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Full Length Research Paper

Calcium channel blocking activities of *Withania coagulans*

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The effects of crude methanolic extract of *Withania coagulans* (Wc.cr) were studied for possible calcium channel blocking activities in rabbit's jejunum preparations at different doses. Potassium chloride (KCl) induced contractions were also inhibited by the Wc.cr extract suggesting calcium channel blocking activity. Calcium channel blocking activity was confirmed by the right shift in the dose response curves of the tissues in calcium free tyrode's solution. The dose response curves of the Wc.cr resemble the curves produced by verapamil, a standard calcium channel blocker. The results confirm the presence of calcium channel blocker(s) in the extract and the plant species could be a potential target for activity-guided isolation of the active moieties.

Key words: *Withania coagulans,* spasmolytic, verapamil, calcium channel-blocking activity.

INTRODUCTION

Withania coagulans (Solinaceae) is a shrub distributed in paleotropical regions of Pakistan. Traditionally the plant is used for the control of diabetes mellitus. Reported activities of the plant are antibacterial (Gaind and Budhiraja, 1967; Khan et al., 1993; Budhiraja et al., 1987), antifungal (Choudhary et al., 1995). Anti-inflammatory property of the plant is well documented (Budhiraja et al., 1984) . Cardiovascular effects in dogs (Hemalatha et al., 2008) and antitumer activity (Budhiraja et al., 1987; Chattopadhyay et al., 2007) have been reported. The fruits are used in Punjab for coagulation of milk in preparing paneer, and also used as blood purifier. Traditionally, the plant is used in dyspepsia, flatulent colic and for diuretic purposes (Hemalatha et al., 2008). Based on the aforementioned reported pharmacological activities and current ethno medical uses (use in flatulent colic), we carried out our screening for possible spasmolytic and calcium channel blocking effects in rabbits' jejunum preparations.

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Studies on isolated organ(s) are a useful tool to evaluate the pharmacological activity of a drug (Enna et al., 2002). The in vitro techniques are preferred to evaluate the phytomedicine, because these techniques are quicker and cheaper and conducted in short span of time (Baker et al., 1995).

MATERIALS AND METHODS

Collection and extraction

The shade dried fruits of the plant were purchased in April - May 2008 from the local market of Chakdara Dir, N.W.F.P Pakistan. Professor Dr. Jehandar Shah, plant taxonomist and Ex-Vice Chancellor of University of Malakand identified the plant. A voucher specimen (Wc-01-2009) has been submitted to the herbarium of the University of Malakand. The clean fruits materials (54.5 g) were grinded in mortar and pestle and soaked in 90 - 95% methanol (commercial grade) with occasional shaking at room temperature. After 3 - 4 days, the methanol soluble materials were filtered off using a filter paper. This process was repeated thrice and then all filtrates were combined and concentrated under vacuum at low temperature (below 40°C) using a rotary evaporator. A brownish-red or scarlet crude extract (8.5 g) was obtained and screened for possible calcium channel blocking activity.

Drugs and standards

All chemicals used were of analytical grade. Acetylcholine was purchased from BDH Chemicals, Poole England and the rest of chemicals were purchased from E. Merck Germany. The drugs used in these experiments were dissolved in distilled water and all solutions were freshly prepared on the same day of experiments.

Animals and data recording

Local breed rabbits (n = 6) of either sex, weighing 1.0 - 1.4 kg, were used in these experiments. The animals were housed at the Animal House of University of Malakand complying with standards mentioned in the Animals (Scientific Procedures) Act 1986, UK. A standard diet and tap water were given to all animals. The animals were kept with out food, 24 h prior to starting of the experiments and they were given only water.

Intestinal responses were recorded using Teaching Force Transducer (Model No: MLT 0210/A Pan Lab S.I.) attached with Power Lab. (Model No: 4/25 T) AD Instruments, Australia. Data were recorded at range 20 mv, Low pass 5Hz X 10 gain using input 1, rate 40/S.

Rabbit's jejunum preparations

Studies on isolated tissue experiments were carried out as following; Rabbits were sacrificed and their abdomen was opened and portion(s) of jejunum were isolated and kept in tyrode's solution aerated with carbogen gas (5% Carbon dioxide and Oxygen mixture) (Qayum A, 2004). Out of the pieces of jejunum already maintained in tyrode's solution, preparations of about 2 cm length were mounted in 10 ml tissue bath containing tyrode's solution at 37°C and aerated with carbogen gas.

Constituents and concentrations (in mM) used in tyrode's solution were KCl 2.68, NaCl 136.9, $MgCl_2$ 1.05, $NaHCO_3$ 11.90, NaH_2PO_4 0.42, $CaCl_2$ 1.8 and glucose 5.55. A one gram pressure per two centimeters was applied to the tissue. Tissues were kept undisturbed and stabilized for a previous equilibrium period of about 30 minutes. This was achieved by giving sub-maximal doses of acetylcholine (0.3 μ M) to the tissue till it produced a reproducible response that was considered as normal response.

Crude plant extract at dose(s) of 0.01, 0.03, 0.1, 0.3, 1, 5 and 10 mg/ml were tried for any possible spasmolytic activity through a series of experiments.

Calcium channel blocking activity

The tissues (n = 6) were pre-treated with high concentration of KCl (80 mM) to depolarize the tissues and prepare them for sustained contractions (Farre et al., 1991). The test material extracts were applied on the tissues mounted in tissue bath, in a cumulative manner (Gilani et al., 2005), to obtain dose response curves and their relaxation was expressed as percent of the K^+ induced contractions (Van-Rossum et al., 1963).

To confirm that this relaxation is due to calcium channel blockade, we performed a series of experiments in calcium free tyrode's solution with EDTA (0.1mM) leading to the removal of calcium from the tissues. Tissues were exposed to calcium free and then to potassium rich tyrode's solution for 30 minutes having following composition (mM): KCl 50, NaCl 91.04, $MgCl_2$ 1.05, $NaHCO_3$ 11.90, NaH_2PO_4 0.42, glucose 5.55 and EDTA 0.1. Earlier all the tissues were stabilized in normal tyrode's solution for at least 30 minutes. Then controlled calcium response curves were obtained in the depolarized tissues with commutative addition of Ca^{++} at concentrations (1×10^{-4} – 256×10^{-4} M). Tissues were exposed to the extracts (in increasing order of their concentration) for one hour to get the concentration response curve. In other series of experiments, tissues were exposed to different concentrations of Verapamil (3×10^{-9} - 1×10^{-6}M), a standard calcium channel blocker, in commutative fashion to confirm the calcium channel blocking activities.

Statistical analysis

A Microsoft-XL Sheet was used to calculate Mean and Standard Error Mean (SEM) of the data (not shown). Student's 't' test was used to compare the data and 'P' value less or equal to 0.05 was considered as significant.

RESULTS AND DISCUSSION

The contractile effects of the smooth muscles of the intestines are due to the cytosolic free calcium levels (Karaki and Wiess, 1983). There is an exchange of calcium between extracellular and intracellular calcium stores. The voltage dependent calcium channels (VDCs) are responsible for the influx of calcium into the sarcoplamic reticulum (Bolton, 1979; Carl et al., 1996; Godfraind et al., 1986). This leads to periodic depolarization and repolarization of the intestinal tissues that accounts for its spontaneous responses. The effects of different concentrations of extract of *W. coagulans* are shown in Figure 1. There is a gradual increasing in the spasmolytic effect of the extract. To see whether the spasmolytic activity is due to possible calcium channel blockade, we in another series of experiments, treated the isolated tissues with KCl (80 mM) that opens the voltage–operated calcium channels and allows the extra-cellular calcium into the cytosol resulting in the depolarization of the tissue (Ghayur and Gilani, 2005; Gilani et al., 2005). The extract of *W. coagulans* showed to relax the tissues depolarized by KCl (80 mM) and was therefore, considered to have calcium channel blocking activity at a dose range of 0.1 - 3.0 mg/ml (Figure 1). However positive relaxing effect on the KCl- induced contraction in all tissues does not imply that the test substance is having a calcium channel blocking activity (Kobayashi et al., 1989); therefore, we carried out experiments on rabbit's jejunum with plant extract to confirm the calcium channel blocking activity that shifted the calcium dose response curve to the right. Tissue contractile response was relaxed by fifty percent of control at dose of 0.3 mg/ml in constructed calcium chloride curve ([log Ca++] M = -2.2) . Similarly, verapamil at a dose of 0.1 micromolar (μM) relaxed the normal contractile response by fifty percent in the presence of [log Ca++] M = -1.75 (Figure 2B). Thus effects of the extract resemble the effects of verapamil that is used in different cardiovascular disorders (Triggle, 1992). As the entry of calcium through VDCs is predominantly inhibited by verapamil, a standard calcium channel blocker (Fleckenstein, 1997; Cortes AR et al., 2006), and the effects of the extract resemble the effects of verapamil.

Therefore, it strongly suggests the presence of calcium

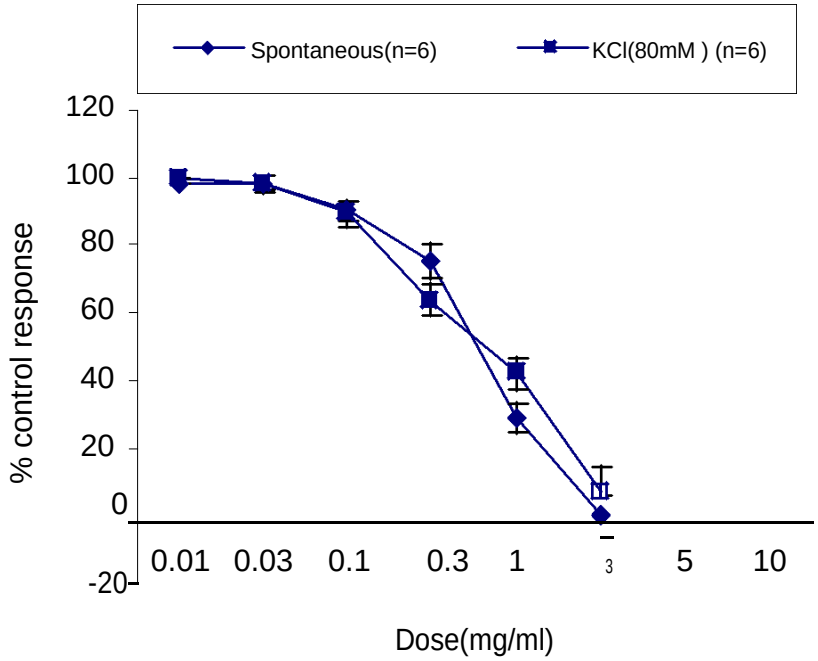

Figure 1. Spasmolytic effects of the crude extract of *Withania coagulans* on isolated rabbit's jejunum preparations (spontaneous) and KCl induced contractions. All values are mean ± SEM (n = 6).

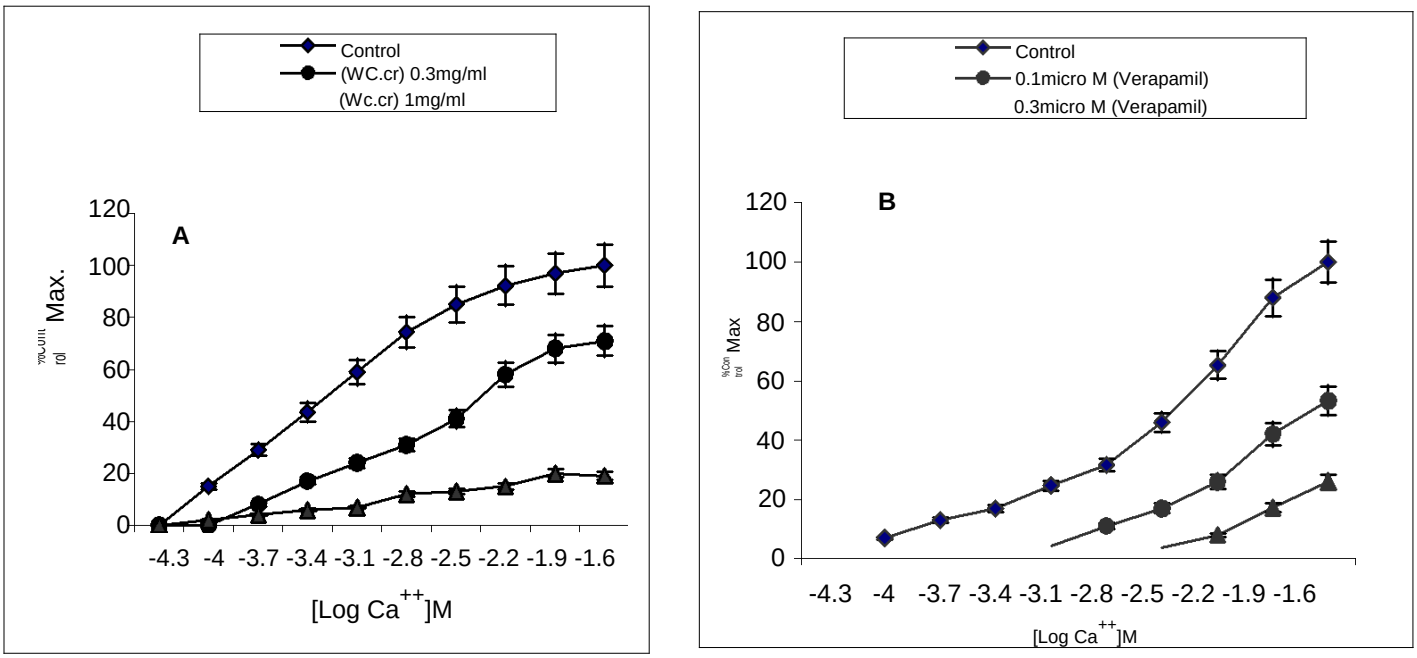

Figure 2. Dose response curves of Ca^{++} in absence and presence of increasing doses of (A) Extract of *Withania coagulans* (Wc.cr) and (B) verapamil in isolated rabbit's jejunum preparations. All values are Mean ± SEM (n = 6).

antagonist(s). Hence, the plant specie could be a potential target for activity guided isolation of calcium channel antagonist(s) or to standardize the crude extract to use and promote it for traditional uses on scientific grounds as WHO is giving more emphasis on promoting traditional medicine in Third World Countries

(Olayiowola, 1984).

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