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**Pharmacological evaluation of *Dactyloctenium aegyptium*: An indigenous plant used to manage gastrointestinal ailments**

## Pharmacological evaluation of *Dactyloctenium aegyptium*: An indigenous plant used to manage gastrointestinal ailments

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### Abstract

Crude extract of *Dactyloctenium aegyptium* and its fractions were evaluated to rationalize its folkloric use in gastrointestinal ailments. In spontaneous contracting rabbit jejunum preparation, *D. aegyptium* exert concentration-dependent spasmogenic effect (0.01-0.1 mg/mL) followed by spasmolytic effect at higher doses (0.3-3.0 mg/mL). Pretreatment of the tissue preparations with atropine resulted in suppression of the spasmogenic response. Further-more, *D. aegyptium* (1.0 mg/mL) caused relaxation of K<sup>+</sup> (80 mM)-induced spastic contractions in isolated rabbit jejunum preparations and there was non-parallel shift in Ca<sup>++</sup> dose response curves towards right (0.1-0.3 mg/mL). These findings were found to be comparable with verapamil, a standard Ca<sup>++</sup> channel blocker. The solvent-solvents fractionation reflected segregations of spasmogenic and spasmolytic effects in respective aqueous and dichloro-methane fractions. The above-mentioned findings reflected, presence of cholinergic as well as Ca<sup>++</sup> channel blocking activity in ethanolic extract of *D. aegyptium* thus providing scientific basis for its folkloric use in constipation and diarrhea.

### Introduction

*Dactyloctenium aegyptium* (L.) Beauv (Synonyms: *Eleusine aegyptia*; Family: *Gramineae*) is a common coarse grass; known by multiple vernacular names including madhana, bhobra, chimbari, takraa, makaraa, timida (Khare, 2007), beach wire grass, coast button grass, duck grass, durban crowfoot, finger comb grass, four-finger grass (Kirtikar and Basu, 1987), Egyptian finger grass and Egyptian crowfoot grass (Quattrocchi, 2006). It is native to Asia and widely distributed throughout the plains of Pakistan, Afghanistan, Israel, Lebanon, Turkey, India, Nepal, Sri Lanka, Malaysia, Myanmar, Philippines, China, Japan, Singapore, Thailand, Vietnam, New Guinea, Algeria, Morocco, Egypt, Sudan, Tunisia and Libya (Clayton et al., 2006). Its flowering time is from July to August (Baquar, 1989).

Phytochemical investigations resulted in isolation of

oxalic acid (Khan, 2002) and oxalates, glutamic and aspartic acids, cystine and tyrosine from aerial parts, while roots contain saponins and carbohydrates (Ghani, 2003). In some areas grass is also reported to contain glycosides (Bor, 1960; Holm et al., 1977; Randall, 2002; Khan, 2002).

*D. aegyptium* has folkloric reputed as astringent, bitter tonic, anti-anthelmintic and used to treat gastrointestinal, biliary and urinary ailments, polyurea (Khare, 2007), fevers (Choudhury et al., 2010), small pox (Sanglakpam et al., 2012), heart burn, immunodeficiency (Kipkore et al., 2014), urinary lithiasis, spasm of maternity and renal infections (Gupta et al., 1966; Kirtikar and Basu, 1987; Robbins., 2002), gastric ulcers and wounds healing (Nadkarni, 2002; Khare, 2007; Kirtikar and Basu, 1987). The seeds are used by tribesmen to prepare liquor as well as famine food with unpleasant taste (Sahal et al., 2014; Bor, 1960). The screening for anticancer potential of *D. aegyptium*



revealed apoptotic inducing capability of plant extract on human lung and cervical cancer cell lines (Hansakul et al., 2009). The plant extracts has been reported to exert moderate antimicrobial activity against some strains of pathogenic bacteria (i.e., *Proteus vulgaris*, *Escherichia coli*, *Bacillus cereus*, *Salmonella typhi*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*) and yeast (i.e., *Candida albicans*) (Abdallah and El-Ghazali, 2013).

Although *D. aegyptium* has traditionally been used to manage gastrointestinal ailments i.e constipation and diarrhea but it was not previously pharmacologically evaluated for the possible mode(s) of action. Present study was undertaken to explore the pharmacological potential of the crude ethanolic extract and its solvent fractions to provide mechanistic basis for its folkloric uses in gastrointestinal ailments.

## Materials and Methods

### Collection and extraction of plant material

Aerial parts of *D. aegyptium* were collected from the botanical garden of Bahauddin Zakariya University, Multan Pakistan in June 2013 and authenticated with the kind cooperation of Prof. Dr. Altaf Ahmad Dasti, an expert taxonomist from Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan, Pakistan vide voucher number Fl. P 144-5, deposited in the herbarium of the same institution. The plant material was subjected to shade drying, rendered free of foreign material through manual picking and grinded with the help of herbal grinder to a coarse powder. About 1 kg of powdered plant material was macerated with 70% aqueous etha-nol at 25°C for 7 days in a wide- mouth amber colored glass container for 7 days with occasional shaking. The soaked material was passed through muslin cloth to remove the vegetative debris and obtained fluid was then filtered through Whatman 1 filter paper. The above-mentioned extraction procedure was repeated thrice and filtrates of these three macerations were mixed. The solvent was subjected to evaporation at 37°C under reduced pressure on rotary evaporator (Rotavapor, BUCHI Labortechnik AG, Model 9230, Switzerland) attached with a vacuum pump and a recirculation chiller to obtain a dark green extract (Da.Cr) with approximate yield of 31%.

Solvent-solvent extraction (fractionation) was performed following dissolution of 20 g of crude ethanol extract of *D. aegyptium* in about 200 mL of distilled water to which 200 mL of dichloromethane (DCM) was added and shaken vigorously in a separating funnel and organic layer was separated. The procedure was repeated thrice and individually collected DCM fractions were pooled. The organic

portion was evaporated on rotary evaporator to obtain the dichloromethane fraction (Da.DCM), while the aqueous portion was lyophilized to remove moisture contents (Da.aq) with respective yields 18 and 27% (Bashir et al., 2006). Ethanol crude extract of *D. aegyptium* and its fractions were stored in amber glass container at -20°C.

### Chemicals and drugs

The chemical used in these experiments were of reagent analytical research grade. Potassium chloride, acetylcholine chloride, ethylene diamine tetra-acetic acid (EDTA) and atropine sulfate were purchased from Sigma Chemical Co. St Louis, MO, USA, calcium chloride, glucose, magnesium sulfate, potassium dihydrogen phosphate, sodium bicarbonate, sodium dihydrogen phosphate, and ethanol were obtained from Merck, Darmstadt, Germany. Ammonium hydroxide, sodium chloride and sodium hydroxide were purchased from BDH Laboratory Supplies, Poole, England. Stock solution, subsequent dilutions and physiological salt solutions were made fresh in distilled water on the day of experiment.

### Extract solution preparation

About 3 g of the crude ethanolic extract of *D. aegyptium* was dissolved in 10 mL of distilled water to prepare 0.3 g/mL w/v (300 mg/mL) of stock solution. The serial dilutions to 30 mg/mL, 3 mg/mL and 0.3 mg/mL of Da.Cr were made fresh on the day of experiment from the stock solution.

### Animals and housing conditions

The local breed adult (male/female) albino rabbits (1.0-1.5 kg) of 6-7 months in age, purchased from local market were maintained in animal house of Faculty of Pharmacy, Bahauddin Zakaryia University, Multan under controlled environmental condition (23-25°C), and provided with fresh green fodder and tap water *ad libitum*. The animals were subjected to overnight fasting but provided free access to H<sub>2</sub>O prior to experiments. All the experiments performed were in compliance with the rulings of Institute of Laboratory Animal Resources Commission on Life Sciences (NRC, 1996), and was approved by the Ethical Committee of Bahauddin Zakaryia University, Multan vide letter No. EC /01PhDL/2013 dated 4<sup>th</sup> September, 2013.

### Preliminary phytochemical analysis

The crude ethanolic extract of *D. aegyptium* was subjected to phytochemical analysis for the detection of alkaloids, saponins, tannins, Phenol, anthraquinones and flavonoids (Evans, 2006). Appearance of yellowish brown color on mixing of Dragendorff's reagent with HCl treated aqueous plant extract solution, confirmed the presence of alkaloids in extract. Formation of froth on vigorous shaking of the aqueous extract solution, confirmed the presence of saponin. Development of

bluish green color on mixing of aqueous  $\text{FeCl}_3$  with extract solution indicated presence of phenols and tannins. The appearance of pinkish violet or red color on exposure to  $\text{NH}_4\text{OH}$  of the mixture of benzene with aqueous solution of plant extract already acidified with 1% HCl confirmed presence of anthraquinones among the plant constituents. The plant material was confirmed positive for flavonoids on appearance of yellow color with  $\text{AlCl}_3$  reagent.

#### *In vitro experiments on isolated rabbit jejunum preparations*

The rabbits were sacrificed subsequent to a blow on the head, abdomens incised and jejunums dissected out. The mesenteries were removed and cut into segments of about 2 cm in length. The segments were mounted between two stainless steel hooks in 10 mL tissue bath containing normal Tyrode solution (pH 7.4) maintained at  $37^\circ\text{C}$  by means of circulating thermo regulator and aerated with carbogen (95%  $\text{O}_2$  + 5%  $\text{CO}_2$ ). A preload of 1 g was applied and the tissue was allowed to be equilibrated for a period of 30 min during which the tissue was washed with fresh fluid after every 10 min intervals prior to exposure to any test material. Subsequently, the tissue preparations were repeatedly exposed to acetylcholine (30  $\mu\text{M}$ ), followed by washing with Tyrode solution to stabilize tissue preparations as well as recording of the sub-maximal response in order to compare magnitude of the observed spasmogenic effect of test material with acetylcholine-induced contractile response. The spontaneous contractions were recorded isotonicly through a Power Lab Data Acquisition System (AD Instruments, Australia) (Janbaz et al., 2014). The spontaneous rhythmic contractions in isolated rabbit jejunum preparations, allows testing of relaxant and/or contractile activities (Gilani et al., 2005). The contractile activity of test materials on isolated rabbit jejunum preparations is comparable to that of acetylcholine (ACh), which can be suppressed on pretreatment with atropine (0.1  $\mu\text{M}$ ) (Janbaz et al., 2013).

#### *Determination of calcium channel blocking activity*

The  $\text{Ca}^{++}$  channel blocking activity of the test material was determined following application on  $\text{K}^+$  (80 mM)-induced spastic contractions in isolated rabbit jejunum preparations (Farre et al., 1991). The isolated rabbit jejunum preparations following exposure to  $\text{K}^+$  (80 mM) exhibited a sustained contraction, on which test material was applied by addition to the isolated tissue bath in a cumulative fashion (Van-Rossum, 1963). The  $\text{K}^+$  (80 mM)-induced contractions in isolated smooth muscle preparations has been reported to be mediated through influx of  $\text{Ca}^{++}$  from extracellular fluid, and the substances capable to inhibit such types of contractions are speculated to be acting through blockade of the  $\text{Ca}^{++}$  channels (Bolton, 1979). The  $\text{Ca}^{++}$  channel blocking effect of the test material was confirmed further by the

previously reported method (Saqib et al., 2012). The isolated rabbit jejunum preparations were allowed to be stabilized in normal Tyrode solution, which was subsequently replaced for 45 min with a solution containing normal concentration of  $\text{K}^+$  but  $\text{Ca}^{++}$  was removed and EDTA (0.1 mM) was added to deplete  $\text{Ca}^{++}$  from the tissues. The isolated tissue preparation was further replaced by a  $\text{K}^+$ -rich and  $\text{Ca}^{++}$ -free Tyrode's solution having the following composition (mM): KCl (50), NaCl (91.04),  $\text{MgCl}_2$  (1.05),  $\text{NaHCO}_3$  (11.90),  $\text{NaH}_2\text{PO}_4$  (0.42), glucose (5.55), and EDTA (0.1). After an incubation period of 30 min, the  $\text{Ca}^{++}$  was added to tissue bath in a cumulative manner to obtain control  $\text{Ca}^{++}$  dose-response curves (CDRCs). The stepwise increase in contractile activity of the tissue indicated that the extent of contractions was dependant on the availability of extracellular  $\text{Ca}^{++}$  for  $\text{K}^+$ -induced influx of  $\text{Ca}^{++}$ . When successive CDRCs for  $\text{Ca}^{++}$  were found to be superimposable, the tissue was washed and was allowed to be equilibrated with the crude ethanolic extract of *D. aegyptium* for 60 min, and then concentration response curves of  $\text{Ca}^{++}$  were reconstructed and compared to the CDRCs in absence of extract. The concentration response curves for  $\text{Ca}^{++}$  were developed in the presence of various concentrations of the extract to assess a possible  $\text{Ca}^{++}$  channel blocking effect. The  $\text{Ca}^{++}$  channel blocking activity on the part of plant was confirmed as *D. aegyptium* caused shifting of the CDRCs constructed in calcium-free medium towards right in a concentration-dependent manner (Bolton, 1979).

#### *Statistical analysis*

The results for spasmolytic and spasmogenic activities are expressed as the mean  $\pm$  SEM. The  $\text{EC}_{50}$  (median effective concentration) values with 95% confidence interval were calculated using the computer software GraphPad Prism program version 5.0 for Windows, (Graph-Pad, San Diego, USA). Dose-response curves were analyzed by nonlinear regression sigmoidal response curve (variable slope).

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## Results

Preliminary phytochemical investigations on *D. aegyptium* revealed presence of alkaloids, anthraquinones, flavonoids, saponins and tannins among the ethanol soluble extractable constituents.

The crude ethanol extract of *D. aegyptium* (Da.Cr) exhibited concentration dependent (0.01-0.1 mg/mL) contractile effect in isolated rabbit jejunum preparations, but paradoxically it exerted relaxant effect on further increase in tissue bath concentrations (0.3-3.0 mg/mL),  $\text{EC}_{50}$  value of 0.5071 mg/mL (95% CI: 0.2011-0.7539 mg/mL; n=5) (Figure 1B, 3A). However, pretreatment of the tissue preparations with atropine (0.1  $\mu\text{M}$ ) resulted in blockade of the contractile response and relaxation of the spontaneous activity was

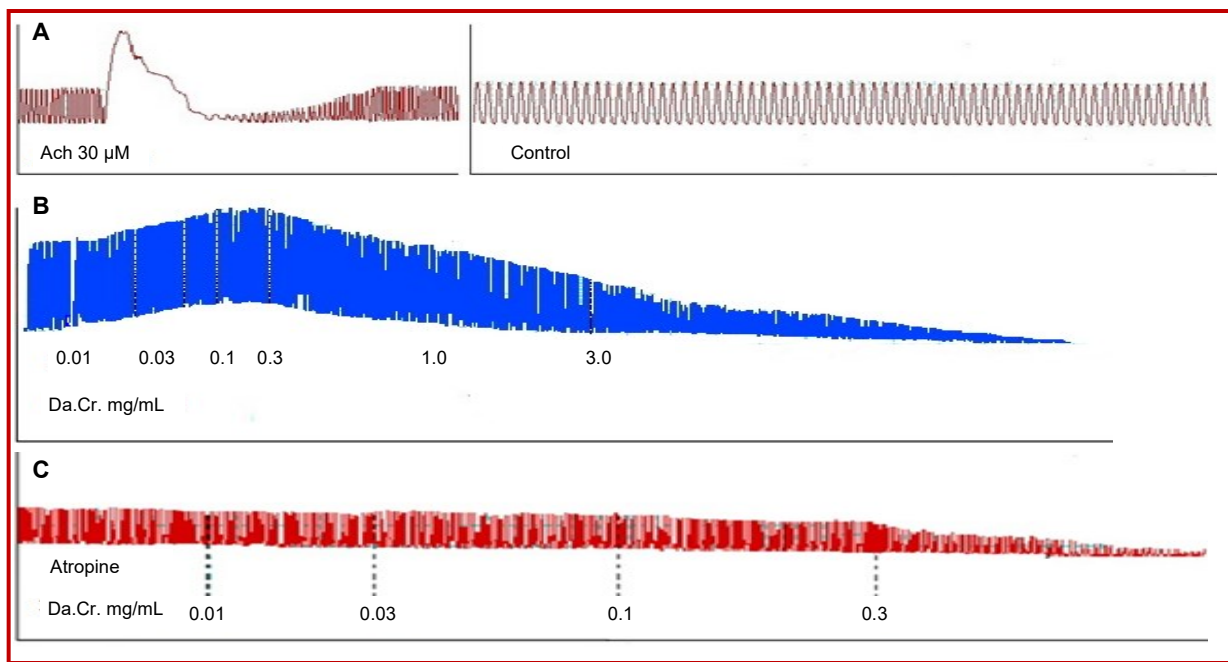


Figure 1: (A) Spontaneous contractions of isolated rabbit jejunum preparations and effect of acetylcholine (30  $\mu$ M) on spontaneous contractions. (B) Effect of *D. aegyptium* (Da.Cr) on spontaneous contractions of isolated rabbit jejunum preparations. (C) Effect of Da.Cr on spontaneous contractions of isolated rabbit jejunum preparations pretreated with atropine (0.1  $\mu$ M)

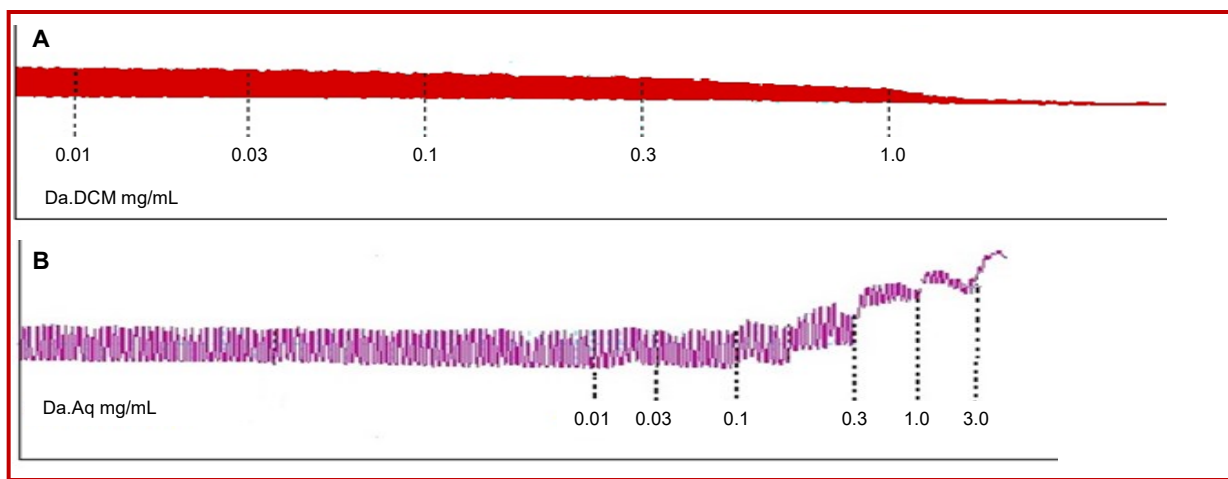


Figure 2: A) Effect of dichloromethane fraction (Da.Dcm) of *D. aegyptium* on spontaneous contractions of isolated rabbit jejunum preparations. B) Effect of aqueous fraction (Da.Aq) of Da.Cr on spontaneous contractions of isolated rabbit jejunum preparations

observed at tissue bath concentration of 0.01-0.3 mg/mL with  $EC_{50}$  value of 0.0882 mg/mL (95% CI: 0.1011-0.6539 mg/mL; n=5) (Figure 1C, 3A). Dichloromethane fraction of crude ethanolic extract of *D. aegyptium* inhibited spontaneous contraction at a lower tissue bath concentrations than Da.Cr with  $EC_{50}$  value of 0.1801 mg/mL (95% CI: 0.1107-0.6081 mg/mL; n=5) (Figure 2A, 3A), whereas aqueous fraction of crude extract of *D. aegyptium* exhibited spasmogenic effect on spontaneous contractions (Figure 2B, 3A).

The Da.Cr caused complete relaxation of  $K^+$  (80 mM)-induced contractions at tissue bath concentration of 0.01

-1.0 mg/mL, with  $EC_{50}$  value of 0.1061 mg/mL (95% CI: 0.1021-0.6892 mg/mL; n=5) (Figure 3B). Dichloromethane fraction of crude ethanolic extract of *D. aegyptium* inhibited  $K^+$  (80 mM)-induced contractions at a lower tissue bath concentrations than Da.Cr with  $EC_{50}$  value of 0.0491 mg/mL (95% CI: 0.0194-0.0650 mg/mL; n=5) (Figure 3b), whereas aqueous fraction of crude extract of *D. aegyptium* did not produce relaxation of  $K^+$  (80 mM)-induced contractions (Figure 3B).

Verapamil (standard drug), exhibited a similar pattern of suppression of the spontaneous and  $K^+$  (80

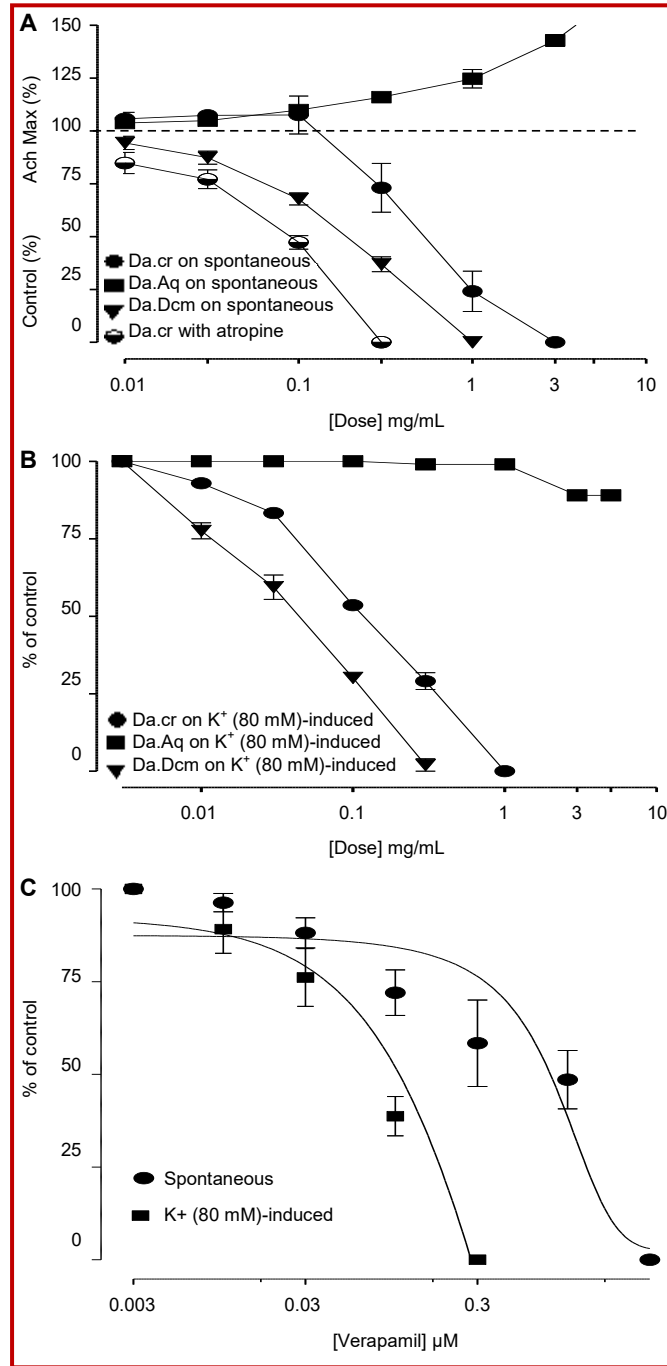


Figure 3: A) Effect of Da.Cr, Da.Dcm and Da.Aq on spontaneous contractions of isolated rabbit jejunum preparations. B) Effect of Da.Cr, Da.Dcm and Da.Aq on K<sup>+</sup> (80 mM)-induced contractions of isolated rabbit jejunum preparations. C) Effect of verapamil on spontaneous and K<sup>+</sup> (80 mM)-induced contractions in isolated rabbit jejunum preparations (values shown are mean  $\pm$  SEM, n=5)

mM)-induced contractions with respective EC<sub>50</sub> value of 0.385  $\mu$ M/mL (95% CI: 0.2493-0.6394 mg/mL; n=5) and 0.1320  $\mu$ M/mL (95% CI: 0.0899- 0.1935 mg/mL; n=5) (Figure 3C).

The Da.Cr and Da.Dcm on application to the isolated rabbit jejunum preparations at respective tissue bath concentrations of (0.1-0.3 mg/mL) and (0.01-0.03

mg/mL) caused shifting of the Ca<sup>++</sup> concentration response curves towards right in a manner comparable to verapamil (0.03-0.1  $\mu$ M) (Figure 4A,B,C).

## Discussion

*D. aegyptium* has traditionally been used to manage

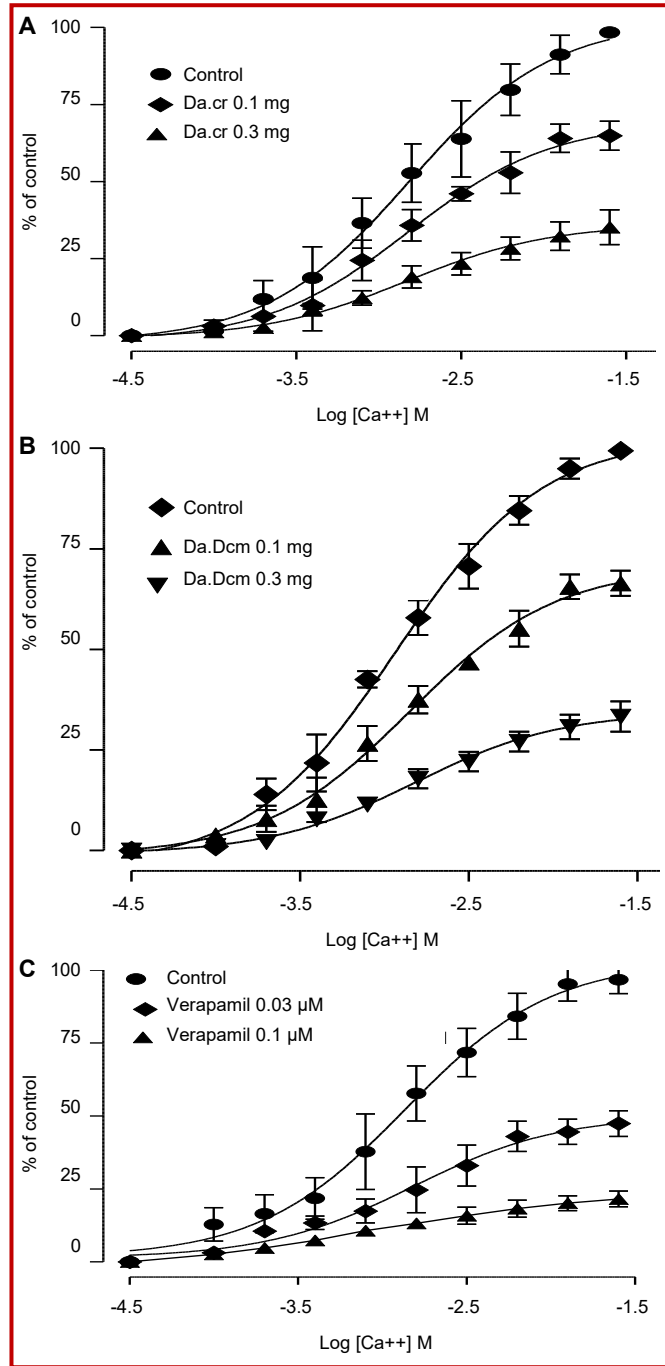


Figure 4: A) Effect of Da. Cr on concentration response curve (CRC) of Ca<sup>++</sup> in isolated rabbit jejunum preparations. B) Effect of Da. Dcm on CRC of Ca<sup>++</sup> in isolated rabbit jejunum preparations. C) Effect of verapamil on CRC of Ca<sup>++</sup> in isolated rabbit jejunum preparations (values shown are mean  $\pm$  SEM, n=5)

gastrointestinal ailments i.e. constipation and diarrhea and present study was undertaken to provide scientific basis for its folkloric uses. The ethanolic extract of *D. aegyptium* (Da.Cr) exerted spasmogenic effect on application to the spontaneously contracting isolated rabbit jejunum preparations at low tissue bath concentrations, while exhibited spasmolytic activity on

increasing the bath concentrations, indicating presence of both spas-mogenic and spasmolytic constituents. The spasmogenic response on the part of Da.Cr may possibly due to the presence of some cholinergic muscarinic constituent(s) (Brown and Taylor., 1996), which was confirmed further as pretreatment of isolated rabbit jejunum preparations with atropine (0.1

$\mu\text{M}$ ) resulted in suppression of the spasmogenic activity (Arunlakshana and Schild., 1959) and exhibiting dominant spasmolytic effect. The *D. aegyptium*-induced increase in contractile activity of gastrointestinal tract may provide a scientific basis for its folkloric uses in constipation.

The *D. aegyptium* induced spasmolytic activity was speculated to be due to the presence of  $\text{Ca}^{++}$  channel blocking activity (Janbaz et al., 2013a), since increase in free cytoplasmic  $\text{Ca}^{++}$  concentrations is essential for activation of contractile elements in smooth muscle preparations (Karaki and Weiss, 1983), due either by influx through the L-type voltage-dependant  $\text{Ca}^{++}$  channels (VDCs) or release from intracellular  $\text{Ca}^{++}$  stores in sarcoplasmic reticulum (Godfraind et al., 1986). The periodic depolarization followed by repolarization is responsible for spontaneous activity in isolated rabbit jejunum preparation. In a state of maximum depolarization, there is rapid influx of  $\text{Ca}^{++}$  through VDCs and is responsible for the generation of an action potential (Brading, 1981). The observed spasmolytic effect of Da.Cr on isolated rabbit jejunum preparation may possibly be mediated either through blockade of VDCs or through inhibition of  $\text{Ca}^{++}$  released from sarcoplasmic reticulum. The isolated rabbit jejunum preparations on exposure to  $\text{K}^+$  (80 mM), resulted in opening of VDCs, influx of extracellular  $\text{Ca}^{++}$  and contraction of smooth muscles (Bolton, 1979; Karaki and Weiss, 1983). The Da.Cr caused relaxation of  $\text{K}^+$  (80 mM)-induced contractions in isolated rabbit jejunum preparations, possibly due to blockade of  $\text{Ca}^{++}$  channels, which was confirmed further as Da.Cr applied to isolated rabbit jejunum preparations caused not only decrease in tissue response to  $\text{Ca}^{++}$ , but also rightward shifting in  $\text{Ca}^{++}$  concentration response curves, in a manner comparable to verapamil (Janbaz et al., 2014). The  $\text{Ca}^{++}$  channel blocking activity on the part of *D. aegyptium* can be attributed to the observed presence of alkaloids (Gilani et al., 2005b) and flavonoids (Revelta et al., 1997; Ghayur et al., 2007) among the plant constituents.

The  $\text{Ca}^{++}$  channel blockers are known to be effective in hyperactive gut diseases, i.e., diarrhea and abdominal cramps (Brunton., 1996) and presence of such activity in *D. aegyptium* may provide a scientific basis for its traditional use in hyperactive diseases i.e. diarrhea. The co-existence of laxative and anti-diarrheal activity is reported to be widely distributed among plant kingdom, i.e., *Lactuca serriola* (Janbaz et al., 2013b), *Cletis africana* (Khan et al., 2012), *Carthamus oxycantha* (Gilani et al., 2005a), *Calendula officinalis* (Bashir et al., 2006) apparently to keep spasmogenic and spasmolytic effects within tolerable limits by the nature.

The liquid-liquid extraction was performed on *D. aegyptium* by using dichloromethane and water. The dichloromethane fraction (Da.DCM) exerted spasmolytic effect on spontaneous as well as  $\text{K}^+$  (80 mM)-

induced contractions, whereas aqueous fraction (Da.Aq) exhibited spasmogenic activity on spontaneous contractions and was without any relaxant effect on  $\text{K}^+$  (80 mM)-induced contractions, such separation of biological activities on liquid-liquid fractionation has already been reported (Bashir et al., 2006).

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## Conclusion

This study indicates the presence of two components (cholinomimetic and calcium antagonistic) in crude extract of *D. aegyptium*. The spasmogenic activity may be attributed to some cholinergic constituents which provide mechanistic basis for its use in constipation. The spasmolytic effect may be due to  $\text{Ca}^{++}$  channel blocking components that may cause relaxation of gastrointestinal smooth muscles validating its folkloric use in diarrhea.

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## Conflict of Interest

Authors declare no conflict of interest

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