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Gastrointestinal and respiratory activities of *Acacia leucophloea*

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**Abstract**

*Ethnopharmacological relevance:* The barks of *Acacia leucophloea* (Fabaceae) are used in Pakistan traditional medicine as an astringent, a bitter, a thermogenic, a styptic, a preventive of infections, an anthelmintic, a vulnerary, a demulcent, an expectorant, an antipyretic, an antidote for snake bites and in the treatment of bronchitis, cough, vomiting, wounds, ulcers, diarrhea, dysentery, internal and external hemorrhages, dental caries, stomatitis, intermittent fevers and skin diseases (Selvanayagam et al., 1995; Pullaiah, 2002; Purohit et al., 2003; Anjaneyulu et al., 2010). An extract of stem bark and leaves of the plant is applied twice daily to cure psoriasis (Patil and Aher, 2010). Bark and leaves are used for treating renal edema, cardiac edema and indigestion. Leaf juice is administered to treat fever and stomachache and, mixed with cow’s milk, to bleeding piles (Alagesaboopathi, 2009). Traditionally all parts of plants are used against cancer, inflammation, ophthalmia, leprosy and to treat bleeding piles. Leaves are believed to possess hypotensive, CNS-depressant, antisyphilitic and antimicrobial activities while gum possess demulcent properties (Khare, 2007).

Two pimarane derivatives have been isolated from the plant (Bansal et al., 1980; Perales et al., 1980; Apreda Rojas et al., 2001).

**1. Introduction**

*Acacia leucophloea* (Roxb.) Willd. (Fabaceae), commonly known as “safed babul” is a little tree diffused in dry areas throughout Pakistan (Purohit et al., 2003). The barks of plant are used in traditional medicine as an astringent, a bitter, a thermogenic, a styptic, a preventive of infections, an anthelmintic, a vulnerary, a demulcent, an expectorant, an antipyretic, an antidote for snake bites and in the treatment of bronchitis, cough, vomiting, wounds, ulcers, diarrhoea, dysentery, internal and external hemorrhages, dental caries, stomatitis, intermittent fevers and skin diseases (Selvanayagam et al., 1995; Pullaiah, 2002; Purohit et al., 2003; Anjaneyulu et al., 2010). An extract of stem bark and leaves of the plant is applied twice daily to cure psoriasis (Patil and Aher, 2010). Bark and leaves are used for treating renal edema, cardiac edema and indigestion. Leaf juice is administered to treat fever and stomachache and, mixed with cow’s milk, to bleeding piles (Alagesaboopathi, 2009). Traditionally all parts of plants are used against cancer, inflammation, ophthalmia, leprosy and to treat bleeding piles. Leaves are believed to possess hypotensive, CNS-depressant, antisyphilitic and antimicrobial activities while gum possess demulcent properties (Khare, 2007).

Two pimarane derivatives have been isolated from the plant (Bansal et al., 1980; Perales et al., 1980; Apreda Rojas et al., 2001).
Nutritional characteristics of the seeds have been also reported: fatty acids in seed oil (Banerji et al., 1988; Devra et al., 2005), proteins and amino acids in seeds (Vijayakumari et al., 1994; Sidduraju et al., 1997). The roots of Acacia leucophloea contain antraquinones (Saxena and Srivastava, 1986).

Despite the traditional uses of the plant, no pharmacological studies have been reported in the literature about Acacia leucophloea. This study was conducted with the aim to explore the pharmacological bases for the traditional uses of the plant in gastrointestinal and respiratory disorders.

2. Materials and methods

2.1. Plant material and preparation of the crude extract

Barks of Acacia leucophloea were collected in Khanewal, a district of Punjab, Pakistan in June 2008 and authenticated by Miss Saima Shehzadi, at the Institute of Pure and Applied Biology, Bahaud-din Zakariya University, Multan. A voucher specimen of the plant (No.18-04-2008) is kept in the Herbarium of Bahauddin Zakariya University, Multan.

Barks of Acacia leucophloea were cleaned and grounded. The powdered material (1 kg) was soaked in 80% methanol for 8 days with occasional shaking. It was filtered through a muslin cloth and then through a filter paper. Filtrate was evaporated on under reduced pressure to a thick, semi-solid mass of dark red color (60 g). The methanol extract was solubilized in distilled water and DMSO for in vitro and in vivo experiments. All dilutions were prepared at the moment of the experiment.

2.2. Drugs

Acetylcholine chloride (ACh), loperamide, dicyclomine, atropine sulfate, carbachol, verapamil hydrochloride, nifedipine, adenosine 5’-diphosphate (ADP), histamine, were purchased from Sigma Chemicals Co., St. Louis, MO, USA. Castor oil was purchased from KCI Pharma, Karachi, Pakistan. Chemicals used for physiologi-cal salt solutions were potassium chloride, magnesium chloride, EDTA (Sigma Chemicals Co.), calcium chloride, glucose, magnesium sulfate, potassium dihydrogen phosphate, sodium bicarbonate, sodium dihydrogen phosphate and sodium chloride (Merck, Darmstadt, Germany). All reagents used were of analytical grade.

2.3. Animals

Local breed rabbits (1–1.8 kg), Sprague–Dawley rats (200–300 g), Balb-C mice (20–36 g) and guinea-pigs (500–600 g) of either sex were housed at the Animal house of the Aga Khan University, maintained at 23–25 °C. Animals were given tap water and a standard diet. Rabbits and rats had free access to water, but food was withdrawn 24 h prior to experiment. Rabbits and guinea pigs were sacrificed by cervical dislocation.

Experiments performed complied with the rulings of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council (1996) and approved by the Ethical Committee of the Aga Khan University.

2.4. Isolated tissue preparations

2.4.1. Rabbit tissue preparations

The spasmolytic activity of the plant material was studied by using isolated rabbit jejunum as previously described (Gilani et al., 2000). Segments of 2 cm were suspended in 10 ml of Tyrode’s solution and aerated with carbogen at 37 °C. The composition of the Tyrode’s solution (mM) was as follows: KCl 2.68, NaCl 136.9, MgCl2 1.05, NaHCO3 11.90, NaH2PO4 0.42, CaCl2 1.8 and glucose 5.55. A resting tension of 1 g was applied to each tissue and was kept constant throughout the experiment. Intestinal contractile and relaxant responses were recorded isotonically using a Harvard Transducer (Harvard Apparatus, Holliston, MA, USA) coupled with a Harvard Student Oscillograph (Harvard Apparatus, Holliston, MA, USA). Each tissue was allowed to equilibrate for at least 30 min before the addition of the drug. The preparation was stabilized with a sub-maximal concentration of acetylcholine (0.3 μM) at 3 min intervals until constant responses were recorded. Under these experimental conditions, rabbit jejunum exhibits spontaneous rhythmic contractions, allowing directly relaxant (spasmolytic) activity without the use of an agonist. To assess whether the spas-molytic activity of the test substances was through calcium channel blockade, K+ (as KCl) was used to depolarise the preparations (Farre et al., 1991). High K+ concentration (≥30 mM) is known to cause smooth muscle contraction through opening of voltage dependent L-type Ca2+ channels, thus allowing influx of extra cellular Ca2+ causing a contractile effect (Bolton, 1979); a substance causing inhibition of high K+ induced contraction is considered as a blocker of Ca2+ influx (Godfraind et al., 1986). A high K+ solution (80 mM) was added to the tissue bath, to produce a sustained contraction. Nifedipine and dicyclomine were used as positive controls. Test materials were then added in a cumulative fashion to obtain concentration dependent inhibitory responses (Van Rossum, 1963). The relaxation of intestinal preparation, pre contracted with K+ 80 mM was expressed as percent of the control response mediated by K+. To confirm the calcium antagonist activity of test substances, the tissue was allowed to stabilize in normal Tyrode’s solution, which was then replaced with Ca2+ free Tyrode’s solution containing EDTA (0.1 mM) for 30 min in order to remove Ca2+ from the tissues. This solution was further replaced with a K+ rich and a Ca2+ free Tyrode’s solution having the following composition (mM): KCl 50, NaCl 91.04, MgCl2 1.05, NaHCO3 11.90, NaH2PO4 0.42, glucose 5.55, and EDTA 0.1 mM. After an incubation period of 40 min, control concentration response curves (CRCs) of Ca2+ were obtained. When the control CRCs of Ca2+ were found superimposable (usually after two cycles), the tissue was pre-treated with the plant extract for 60 min to test a possible calcium channel blocking effect. The CRCs of Ca2+ were developed in the presence of different concentrations of the test material.

2.4.2. Guinea-pig ileum

The ileum was dissected and kept in Tyrode’s solution. The seg-ments, about 2 cm long, were mounted individually in a 10 ml tissue bath, filled with Tyrode’s solution, at 37 °C and aerated with carbogen. An initial load of 0.7 g was applied to the tissue and isotonic contractions were recorded with a Bioscience Transducer coupled to a Harvard oscillograph (Harvard Apparatus, Holliston, MA, USA). An equilibrium period of 30 min was required before administration of drugs. After this equilibration period, the tissues were repeatedly treated with sub-maximal concentration (0.3 μM) of acetylcholine chloride (Ach) with 3 min interval until constant responses were recorded. The inhibitory effect of the test mate-rial was assessed as percent of the maximum effect produced by acetylcholine.

2.4.3. Rabbit trachea

Trachea was dissected and kept in Kreb’s solution. The tracheal tube was cut into rings, 2–3 mm wide, each containing about three to four cartilages. Each ring was opened by a longitudinal cut on the ventral side opposite to the smooth muscle, forming a tracheal chain with smooth muscle in the centre and cartilaginous portions on the edges. The preparation was mounted in a 20 ml tissue bath containing Kreb’s solution at 37 °C, aerated with carbogen. The composition of Kreb’s solution was (mM): NaCl 118.2, NaHCO3 25.0, CaCl2 2.5, KCl 4.7, KH2PO4 1.3, MgSO4 1.2 and glucose
11.7 (pH 7.4). A tension of 1 g was applied to the tracheal strips continuously throughout the experiment. The tissue was equilibrated for 30 min before the addition of any drug. Carbachol (1.0 μM) or K+ (80 mM) were used to stabilize the respective preparations until constant responses of each agonist were achieved (usually three to four concentrations). When sustained contractions were obtained, the relax fashion isometric responses were registered on a Grass model 7 Polygraph (Grass Instrument Company, Quincy, MA, USA).

2.5. Anti-diarrheal activity

The experiments were carried out by methods employed previously in our laboratory (Gilani et al., 2008). Mice were fasted for 24 h before the experiment. The animals were housed in individual cages and divided in five equal groups of six mice each. The first group received saline as vehicle control (10 ml/kg, orally) and thus acted as negative control. First group of mice was treated with loperamide (10 mg/kg), second group was treated with dicyclomine as positive control while other three groups were treated with test materials, 100, 300 and 500 mg/kg, respectively. One hour after the treatment, each animal received 10 ml/kg of castor oil orally through a feeding needle. Afterwards, the cages were critically inspected for the presence of the diarrheal wet feces and dry feces.

2.6. Statistical analysis

All the data expressed are the mean of three experiments ± standard error of the mean (SEM, n = number of experiments) and the median effective concentrations (EC50) with 95% confidence intervals. p-Value < 0.05 was considered statistically significant. Concentration–response curves were analyzed by non-linear regression (Graph Pad prism 5.04).

3. Results and discussion

Due to the traditional use as a gastrointestinal drug, a methanol extract from barks of Acacia leucophloea was tested for its possible spasmylic effects on spontaneously contracting rabbit jejunum preparation. Fig. 1 shows the inhibition of the spontaneous contractions, suggesting a spasmylic activity. To investigate the possible mechanism behind this effect, a high dose of K+ (>30 mmol/L) is known to cause the smooth muscles contractions through opening of voltage dependent Ca2+ channels, thus allowing an influx of extracellular Ca2+ that causes a contractile effect (Bolton, 1979). A substance provoking the inhibition of high K+-induced contractions, is thought to be a blocker of Ca2+ influx (Godfraind et al., 1986). When tested in isolated rabbit jejunum, the extract showed concentration-dependent (0.1–3 mg/ml) relaxation of spontaneous contractions and high K+ (80 mM)-induced contractions (0.3–5 mg/ml) with EC50 values of 0.6910 mg/ml (0.5551–0.8601, 95% CI n = 10) and 0.8247 mg/ml (0.5590–0.125, n = 09), respectively, as shown in Fig. 2A. Similarly, nifedipine (a Ca channel blocker) potentiated the relaxation effect of extract; this suggests that extract contains substances with Ca channel blocking properties. Nifedipine relaxed the spontaneous contractions and high K+ (80 mM)-induced contractions with respective EC50 values of 0.13 μM (0.104–0.164, n = 4) and 0.013 μM (0.0096–0.0179, n = 4) (Fig. 2B). The total relaxation by the combined effects of Nifedipine and Acacia leucophloea extract may be due to a synergistic blockage of calcium channels, hence producing a relaxation that could not be produced by the extract alone. The blockage of the calcium channels would result in a reduced influx of calcium ions into the sarcoplasmatic reticulum, thus causing a reduction in cytosolic calcium ion concentration which in turn causes a reduced binding of calcium to the protein calmodulin. The calcium calmodulin complex should activate myosin light chain kinase with the resultant phosphorylation of the light chains. If such phosphorylation occurs, interaction between actin and myosin is promoted, resulting in smooth muscle contraction. Hence inhibition of calcium would result in a break in the cascade producing relaxation. Dicyclomine, a standard inhibitor of muscarinic receptors and Ca2+ influx (Downie and Twiddy, 1977) used as a positive control, also showed a similar inhibitory pattern against the spontaneous and high K+ (80 mM-induced contractions with respective EC50 values of 0.1.158 μM (0.8721–4.538, n = 3) and 4.332 μM (3.287–5.109, n = 3) (Fig. 2C). Dicyclomine, a dual blocker of muscarinic receptors and Ca2+ influx (McGrath et al., 1964; Downie and Twiddy, 1977), showed a similar pattern of inhibition, while nifedipine, a standard Ca2+ channel blocker (Fleckenstein, 1977) was a more potent against the high K+-induced contractions. Comparing our data, it can be hypothesized that the methanol extract of the plant acts through a combined blockade of Ca2+ influx and muscarinic receptors. However, positive relaxing effect on the KCl-induced contraction does not imply that the test substance possess a calcium channel blocking activity; therefore, the presence of dual mode of inhibitory activity, involving both Ca2+ antagonists and an anticholinergic activity, was confirmed considering tests carried out on rabbit jejunum and guinea pig ileum.

In jejunum, the methanol extract of Acacia leucophloea shifted Ca2+ curves to the right with the suppression of maximum response, similar to that caused by nifedipine and dicyclomine. Calcium channel blocking activity was confirmed when pretreatment of tissue with the methanol extract of Acacia leucophloea (0.3–1 mg/ml) caused a rightward shift of concentration response curves (CRCs) of Ca2+ with a significant suppression (p < 0.01) of the maximum response of Ca2+ (taken as 100%) to a level of 66.9 ± 8.5% (n = 5) and 30.4 ± 3.8% (n = 4), at 1 and 3 mg/ml, respectively (Fig. 3A). Nifedipine shifted the CRCs of Ca2+ to the right with a significant suppression (p < 0.001) of the maximum effect to a level of 73.5 ± 5.3% (n = 4) and 36.2 ± 5.1% (n = 5) at 0.03 and 0.1 μM, respectively (Fig. 3B). Similarly, dicyclomine also produced a rightward shift in the CRCs of Ca2+ and attenuated (p < 0.01) its maximum response to 56.4 ± 3.7% (n = 5) and 27.3 ± 6.8% (n = 5), at tested concentrations of 3 and 10 μM, respectively (Fig. 3C). In jejunum, the plant extract inhibited high K+-induced contractions, similar to dicyclomine, while nifedipine, a known Ca2+ antagonist was more potent against K+-induced contractions, a typical characteristic of Ca2+ antagonist (Fleckenstein, 1977).

A similar pattern of inhibition suggested its possible anticholinergic action. For this purpose acetylcholine concentration response
Fig. 2. Concentration dependent inhibitory effects of: (A) a methanol extract of *Acacia leucophloea* (Al.Cr.), (B) nifedipine and (C) dicyclomine on spontaneous and high K⁺-(80 mM) induced contractions in isolated rabbit jejunum preparations (values are expressed as the mean ± SEM, n = 3–5).

Fig. 3. Concentration response curves of Ca²⁺ in the absence and presence of increasing concentrations of: (A) a crude extract of *Acacia leucophloea* (Al.Cr.), (B) a nifedipine and (C) dicyclomine in isolated rabbit jejunum preparations (values are expressed as the mean ± SEM, n = 3–5).
curves were constructed in guinea pig ileum. Guinea-pig ileum is known to be a quiescent preparation and is considered suitable to construct CRCs for the stimulant effect and to quantify contractile responses of an agonist in the presence of an inhibitor, thus allowing exploration of the nature of antispasmodic effect (Mehmood et al., 2011). The CRCs of ACh were constructed in the absence and presence of the test material to assess the nature of interaction with ACh receptors. The pretreatment of the tissue with the extract at 0.3 mg/mL caused a rightward parallel shift in the control CRCs of ACh (maximum contractile response of ACh was taken 100%), without suppression (p > 0.05) of the maximum contractile response (97.0 ± 2.3% vs. 100%) (mean ± SEM, n = 4), a typical characteristic of anticholinergic drug. Whereas, at the next tested concentration (1 mg/mL), the extract produced a non-parallel shift with a significant suppression (p < 0.001) of the maximum response (55.3 ± 6.1% vs. 100%) (n = 4) (Fig. 4A). A parallel displacement of ACh-curves without the suppression of maximum response was observed at lower concentrations, a characteristic of a competitive or specific antagonist, like atropine (Gilani and Gobbin, 1986; Eglen and Harris, 1993). This displacement was followed by a non-parallel shift with the suppression of maximum response at the next higher concentrations, suggesting a non-competitive inhibition (Vanden Brink, 1973), known with Ca\(^{2+}\) antagonist (Irie et al., 2000). Similarly, dicyclomine shifted the CRCs of ACh to the right in a parallel manner without suppression (p > 0.05) of the maximum response (94.4 ± 5.4%) (n = 5) at 0.03 µM, while, at concentration of 0.1 µM, it caused a rightward non-parallel shift in the ACh CRCs with a marked suppression (p < 0.001) of the maximum effect (57.8 ± 5.1%) (n = 5) (Fig. 4B). This confirmed that the presence of spasmylic effect in Acacia leucophloea is mediated through dual inhibition of muscarinic receptors and Ca\(^{2+}\) channels.

Experiments were carried on the rabbit tracheal preparations to explain the bronchodilator activity of the methanol extract from Acacia leucophloea bark. The methanol extract caused complete relaxation of carbachol (1 µM) and high K\(^{+}\) (80 mM)-induced contractions in rabbit tracheal preparation in a concentration-dependent way with EC\(_{50}\) values of 0.219 mg/ml (0.0.1578–0.734, n = 5) and 0.6589 mg/ml (0.46–0.94, n = 5), respectively (Fig. 5A). Similarly, dicyclomine also caused the relaxation of carbachol (1 µM) and high K\(^{+}\) (80 mM)-induced contractions with EC\(_{50}\) values of 0.339 (0.272–0.420, n = 7) and 3.30 µM (2.399–4.54, n = 5), respectively (Fig. 5B). Carbachol, being a cholinomimetic drug, increases free intracellular calcium ions (Jan et al., 2004). Pretreatment of tracheal preparation with the methanol extract of Acacia leucophloea, at a concentration range of 0.3–3.0 mg/ml, shifted the control concentration–response curves to the right (Fig. 6A) in a manner similar to that of dicyclomine (Fig. 6B). Similarly to the gut, the crude extract caused the inhibition of the high K\(^{+}\) and carbachol-induced contractions and displaced the Ca\(^{2+}\)-curves to the right with in a parallel fashion without suppression of the
maximum response at low concentration, followed by non-parallel shift with suppression of the maximum effect at the next higher concentration. The extract provoked a relaxation of carbachol-induced contractions at lower concentrations, but high K⁺-induced contractions at a little higher concentrations, suggesting the co-existence of anticholinergic and Ca²⁺ antagonist properties, like dicyclomine.

Both the crude extract and dicyclomine were slightly more effective in tracheal preparations than in the gut preparation. This could be possibly due to a difference in the physiological modulators among various tissues (Gayton and Hall, 1996) causing a better synergistic interaction between different spasmylic mechanisms in the trachea compared to the gut although species difference cannot be ruled out (Ghayur et al., 2005).

Acacia leucophloea methanol extract exhibited dose-dependent (100–500 mg/ml) protective effect against castor oil induced diarrhea in mice as shown by decreased in the total number of faeces (Fig. 7). The extract probably stimulated the re-absorption of water from the intestinal lumen, resulting in the normalization of the deranged water transport across the mucosal cells, probably by the ability of the extract to inhibit intestinal motility. The negative control group (saline treated) did not show any protection against the castor oil-induced diarrhea. Pretreatment of animals with the plant extract resulted in the protection from diarrhea (p < 0.05 vs saline group). The induction of diarrhea with castor oil resulted from the action of ricinoleic acid formed in the hydrolysis of the oil (Iwao and Terada, 1962) which produces changes in transport of electrolyte and water, resulting in the generation of maximum contractions of transverse and distal colon (Izzo et al., 1994). Thus, a potential anti-diarrheal agent may exhibit its effect by the inhibition of the bowl contractions (Di Carlo et al., 1993). Acacia leucophloea methanol extract showed significant anti-diarrheal activity. This was expected, as anticholinergic drugs and Ca²⁺ antagonists, possess anti-diarrheal activity (Reynolds et al., 1984; Rang et al., 2007).

From the above results, it is possible to hypothesize that the antispasmodic effect of barks of Acacia leucophloea can be mediated through a combination of anticholinergic as well as Ca²⁺ channel-blocking actions. This study offers rational hypotheses for the medicinal uses of Acacia leucophloea bark in treating gastrointestinal and respiratory disorders.

References