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Gastrointestinal Stimulant Effect of *Urginea indica* Kunth. and Involvement of Muscarinic Receptors

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**INTRODUCTION**

Digestive diseases, affecting 70% of the general population, are the cause of considerable social and economical impact (Ouyang and Chen, 2004). Constipation, diarrhea and dyspepsia, in particular, are commonly prevailing disorders (Mehmood *et al*., 2010). Medicinal plants are usually preferred to treat these gastrointestinal disorders, because they contain multiple constituents with effect enhancing and/or side-effect-neutralizing potential (Gilani and Rahman, 2005), and, hence, are considered relatively safe in prolonged use. Seed husk of *Plantago ovata* (psyllium husk), for example, is a widely used remedy equally popular among traditional healers and modern physicians for digestive disorders and has been reported to be used as an anthelmintic, cardiac stimulant, deobstruent, diuretic, emmenagogue and for the treatment of calculous affections, cough, bronchitis, asthma, paralytic affection, rheumatism, leprosy, skin diseases, internal pain and scabies (Kirtikar and Basu, 1988; Baquar, 1989; Prajapati *et al*., 2003). Bulbs crushed or sliced are also applied under the sole of the feet to prevent burning sensation (Kapoor, 1990; Usmanghani *et al*., 1997) and are used externally for removing corns and warts (Kapoor, 1990; Prajapati *et al*., 2003).

The plant has been reported to contain glycosides including scillaren-A and scillaren-B (Prajapati *et al*., 2003). *Urginea indica* has been studied for its medicinal effect as an antifungal (Shenoy *et al*., 2006), antiangiogenic and pro-apoptotic (Deepak and Salimath, 2006).

Despite its wide medicinal application, *Urginea indica* has not been studied extensively to evaluate its medicinal uses pertaining to the gastrointestinal stimulant effect. This study was therefore undertaken to provide a pharmacological rationale for the use of *Urginea indica* bulb as a laxative, digestive and stomachic.

**MATERIALS AND METHODS**

Plant material and preparation of crude extract. The bulbs of *Urginea indica* were collected fresh from fields of Mianwali subsequent to the identification of the plant by an expert taxonomist at the Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan. A specimen of the plant has been deposited at...
the 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 an electrically driven device and the powder was soaked at room temperature in 70% aqueous methanol (v/v) for 3 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 the next 3 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 *Urginea indica* bulb (Ui.Cr) yielding 10%. Ui.Cr was solubilized in distilled water for all *in vitro* experiments.

**Preliminary phytochemical analysis.** Qualitative phytochemical analysis of Ui.Cr was done for the detection of alkaloids, saponins, anthraquinones, coumarins, sterols, terpenes and flavonoids by previously described methods (Evans, 2006).

**Drugs and animals.** Acetylcholine chloride (ACh), atropine sulphate and carbachol (CCh) were purchased from Sigma Chemical Co., St Louis, MO, USA. Chemicals used for making physiological salt solutions were ethylenediamine tetra-acetic acid (EDTA), potassium chloride (Sigma Chemical Co.), calcium chloride, glucose, magnesium chloride, magnesium sulphate, potassium dihydrogen phosphate, sodium dihydrogen phosphate sodium chloride and sodium bicarbonate (Merck, Darmstadt, Germany). All chemicals 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.

Animals used in the study were rabbits, guinea-pigs and mice of local breed and either sex; housed at the Animal House of the Aga Khan University, Karachi, maintained at 23–25°C with 12 h light/dark cycle. Animals had free access to standard diet and tap water except that 24 h before commencement of the experiments, food was withdrawn but water was available *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, 1996) and approved by the Ethical Committee of the Aga Khan University, Karachi.

**Charcoal meal gastrointestinal transit test.** The method previously followed by Gaginella et al. (1994) was used with slight modifications. Mice (20–30 g) were divided into four groups of six mice each and fasted for 24 h before the experiment. One group serving as the negative control was treated with normal saline (10 mL/kg, p.o.). Two of the groups were treated with two increasing doses of the extract (6 and 12 mg/kg), serving as the test groups and the last group was administered carbachol (10 mg/kg), a standard cholinergic agent, as the positive control. After 15 min, the animals were given orally 0.3 mL of freshly prepared charcoal meal (distilled water suspension containing 10% gum acacia, 10% vegetable charcoal and 20% starch). Following 30 min of charcoal administration, the mice were killed by cervical dislocation and the abdomen immediately cut open, to excise the whole small intestine (pylorus region to caecum). The length of the small intestine and the distance between the pylorus region and the front of the charcoal meal was measured to obtain the charcoal transport ratio or percentage.

**Laxative activity.** The method of Haruna (1997) was followed for this activity. Mice (20–25 g), fasted for 24 h before the experiment, were placed individually in cages lined with clean filter paper and divided into seven groups of six animals each to receive the following treatments. The first group, acting as the negative control, was administered with saline (10 mL/kg, p.o.). The second and third groups, acting as the test groups, received, orally, 6 and 12 mg/kg of the Ui.Cr. The fourth group, serving as the positive control, received carbachol (10 mg/kg, p.o.). The fifth, sixth and seventh groups received atropine (10 mg/kg) 30 min prior to administering the above said doses of Ui.Cr and carbachol, respectively, for their laxative effect. The feces production was monitored for 6 h in all seven groups for the total number as well as for consistency. The laxative effect of a given treatment was measured as an increase in the number of total feces over that of the negative control.

**Isolated tissue experiments.** Isolated tissue experiments were performed following the methods previously employed in our laboratory (Gilani et al., 2007, 2009).

**Guinea-pig ileum.** The ileum was dissected and kept in Tyrode’s solution. The segments, about 2 cm long, were suspended individually in a 10 mL tissue bath, filled with Tyrode’s solution, aerated with carbogen and maintained at 37°C. The initial tension of 0.7 g was applied and each tissue was allowed to equilibrate for at least 30 min before the addition of any drug. Under these experimental conditions, guinea-pig ileum behaves as a quiescent preparation and is considered more useful for measuring spasmodic activity (Gilani et al., 2004). After equilibration, each tissue preparation was repeatedly treated with sub-maximal concentrations (0.3 μM) of ACh at 3 min intervals until constant responses were recorded. Stimulant effect of the extract was determined on resting baseline of the tissue and was assessed as the percent of the maximum effect produced by the control drug, ACh (1 μM).

**Rabbit jejunum.** To test the gut modulatory effects of the plant material, the rabbit jejunum was dissected out, kept in Tyrode’s solution and cleaned of mesenteries. Respective segments of approximately 2 cm length were suspended in tissue baths containing Tyrode’s solution maintained at 37°C and aerated with carbogen (95% O₂ and 5% CO₂). The composition of the Tyrode’s solution in mM was: KC1 2.68, NaCl 136.9, MgCl₂ 1.05, NaHCO₃ 11.90, NaH₂PO₄ 0.42, CaCl₂ 1.8 and glucose 5.55. Intestinal responses were recorded isotonically using a transducer (Model 50–6360, Harvard Apparatus, Holliston, USA) coupled with a bridge amplifier and PowerLab 4/25 data acquisition system attached.
to a computer running Chart 6 software (AD Instruments, Sydney, Australia). A preload of 1 g was applied. The tissues were allowed to equilibrate for at least 30 min before addition of any drug. After equilibration, each preparation was repeatedly treated with sub-maximal concentrations (0.3 mM) of ACh with a 5–10 min interval between doses to stabilize the preparation. The preparation was considered stable when three responses of the same concentration of ACh were found identical. Spasmogenic effects of the test material were compared with the ACh evoked maximum contractile responses.

For the determination of Ca²⁺ channel blocking (CCB) activity, high K⁺ (80 mM) was used to depolarize the preparations as described by Farre et al. (1991); K⁺ (80 mM) was added to the tissue bath, which produced a sustained contraction. Once a plateau of the induced contraction was achieved, the test material was added in a cumulative fashion to obtain the concentration-dependent inhibitory response (Van-Rossum, 1963). To confirm the Ca²⁺ antagonist activity of the 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 K⁺-rich and Ca²⁺-free Tyrode’s solution, of 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. Following an incubation period of 30 min, control concentration-response curves (CRCs) of Ca²⁺ were obtained. When the CRCs of Ca²⁺ were found to be superimposable (usually after two cycles), the tissue was pretreated with the crude extract for 60 min to test the possible CCB effect. The CRCs of Ca²⁺ were reconstructed in the presence of different concentrations of the test material.

Statistical analysis. All the data expressed are mean ± standard error of mean (SEM, n = number of experiments) and the median effective concentration (EC₅₀) with 95% confidence intervals (CI). The CRCs were analysed by non-linear regression using the GraphPad program (GraphPad, SanDiego, CA, USA). The statistical parameters applied are the Student's t-test and one-way analysis of variance (ANOVA) with post hoc Dunnett’s or Tukey’s test. A value of p < 0.05 was considered significantly different.

RESULTS

Preliminary phytochemical analysis

The Ui.Cr was found to contain alkaloids, tannins and coumarins, while other classes of phytochemicals were absent.

Effect on charcoal meal transit

The prokinetic effect of Ui.Cr was measured in terms of its effect on charcoal meal transit in the small intestine of mice (Fig. 1). Ui.Cr, dose-dependently (6 and 12 mg/kg), accelerated charcoal meal travel through the small intestine, similar to that caused by carbachol. The distance travelled by the charcoal meal in the Ui.Cr treated mice, measured as % of total length of small intestine, was 80.2 ± 5.2 and 87.9 ± 4 with 6 and 12 mg/kg, respectively, as opposed to 49.9 ± 6.1 with saline. Carbachol, similarly, enhanced the proportion of intestine travelled by charcoal meal to 74.9% ± 6.4.

Laxative effect

The Ui.Cr, when administered orally, showed a laxative effect in mice as reflected by the increase in the number of feces (Fig. 2). The laxative effect was dose-dependently mediated at 6 and 12 mg/kg and was comparable to carbachol (10 mg/kg). The respective total number of feces passed by the groups of mice treated with Ui.Cr (6 and 12 mg/kg) and carbachol (10 mg/kg) over 6 h were 4.3 ± 0.9, 9.2 ± 1.4 and 11 ± 1.7. Pretreatment of the animals with atropine (10 mg/kg) blocked the laxative effect of both Ui.Cr and carbachol.
The magnitude of the contractile effect was 3.86 (1.0 mg/mL, respectively, compared with the ACh/C6 mogenic effect of crude extract of Urginea indica (Ui.Cr) in the absence and presence of atropine (0.1 μM) in isolated guinea-pig ileum. The responses are given as % of acetylcholine (10 μM)-evoked maximum contraction (Ach Max.). The values are shown as mean ± SEM, n = 4.

**Figure 3.** Bar-chart showing the concentration-dependent spasmogenic effect of crude extract of Urginea indica (Ui.Cr) in the absence and presence of atropine (0.1 μM) in isolated guinea-pig ileum. The responses are given as % of acetylcholine (10 μM)-evoked maximum contraction (Ach Max.). The values are shown as mean ± SEM, n = 4.

**Figure 4.** The effect of crude extract of Urginea indica (Ui.Cr) and verapamil, respectively, on spontaneous and K⁺ (80 mM)-induced contractions (A and B) and Ca²⁺ concentration-response curves (C and D) in isolated rabbit jejunum. Values are presented as mean ± SEM, n = 4–7.

**Effect on jejunum**

The Ui.Cr caused a concentration-dependent (0.01–0.3 mg/mL) stimulant effect in spontaneously contacting rabbit jejunum followed by relaxation at a higher concentration as shown in Fig. 4A. The magnitude of the highest contraction caused by Ui.Cr (0.3 mg/mL) in this preparation was 22.86 ± 4.10% (mean ± SEM, n = 4) of ACh (1 μM)-induced maximum response. Pretreatment of the tissue with atropine (0.1 μM) abolished the contractile effect and the relaxant effect became dominant with an EC₅₀ value of 1.01 mg/mL (95% CI, 0.72–1.41, n = 3) as shown in Fig. 4A.

When tested against high K⁺-induced contractions, Ui.Cr caused relaxation (Fig. 4A) with an EC₅₀ value of 0.32 mg/mL (0.26–0.39 n = 6). Similarly, verapamil relaxed both spontaneous and K⁺-induced contractions with EC₅₀ values of 0.035 (0.03–0.4, n = 4) and 0.14 μM (0.10–0.19, n = 4), respectively (Fig. 4B). The Ui.Cr also produced a concentration-dependent (0.3–1.0 mg/mL) rightward shift in the Ca²⁺ CRCs, similar to that caused by verapamil at 0.1 and 0.3 μM (Fig. 4C and D).

**DISCUSSION**

_Urginea indica_ is used in traditional medicine as a digestive, stomachic and laxative; therefore the current study was undertaken to determine its gut stimulatory effect using the _in vivo_ and _in vitro_ models to rationalize the medicinal uses. The Ui.Cr was studied on mice for its effect on the charcoal meal transit through the small gut and the laxative activity. The Ui.Cr dose-dependently enhanced the intestinal transit of charcoal and caused a significant increase in the number of feces when administered orally to mice. The Ui.Cr was found distinctly potent in its gastrointestinal stimulant effect with comparable potency to carbachol, a standard muscarinic agonist and gastrointestinal stimulant (Brown and Taylor, 1996). In our previous studies it was observed that the laxative effect of natural products is usually mediated through an ACh like mechanism (Gilani et al., 2000, 2004). To evaluate if the gut stimulating effect of this plant was also mediated by the involvement of a similar pathway, the laxative effect of Ui.Cr was challenged with atropine. Pretreatment of mice with atropine blocked the laxative effect of Ui.Cr similar to carbachol, thus suggesting that the gastrointestinal stimulatory effect of Ui.Cr observed _in vivo_ is mediated through activation of muscarinic receptors. Acetylcholine is a neurotransmitter released by the parasympathetic nervous system. It plays an important physiological role to regulate the peristaltic movements of the gut and to release of digestive juices mediating this action by stimulation of M₃ muscarinic receptor subtypes and atropine blocks all muscarinic receptor sites (Brown and Taylor, 1996).

To confirm the mode of action, Ui.Cr was further studied on isolated tissue preparations. When tested on guinea-pig ileum, a quiescent preparation considered useful for this purpose (Bashir et al., 2006), Ui.Cr produced a spasmodic effect, which was blocked by atropine like that of ACh, thus confirming that the stimulatory effect of Ui.Cr was mediated through muscarinic receptor activation. The spasmodic effect of _Urginea indica_ was
been otherwise harmful. The relaxant effect of Ui.Cr observed at a high concentration was mediated through a Ca\(^{2+}\) antagonist like effect, reflected by the inhibitory effect on K\(^{+}\)-induced contractions and displacement of Ca\(^{2+}\) curves to the right, similar to that of verapamil, a standard Ca\(^{2+}\)-channel blocker (Hamilton et al., 1986). The potency of Ui.Cr for its in vivo gut stimulant effect was comparable to carbachol, but the maximum stimulant effect as observed in vitro was moderate (approximately 40% of ACh induced maximum response), presenting an interesting picture. Acetylcholine is one of the most important neurotransmitters exhibiting normal function in gut motility but cholinergic drugs are not used clinically to relieve constipation because of their strong effect as opposed to a physiological effect as they cause side effects such as abdominal cramps due to their strong effect as opposed to a physiological effect which is short lived (Brown and Taylor, 1996). The moderate effect of Ui.Cr is considered valuable as beyond this it could have caused abdominal cramps but the coexistence of a spasmylocytic component is probably meant by nature to offset the excessive stimulation which could have been otherwise harmful.

These gastrointestinal stimulant effects of the *Urginea indica* extract correlate well with its traditional use in indigestion and constipation (Kirtikar and Basu, 1988; Baquar, 1989; Prajapati et al., 2003), the conditions resulting from delayed gastrointestinal transit (Tatsuta and Ishi, 1993).

The results of the preliminary phytochemical analysis of the crude extract of *Urginea indica* revealed the presence of alkaloids, tannins and coumarins compounds in the plant. Alkaloids have shown a spasmylocytic effect through blockade of voltage-dependent Ca\(^{2+}\) channels (Khalid et al., 2004; Gilani et al., 2005), but the possible nature of the cholinergic and anticholinergic constituents in this plant is uncertain.

These investigations indicate that the crude extract *Urginea indica* possesses gut stimulatory activity mediated through a cholinergic mechanism, which could be the possible reason for its use in disorders resulting from delayed gastrointestinal transit such as constipation and indigestion.

**Conflict of Interest**

The authors have declared that there is no conflict of interest.

**REFERENCES**


