) was selected on trial basis and was given orally to a group. Another group of mice was treated with loperamide (10 mg/kg, p.o.), as a positive control. One hour after treatment, each animal received 10 mL/kg of castor oil orally through a feeding needle. Afterward, the cages were inspected for the presence of diarrhea droppings; their absence was noted as a positive result, indicating protection from diarrhea at that time (Shah et al., 2011c).

Rabbit jejunum

The rabbit abdomen was cut opened and jejunum was dissected out, kept in normal Tyrode's solution as described previously (Gilani et al., 2005; Khan et al., 2011a). Each segment of about 2 cm length was suspended in 10 mL tissue bath containing Tyrode's solution (pH 7.4), maintained at 37°C and aerated with a mixture of 95%O_2 and 5%CO_2 (carbogen). The composition of Tyrode's solution was (mM): NaCl: 136.9, KCl:

2.7, $MgCl_2.6H_2O$: 0.5, $NaHCO_3$: 11.9, $NaH_2PO_4.2H_2O$: 0.32, $CaCl_2$: 1.8, and glucose: 5.05. One end of the segment was attached to the metallic tissue-hook and the other was attached by a cotton thread to an isotonic Bioscience transducer, connected to a Student oscillograph (Harvard Apparatus, Holliston, MA, USA). An initial load of 1 g was applied to each tissue and was allowed to equilibrate for 30 min before the addition of any drug. Following equilibration period, each preparation was then stabilized with sub-maximal concentration of ACh (0.3 µM) at 3 min interval until constant responses were recorded. Under these experimental conditions, rabbit jejunum exhibits spontaneous rhythmic contractions, allowing testing of the relaxant (spasmolytic) activity directly without the use of any agonist. For elucidation of mechanisms of spasmolytic activity, high K^+ (80 mM) and low K^+ (20 mM) concentrations were used to depolarize the isolated tissues that lead to produce sustained contractions. The plant material was then added in a cumulative fashion to obtain concentration-dependent inhibitory responses (Shah et al., 2011a). The relaxation of isolated tissue preparations was expressed as percent of the control response mediated by added low and high K^+ concentrations.

Bronchodilator activity

Rats were anaesthetized with sodium thiopental (Pentothal, 80-100 mg/kg, i.p.), then cannulated the tracheal tube and ventilated with volume ventilator (Miniature ideal pump, Bioscience, UK) adjusted at rate of 70-80 strokes/min to deliver 7-10 mL/kg of carbogen gas (Khan et al., 2011b; Khan et al., 2012a). A polyethylene catheter was inserted into the jugular vein for drugs administration. Changes in airways resistance (mmHg) were measured by pressure transducer (MLT-1199) connected to side arm of tracheal cannula and recorded by PowerLab 4/25 with running chart software via Quad bridge amplifier (AD Instruments, Bella Vista, NSW, Australia). Bronchoconstriction was induced with carbachol (100 µg/kg), which was reversed within 7-10 min. The test drug was given to the animals 5-8 min prior to administration of carbachol. The responses were expressed as the percent reduction of the carbachol-induced bronchospasm.

Guinea-pig trachea

Trachea was dissected from guinea-pig, sacrificed by cervical dislocation and kept in normal Kreb's solution. The tracheal tube was cut into rings, 2-3 mm wide, each containing about two cartilages. Each ring was opened by a longitudinal cut on the ventral side opposite the smooth muscle, forming a strip with smooth muscle in the center and cartilaginous portions on the edges (Khan and Gilani, 2006). The preparation was mounted in 20 mL tissue bath containing Kreb's solution (pH 7.4), at 37°C and aerated with carbogen. The composition of Kreb's solution was (mM): NaCl: 118.2, $NaHCO_3$: 25.0, $CaCl_2$: 2.5, KCl: 4.7, KH_2PO_4: 1.2, $MgSO_4.7H_2O$: 1.2, and glucose: 11.7. A tension of 1 g was applied to the tracheal strips continuously throughout the experiment. The tissues were allowed to equilibrate for 1 hour before the addition of any drug. The carbachol (1 µM) was used to obtain sustained contractions in respective preparations and the inhibitory effect of test substance was assessed by adding in a cumulative fashion. The presence of PDE inhibitory effect was evaluated indirectly through constructing isoprenaline-induced inhibitory concentration-response curves (CRCs) against carbachol-induced contractions in absence (control) and presence of plant material, as described previously (Gilani et al., 2009). Isometric responses were recorded on a PowerLab (AD Instruments, Australia).

Acute toxicity test

Mice were divided in groups of five mice each. The test was performed using increasing doses of the plant extract, given orally in 10 mL/kg volume to different groups serving as test groups. Another group of mice was administered saline (10 mL/kg, p.o.) as negative control. The mice were allowed food *ad libitum* and kept under regular observation for lethality recorded after 24 hours.

Statistical analysis

The data expressed are mean ± SEM and median effective concentrations (EC_{50}) with 95% confidence intervals (CI). The statistical parameters applied were Chi-square test for antidiarrheal assay and one-way analysis of variance followed by Dunnet's test for bronchodilatory activity. Difference of $p<0.05$ was considered statistically significant. Concentration-response curves were analyzed by non-linear regression using GraphPad program (GraphPAD, San Diego, CA, USA).

Results

M. alba was found to contain flavonoids, sterols, tannins and terpenes, while tested negative for rest of the classes. It exhibited protective effect against castor oil-induced diarrhea in mice. The negative control group (saline treated) did not show any protection against castor oil-induced diarrhea. Pretreatment of animals with plant extract showed 60% protection from diarrhea at 100 mg/kg ($p<0.05$ vs. saline group). Loperamide (10 mg/kg) showed complete (100%) protection from diarrhea ($p<0.01$ versus saline group) in positive control group (Table I). *M. alba* and cromakalim caused concentration-dependent relaxation of spontaneous contractions of rabbit jejunum preparations (Figure 1) with respective EC_{50} values of 5.7 mg/mL (4.1-7.9, 95% CI, n = 5) and 34.2 µM (30.1-38.9; n = 4). When tested

Table I

Effect of the crude extract of *M. alba* on castor oil-induced diarrhea in mice

Treatment (p.o.)	n	Mice with diarrhea	% Protection
Saline (10 mL/kg) + castor oil (10 mL/kg)	10	10	0
M. alba (100 mg/kg) + castor oil (10 mL/kg)	10	4[a]	60
Loperamide (10 mg/kg) + castor oil (10 mL/kg)	10	0[b]	100

[a]p<0.05, [b]p<0.01 compared to saline group, Chi-square test

against high K^+ (80 mM)-induced contractions, *M. alba* exerted weak inhibitory effect (27%, n = 4) at highest tested concentration (10 mg/mL), while completely relaxed the contractions induced by low K^+ (20 mM) with EC_{50} value of 7.2 mg/mL (4.0-9.9, n = 3). In presence of glibenclamide (3 µM), the inhibition of low K^+ (20 mM)-induced contractions was prevented (Figure 2A). Similarly, cromakalim also caused glibenclamide-sensitive relaxation of the contractions induced by low K^+ (20 mM) with EC_{50} value of 12.4 µM (11.0-18.5; n = 3), without any effect on high K^+ (80 mM)-induced contractions (Figure 2B), whereas, verapamil inhibited low K^+ (20 mM) and high K^+ (80 mM)-induced contractions at a similar concentration range, with EC_{50} values of 0.3 (0.2-0.6; n = 3) and 0.3 µM (0.2-0.4; n = 3) respectively (Figure 2C). *M. alba* at the doses of 3, 10 and 30 mg/kg caused 19.6 ± 7.1, 30.8 ± 6.9 and 42.5 ± 2.5% (n = 5) respective suppression of carbachol (100 µg/kg)-induced increase in inspiratory pressure of anaesthetized rats (Figure 3A). Cromakalim was used as a positive control, which inhibited the carbachol (100 µg/kg)-mediated bronchoconstriction at 0.1, 0.3 and 1.0 mg/kg by 6.2 ± 3.1, 36.0 ± 4.7 and 50.3 ± 2.7% (n = 4) respectively (Figure 3B). *M. alba* was found devoid of

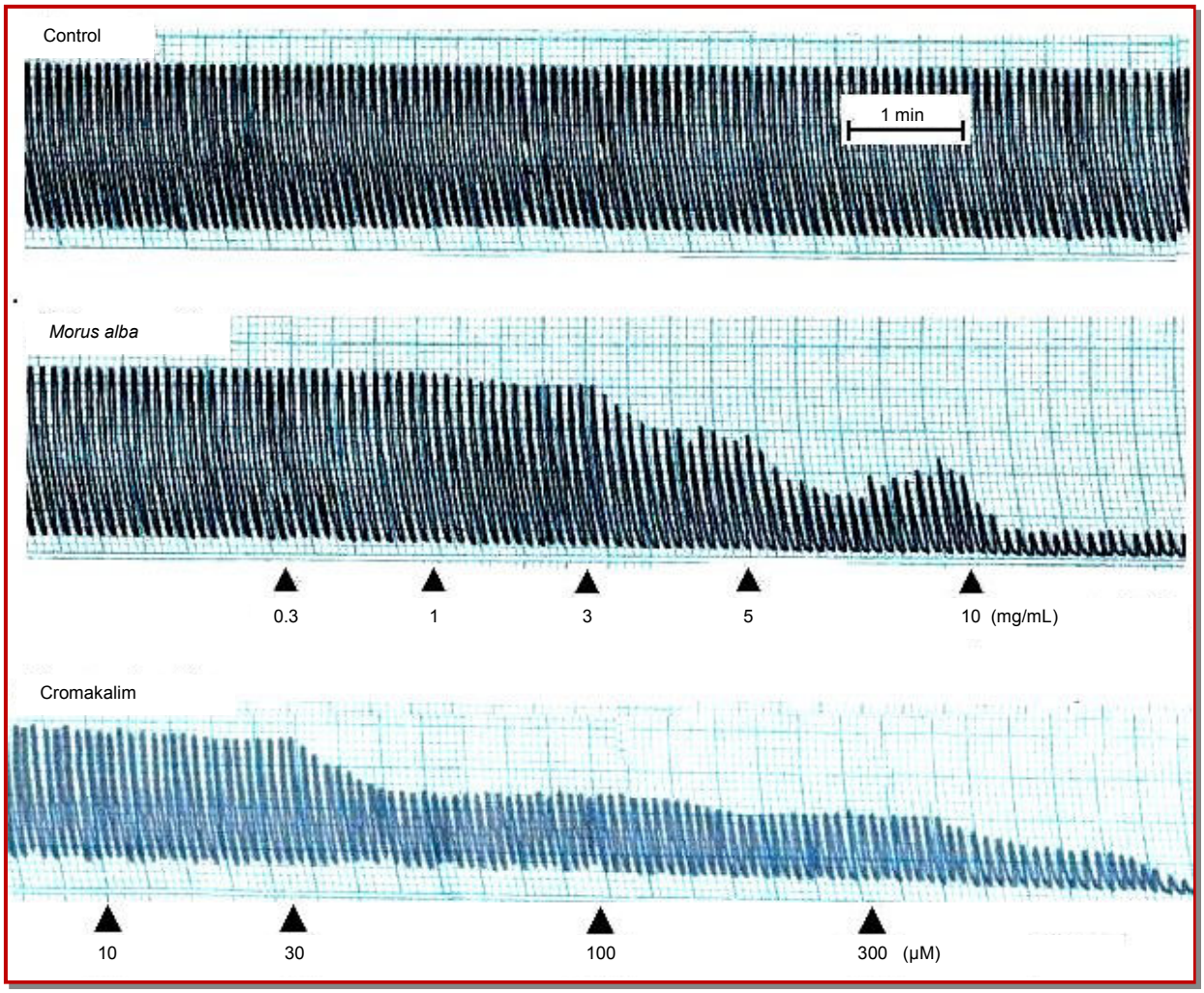

Figure 1: Tracings showing concentration-dependent relaxant effect of the crude extract of *Morus alba* and cromakalim on spontaneously contracting isolated rabbit jejunum preparations. Test material was added to the tissue bath in cumulative fashion and the concentrations shown are the final bath concentration

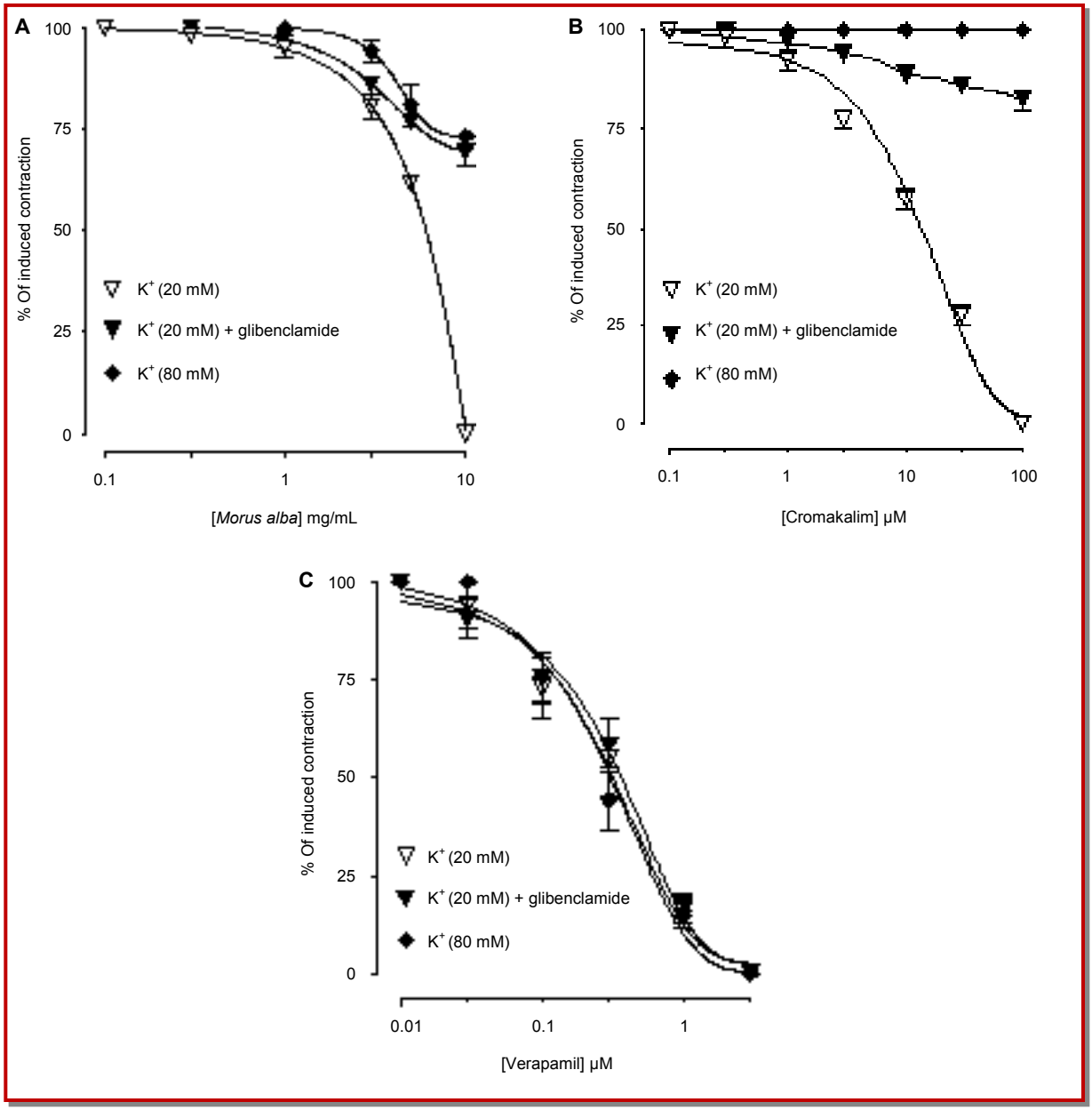

Figure 2: Concentration-response curves showing the comparison of (A) crude extract of *Morus alba*, (B) cromakalim and (C) verapamil for the inhibitory effect against low K^+ (20 mM) in the absence and presence of glibenclamide (3 µM) and high K^+ (80 mM)-induced contractions in isolated rabbit jejunum preparations. Values shown are mean ± SEM, n = 3-4

any stimulant action when screened on the resting base of guinea-pig trachea. When tested against the high K^+ (80 mM)-induced contractions, *M. alba* exerted partial inhibitory effect (37%, n = 4), while produced complete relaxation of low K^+ (20 mM)-induced contractions with EC_{50} value of 1.9 mg/mL (1.2-2.8; n = 3). In presence of glibenclamide (3 µM), the relaxation of low K^+ (20 mM)-induced contractions was prevented (Figure 4). Pretreatment of tracheal preparations with *M. alba* shifted the isoprenaline-induced inhibitory CRCs to left (Figure 5A) in concentration-dependent manner (0.3 and 1.0 mg/mL), similar to that caused by papaverine (1.0 and 3.0 µM) as shown in Figure 5B, exhibits potentiating effect. The three different groups of mice were given *M. alba* in the graded doses of 1, 5 and 10 g/kg respectively and the animals were observed for mortality after 24 hours of drug administration. The extract did not cause any mortality up to the dose of 10 g/kg.

Discussion

In view of *M. alba* medicinal use in hyperactive gut disorder, diarrhea, its extract was evaluated for the

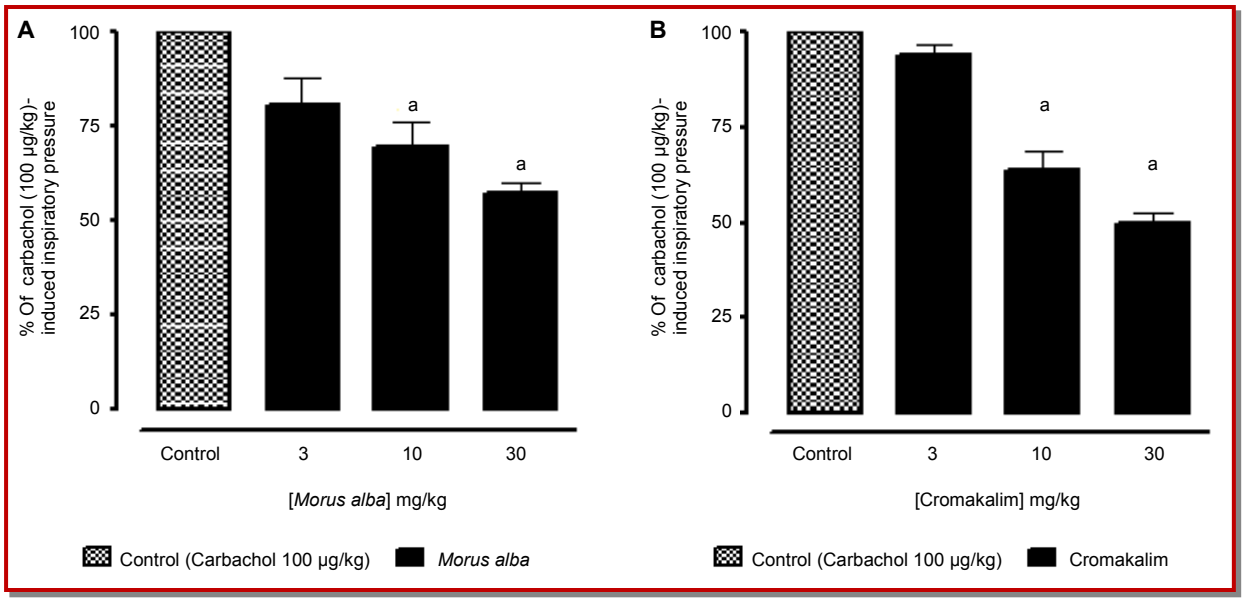

Figure 3: Dose-dependent suppressant effect of (A) crude extract of *Morus alba* and (B) cromakalim on carbachol-mediated bronchoconstriction in anaesthetized rats. Values shown are mean ± SEM, n = 4-5, [a]p<0.01 vs. control (Carbachol), One-way analysis of variance, followed by Dunnett's test

possible antidiarrheal action in mice and the underlying pharmacological mechanism was elucidated using isolated intestinal tissues. In castor oil-induced diarrhea model, *M. alba* extract showed protective effect, like that caused by loperamide, a standard antidiarrheal drug (Reynolds et al., 1984). Castor oil induces diarrhea due to ricinoleic acid, formed during the hydrolysis of oil, which produces changes in transport of electrolytes and water, leading to generation of giant contractions of intestine (Iwao and Terada, 1962; Croci et al., 1997). Thus, a potential antidiarrheal may exhibit its effect by inhibiting bowel contractions. The antidiarrheal activity of *M. alba* following oral administration appears to be virtue of gastrointestinal relaxant components presence in *M. alba*.

To see its possible restrictive effect on gut motility, *M. alba* was studied in gut preparations to elucidate the pharmacodynamic aspects responsible for antidiarrheal effect. In spontaneously beating rabbit jejunum, *M. alba* relaxed the spontaneous contractions, thus showing an antispasmodic action. It is observed that spasmolytic effect of the medicinal plants is usually mediated through Ca^{++} antagonist action (Ghayur and Gilani, 2005; Shah et al., 2011a; 2011b). To see whether the spasmolytic effect of *M. alba* is also mediated via similar mechanism, the extract was tested on high K^+-induced contractions. However, its weak inhibitory effect against high K^+, indicate that the spasmolytic effect is probably mediated through some other mechanism(s). When tested against low K^+-induced contractions, it caused complete inhibition. The substance that selectively relaxes the contractions induced by low K^+ is considered to be potassium channel opener, while Ca^{++} entry blockers inhibit both low and high K^+-induced contractions equally, and these experiments allow distinguishing K^+ channel activation from CCB mechanism (Hamilton et al., 1986; Kishii et al., 1992; Gopalakrishnan et al., 2004). The K^+ channel opening effect was confirmed, when the inhibition of low K^+-induced contractions was prevented in the presence of glibenclamide, a blocker of ATP-dependent K^+ channels (Frank et al., 1994 ; Davies et al., 1996). Cromakalim, a prototypical K_{ATP} channel opener (Brown and Raeburn, 1991; Deitmer et al., 1992; Moura et al., 1993) produced similar results to that of the *M. alba* extract, except that it produced no effect on high K^+-induced contractions, while verapamil, a Ca^{++} antagonist (Fleckenstein, 1977) inhibited low and high K^+-induced contractions at similar concentrations. These results indicate that the antispasmodic effect of the *M. alba* is mediated possibly through a combination of dominant ATP-dependent K^+ channel activation and weak Ca^{++}-antagonist mechanisms. Potassium channel openers are relatively new class of drugs that comprises of a diverse group of molecules with wide range of potential therapeutic uses, like asthma, gastrointestinal spasm, diarrhea, hypertension and urinary incontinence (Empfield et al., 1995; Poggioli et al., 1995; Shieh et al., 2000). These compounds open K^+ channels, cause membrane hyperpolarization through the increase in K^+ efflux, thus causing decrease in the intracellular free Ca^{++} leading to smooth muscle relaxation (Cook, 1988; Quest, 1992).

Based on the medicinal use of *M. alba* in asthma and potential therapeutic use of K^+ channel openers in this disorder (Buchheit and Fozard, 1999; Pelaia et al., 2002;

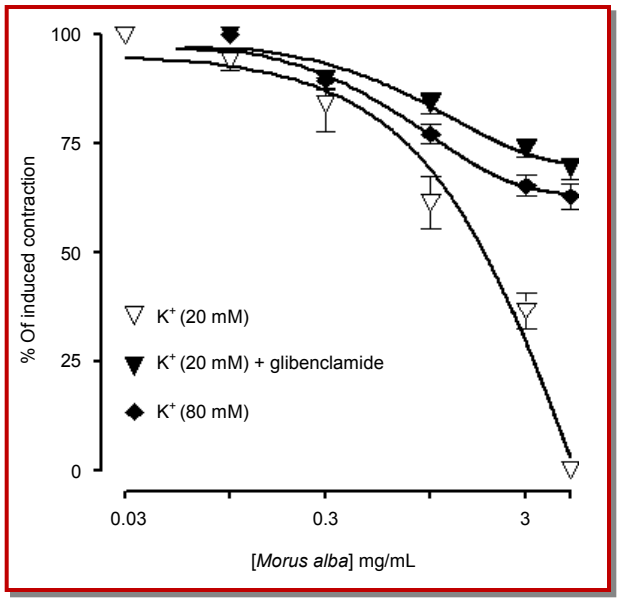

Figure 4: Concentration-response curves showing effect of the crude extract of *Morus alba* on low K+ (20 mM) in the absence and presence of glibenclamide (3 µM) and high K+ (80 mM)-induced contractions in isolated guinea-pig tracheal preparations. Values shown are mean ± SEM, n = 3-4

Sheng and Jian, 2005), the extract was studied for possible bronchodilatory effect in anaesthetized rats, where *M. alba* dose-dependently suppressed the carbachol-evoked bronchospasm, like that caused by cromakalim. The plant extract was then studied in isolated trachea to investigate the possible modes of brochodilatory action. In guinea-pig tracheal preparations, like in jejunum, the *M. alba* extract caused glibenclamide-sensitive relaxation of low K^+-induced contractions with partial effect on the contractions induced by high K^+, indicating the involvement of K_{ATP} channel opening and partial CCB mechanisms in the *M. alba* bronchodilatory effect. Interestingly, the plant extract was found more effective in the trachea than the jejunum. Tissue selective behavior of *M. alba* could be due to K^+ gates activating component(s), as K_{ATP} channels are known to be heterogeneous (Rudy, 1988; Aguilar-Bryan et al., 1998; Jenkinson, 2006) and some K_{ATP} channel openers are tissue selective. For example, a second generation K_{ATP} channel activator, rilmakalim is bronchial smooth muscle selective, while most of the first generation molecules, such as lemakalim and bimakalim seem to exhibit a greater potency in the vasculature (Weston and Edwards, 1992; Jacoby et al., 1993). We have experienced that plants with medicinal use in the overactive airways disorders, usually possess PDE inhibitory effect, co-exist with other spasmolytic mechanisms (Shah and Gilani, 2010; Khan et al., 2012b; Rehman et al., 2012). To investigate whether *M. alba* also exhibits PDE enzyme inhibition component(s), isoprenaline-inhibitory-CRCs were constructed against carbachol-induced contractions by pretreatment of tissues with *M. alba*, as PDE-inhibitors are known to potentiate the isoprenaline-inhibitory effect (Lorenz and Wells, 1983; Abdel-Haq et al., 2000). The presence of PDE-inhibitory effect in *M. alba* was confirmed when the plant extract potentiated isoprenaline relaxant effect by causing leftward shift of isoprenaline-induced inhibitory curves, similar to that caused by papaverine, a standard PDE inhibitor (Rang et al., 1999). These findings indicate that bronchodilator effect of *M. alba* is mediated through combined K_{ATP} channel opening, PDE inhibition and weak CCB pathways, hence provides sound mechanistic basis for its application in airways hyperactivity disorders. The PDE inhibitors are known to cause bronchodilatation by increasing intracellular level of cAMP and have been used for several decades in treatment of asthma (Weinberger and Hendeles, 1996; Barnes, 2006). Another possible explanation for the more affectivity of the plant extract in the trachea could be that K^+ channel activating and PDE-inhibitory components may exert synergistic interaction in airways, though species difference cannot be ruled out.

The results of preliminary phytochemical analysis reveal that *M. alba* extract contains flavonoids, sterols, tannins and terpenes. The flavonoids are well known for their spasmolytic activity (Di Carlo et al., 1993; Pietta, 1998; Ghayur et al., 2007) and the presence of such class of compounds in *M. alba* are likely to contribute in its antispasmodic and bronchodilatory effects, though the likely role of other constituents present in the plant cannot be ignored. In acute toxicity study, the extract was found safe up to the maximum dose (10 g/kg) tested, which is in line with the wide therapeutic and nutritional use of *M. alba.*

In summary these data indicate that *M. alba* possesses combination of spasmolytic activities, channeled through various mechanisms, including K_{ATP} channel activation, weak Ca^{++} antagonism and PDE inhibition. The predominant K^+ channel opening and mild Ca^{++} influx inhibition explains its antispasmodic action and combination of K_{ATP} gates activation, PDE inhibitory and partial CCB effects account for tracheal relaxation. Thus the present study provides pharmacological basis for the medicinal use of *M. alba*in hyperactive gut and respiratory disorders, like diarrhea and asthma. Moreover, the *in vivo* antidiarrheal and bronchodilatory activities prove effectiveness of the plant in such conditions and is a step forward towards the evidence based medicinal use of phytomedicine.

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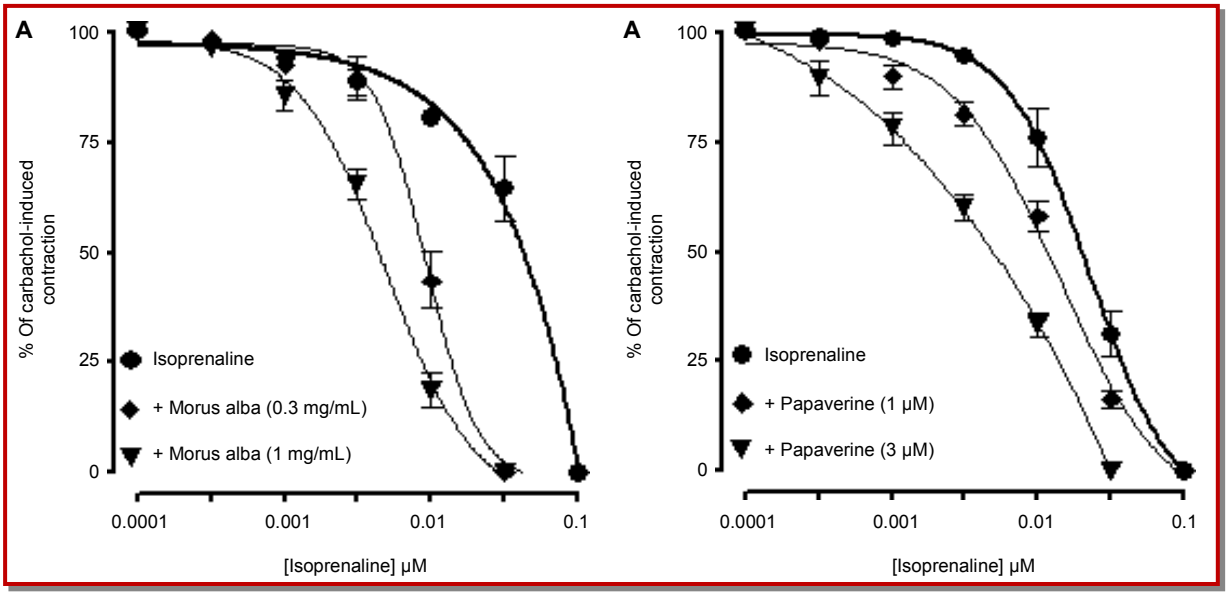

Figure 5: Inhibitory concentration-response curves of isoprenaline against carbachol-induced contractions in the absence and presence of different concentrations of (A) crude extract of *Morus alba* and (B) papaverine in isolated guinea-pig tracheal preparations. Values shown are mean ± SEM, n = 4-5

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