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Abstract

This study was designed to evaluate bronchodilator and cardio-tonic effects of *Urginea indica* to provide rational for these medicinal uses. *U. indica* bulb extract was studied on rabbit tracheal and guinea-pig atrial preparations mounted in tissue baths under simulated physiological conditions. *U. indica* inhibited carbachol (1 μ M) and K⁺ (80 mM)-induced contractions in rabbit trachea, similar to dicyclomine, suggesting the presence of anticholinergic and calcium channel blocking (CCB) mechanisms in *U. indica*. Anticholinergic and CCB effects of *U. indica* were respectively confirmed when it shifted the carbachol and Ca²⁺ concentration-response curves rightwards, similar to dicyclomine. *U. indica* (0.01-1 mg/mL) increased force of guinea-pig atrial contractions without significantly affecting the rate. These data, indicating that *U. indica* possesses the bronchodilator activity possibly mediated through a combination of anticholinergic and Ca²⁺ antagonist mechanisms together with selective positive inotropic effect, provide rational for medicinal applications of *U. indica* in airways and cardiac disorders.

Introduction

Urginea indica Kunth., belonging to the family liliaceae, is a perennial glabrous herb commonly known as "Indian squill" and locally in Pakistan as "Jungli-piyaz", where it grows in Salt Range, Kotli Near Mirpur and Mt.Tilla (Baquar, 1989). In the indigenous traditional system of medicine, *U. indica* is reputed for a number of therapeutic benefits, for which bulb or rhizome are the most commonly employed plant parts. It is chiefly used in chronic bronchitis and asthma. The other actions attributed to *U. indica* are anthelmintic, cardiotoxic in heart insufficiency, deobstruent, digestive, expectorant, stomachic, diuretic, emmenagogue and purgative, in addition to its use in calculous and paralytic affections, rheumatism, leprosy, skin diseases, internal pain and scabies (Baquar, 1989; Kirtikar and Basu, 1988; Prajapati et al., 2003). Bulbs crushed or sliced are also applied under the sole of feet to prevent

burning sensation (Kapoor, 1990; Usmanghani et al., 1997) and are externally used for removing corns and warts (Kapoor, 1990; Prajapati et al., 2003).

Among phytochemical constituents, the glycosides, scillaridin-A and scillaridin-B have been reported to be present in fresh squill (Prajapati et al., 2003). Other constituents found in squill include flavonoids, carbohydrates, antifungal glycoproteins, steroids, alkaloids, tannins, coumarins and saponins (Abbas et al., 2012; Kameshwari et al., 2012).

Pharmacological evaluations have revealed the presence of antibacterial, antifungal (Shenoy et al., 2006), laxative and spasmodic (Abbas et al., 2012), antioxidant, antiangiogenic and pro-apoptotic activities in *U. indica* (Deepak and Salimath, 2006).

Despite of its extensive medicinal application in airways hyperactivity disorders and also in cardiac disor-



ders, *U. indica* has not been studied widely to evaluate these medicinal uses. This study was aimed to provide pharmacological basis for the medicinal use of *U. indica* in broncho-spastic disorders like asthma and as cardiac stimulant.

Material and Methods

Plant material and preparation of crude extract

The bulbs of *U. indica* were collected fresh from fields of Mianwali subsequent to the identification of the plant by an expert taxonomist at the Institute of Pure & Applied Biology, Bahauddin Zakariya University, Multan, Pakistan. A specimen of the plant has been deposited at herbarium of the same institute (voucher no. P. Fl 59-1843). The plant material was washed for any contaminants and subjected to shade drying. The dried plant material (400 g) was ground into coarse powders through electrically driven device and the powder was soaked in 70% aqueous-methanol (v/v) for three days in amber colored glass bottles with occasional shaking. The soaked material was passed through double layered muslin cloth to remove vegetative debris and the obtained fluid was subsequently filtered through filter paper (Williamson et al., 1998). The residue was re-soaked for next three days and the procedure repeated twice. The filtrates were evaporated on a rotary evaporator (R-210, BUCHI, Switzerland) under reduced pressure (-760 mmHg) at 37°C to a thick, semi-solid paste of dark brown colour, the crude extract of *U. indica* bulb yielding 10%. *U. indica* extract was solubilized in distilled water for all *in vitro* experiments.

Animals

Animals used in the study were rabbits of local breed and either sex; housed at the Animal House of the Aga Khan University, Karachi, maintained at 23-25°C. Animals were provided with standard diet and tap water *ad libitum*. Experiments were performed in compliance with the rulings of the Institute of Laboratory Animals Resources, Commission on Life Sciences, National Research Council (NRC, 2011).

Chemicals

Acetylcholine chloride (ACh), carbachol (CCh) and dicyclomine were purchased from Sigma Chemical Co., USA. Chemicals used for preparing physiological salt solutions were ethylenediamine tetra-acetic acid (EDTA), potassium chloride (Sigma Chemical Co.), calcium chloride, glucose, magnesium chloride, magnesium sulfate, potassium dihydrogen phosphate, sodium dihydrogen phosphate, sodium chloride and sodium bicarbonate (Merck, Darmstadt, Germany). All chemical used were of the analytical grade available. Stock solutions of all the chemicals were made in distilled water and their dilutions were made fresh on the day of

experiment. The vehicle used for solubilization of drugs had no effect on tissue contractility in the control experiments.

Isolated tissue experiments

Isolated tissue experiments were performed following the methods previously employed in our laboratory (Gilani et al., 1997; 2007; 2012).

Trachea was dissected out and kept in Kreb's solution. The trachea was cleaned free from the surrounding fatty tissues and cut into rings having 2-3 mm width (containing 2 cartilages). Each ring was then opened by longitudinal cut on the side opposite to the smooth muscle layer in such a way that smooth muscles were in between the C-shaped cartilaginous part. The isolated preparations were then mounted in tissue baths containing Kreb's solution and aerated with carbogen at 37°C. 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 and equilibrated for 1 hour before the addition of any chemical substance. CCh (1 µM) was used to stabilize the preparations until constant responses to successive treatments were achieved (usually after 3-4 exposures). The bronchodilator activity of the plant extract and control drugs was studied on CCh (1 µM) or high K⁺-induced sustained contractions, by adding them in a cumulative manner. Cumulative curves to CCh were constructed using increasing concentration of agonist. When a 3-fold increase in concentration produced no further increment in response, the tissue was washed to re-establish the base-line tension. The CCh CRCs were repeated in the presence of increasing concentrations of *U. indica* and dicyclomine. Isometric responses were recorded by force transducer (model FORT100) coupled to a Transbridge (model TBM4M, World Precision Instruments, Hertfordshire, UK) and PowerLab data acquisition system (model ML845, AD Instruments, Australia) and computer running Chart software (version 6).

Paired atria from healthy guinea-pigs were dissected out, cleaned of fatty tissues and mounted in 15 mL tissue organ baths containing Kreb's solution, at 32°C and the tissues were aerated with carbogen. The tissues exhibited spontaneous beating under the resting tension of 1 g due to the presence of pacemaker cells. An equilibrium period of 30 min was provided before the application of any chemical substance. Control responses of ACh (0.3 µM) and isoprenaline (1 µM) were obtained at least in duplicate. Tension changes in the tissue were obtained via a Grass force-displacement transducer (model FT-03) and were recorded using Grass Model 7 Polygraph.

Statistical analysis

All the data expressed are mean \pm standard error of mean (SEM, n = number of experiments) and the median effective concentration (EC_{50}) with 95% confidence intervals (CI) concentration-response curves (CRCs) were analyzed by non-linear regression using GraphPad program (GraphPAD, San Diego, CA, USA). The statistical parameter applied is the Student's t -test. $p < 0.05$ was considered significantly different.

Results

U. indica caused relaxation of tracheal preparations pre-contracted with CCh ($1 \mu\text{M}$) and K^+ (80 mM) with respective EC_{50} values of 0.1 mg/mL ($0.1\text{-}0.2$, $n = 6$) and 0.8 mg/mL ($0.5\text{-}1.2$, $n = 6$; Figure 1A). Dicyclomine

similarly was found to be more potent in inhibiting CCh ($1 \mu\text{M}$) induced contractions compared to that against high K^+ with respective EC_{50} values of $0.2 \mu\text{M}$ ($0.1\text{-}0.2$, $n = 5$) and 1.8 ($1.2\text{-}2.5$, $n = 5$; Figure 1B).

When studied for its possible anticholinergic action, *U. indica* produced rightward parallel displacement of the CCh CRCs without suppression of the maximum contractile response at 0.03 mg/mL , followed by non-parallel shift with suppression of the maximum effect at next higher concentration (0.1 mg/mL ; Figure 2A). Dicyclomine (0.03 and $0.1 \mu\text{M}$) exhibited a similar pattern of shift in CCh CRCs (Figure 2B).

When studied for its effect on Ca^{2+} influx, *U. indica* shifted Ca^{2+} CRCs towards right in a concentration-

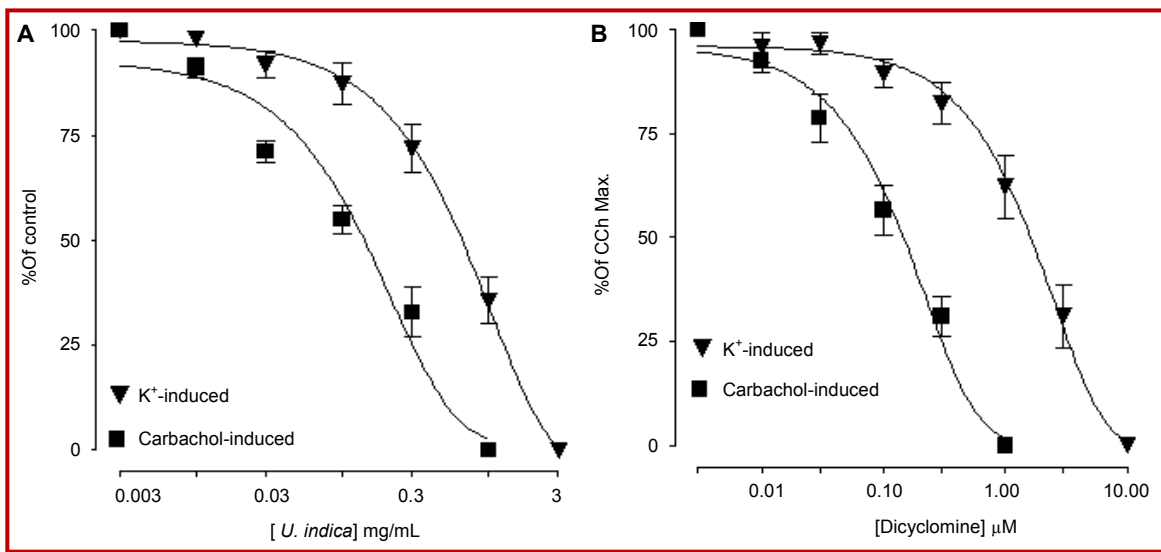


Figure 1: Concentration-dependent inhibitory effects of (A) crude extract of *U. indica* and (B) dicyclomine on K^+ (80 mM) and carbachol ($1 \mu\text{M}$)-induced contractions in isolated rabbit trachea. Values are shown as mean \pm SEM, $n = 5\text{-}6$.

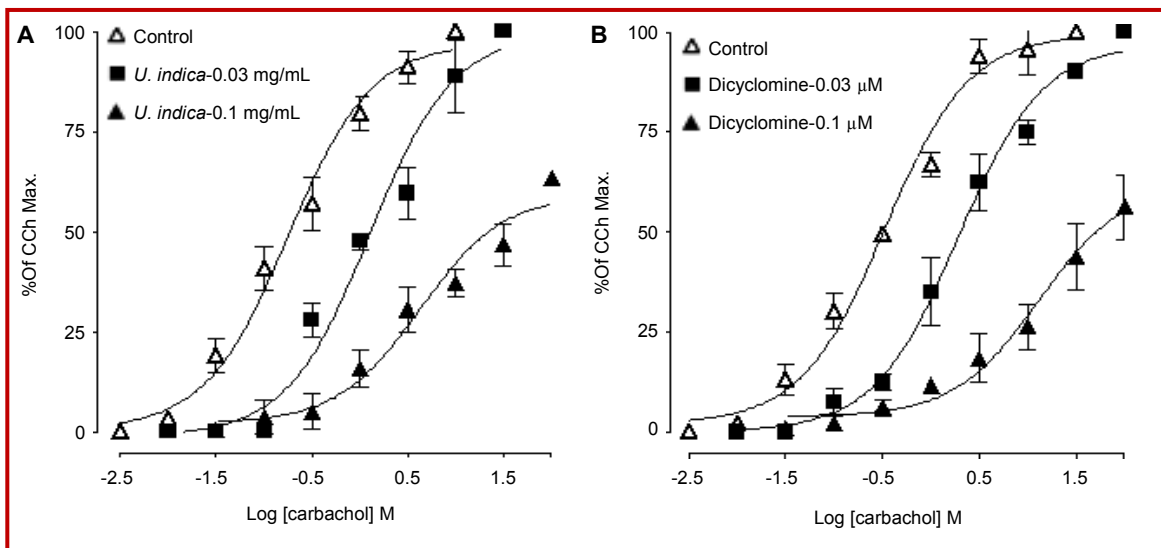


Figure 2: Concentration-response curve of carbachol in the absence and presence of (A) crude extract of *U. indica* and (B) dicyclomine in isolated rabbit tracheal preparations. Values are expressed as mean \pm SEM, $n = 2\text{-}4$.

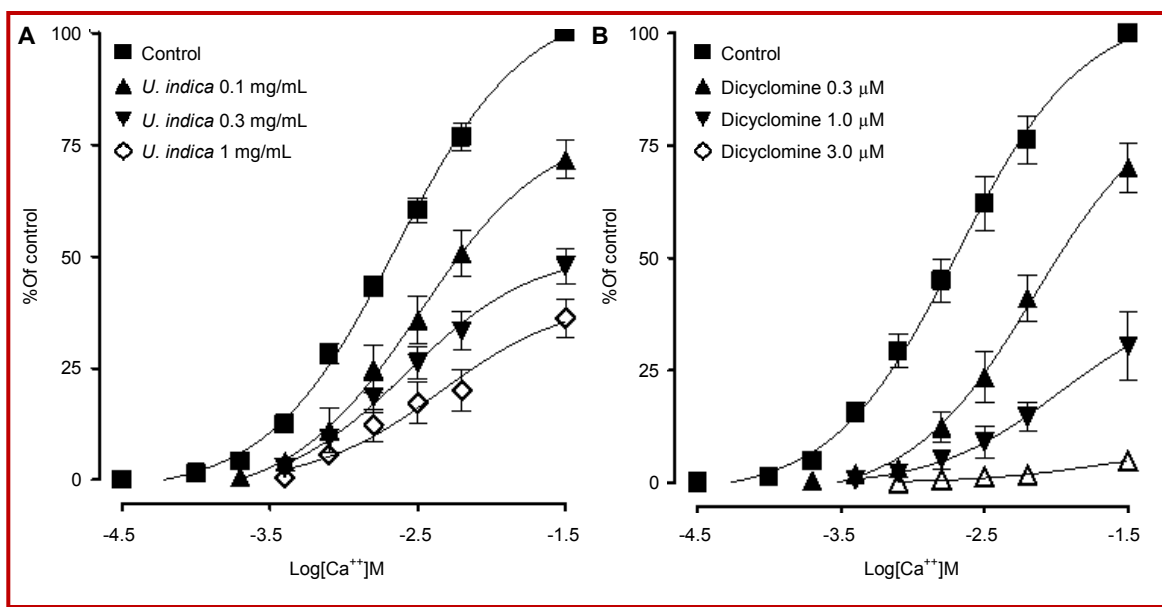


Figure 3: Concentration-response curve of Ca^{2+} in the absence and presence of (A) crude extract of *U. indica* and (B) dicyclomine in isolated rabbit trachea. Values are expressed as mean \pm SEM, $n = 4$

dependent manner (0.1-1 mg/mL), similar to dicyclomine (0.3-3 μM ; Figure 3A and B).

U. indica caused a concentration-dependent (0.01-1.0 mg/mL) increase in the force of spontaneous contractions of paired atria followed by inhibition at the higher concentrations (3.0-10.0 mg/mL) without significantly affecting the rate of contractions (Figure 4).

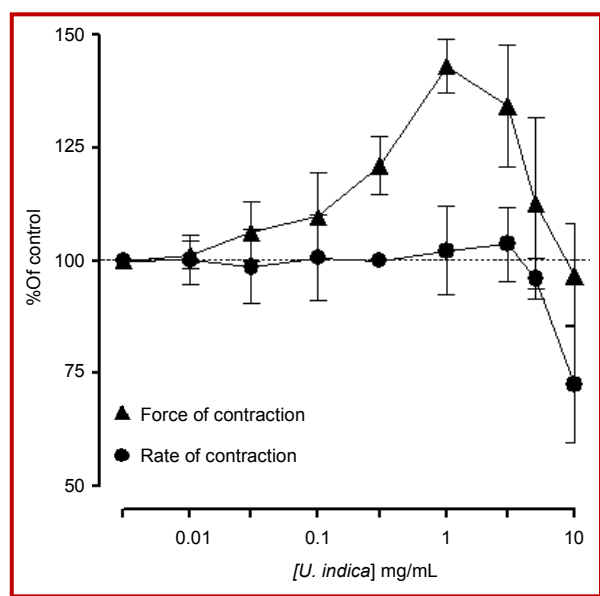


Figure 4: Effect of crude extract of *U. indica* on force and rate of contraction in isolated guinea pig atria. Values are shown as mean \pm SEM, $n = 3$.

Discussion

U. indica plant has traditionally been used to relieve airways disorders including asthma and bronchitis, therefore, the current study was undertaken to see primarily its bronchodilator effect to rationalize the medicinal uses.

The proposed broncho-relaxant activity of *U. indica* was investigated on CCh (1 μM) and high K^{+} -induced contractions in isolated rabbit tracheal preparations. *U. indica* exhibited relaxant effect on both the contractions with higher potency against CCh-induced contraction, in a manner similar to dicyclomine, a dual blocker of muscarinic receptors and Ca^{2+} influx (Downie et al., 1977; McGrath et al., 1964).

To confirm the antimuscarinic activity, effect of the plant extract was studied on CCh-concentration-response curves constructed in the isolated rabbit tracheal preparations. At lower concentration, *U. indica* caused rightward displacement of the curve without suppression of the maximum response and at higher concentrations, it shifted the curve in a non-parallel manner with suppression of the maximum response. The parallel shift of CCh-curves at lower concentration of *U. indica* without suppression of maximum response is indication for blockade of muscarinic receptors in a competitive manner; whereas, non-parallel shift of CCh-curves observed at higher concentration of *U. indica* with suppression of the maximum response can be attributed to the presence of some non-competitive inhibitor like that exhibited by a Ca^{2+} -channel blocker. The CCB effect was confirmed when pretreatment of

the tissue with *U. indica* also produced a concentration-dependent rightward shift in the Ca^{2+} concentration-response curve, similar to dicyclomine.

The Ca^{2+} channel blockers have been found to be useful in broncho-spastic disorders (Ahmed, 1992; Mathewson, 1985) and muscarinic antagonists are also included as present day treatment for the relief from asthma and similar diseases (Boushey, 2006). The tone of broncholar smooth muscles is regulated by the parasympathetic division of autonomic nervous system and reflex increase in parasympathetic activity may contribute toward bronchoconstriction because the respiratory tract is rich in cholinergic innervations through vagal fibers linked to M_1 muscarinic receptors situated in the mucosal surface of the respiratory tract. The mucus secretions are also involved in adding up miseries to the pathology of the respiratory tract particularly sub-mucosal glands are rich in parasympathetic innervations mostly through M_3 receptors and this may be one of the plausible explanations using muscarinic antagonist in chronic obstructive pulmonary disease as well as asthma (Barnes and Hansel, 2004).

In isolated guinea-pig atria, *U. indica* produced positive inotropic effect at lower concentrations followed by inhibition of the contractile force at higher concentrations, suggesting the presence of dose-related cardiotonic effect in *U. indica*. Indian squill is already been used in its crude form for the treatment of cardiac insufficiency but there is not report on cardiac stimulant effect of the plant itself, however the plant is reported to contain scillaren-A and scillaren-B (Prajapati et al., 2003), the cardio-active glycosides producing their effect through inhibition of Na^+/K^+ -ATPase, similar to that of digitalis glycosides (Barceloux, 2008). Cardiac inhibitory effect observed at higher concentrations could possibly be the result of the presence of Ca^{2+} channel blocking activity in *U. indica*. These results, showing cardiac stimulant effect in *U. indica*, explain this use of the plant in traditional medicine for cardiac insufficiency.

These investigations indicate that the crude extract of *U. indica* possesses bronchodilator activity possibly mediated through a dual blockade of Ca^{2+} influx and muscarinic receptors and cardiac stimulant effect through selective inotropic effect, which can be the possible reason for its medicinal use in airways disorders and cardiac insufficiency.

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