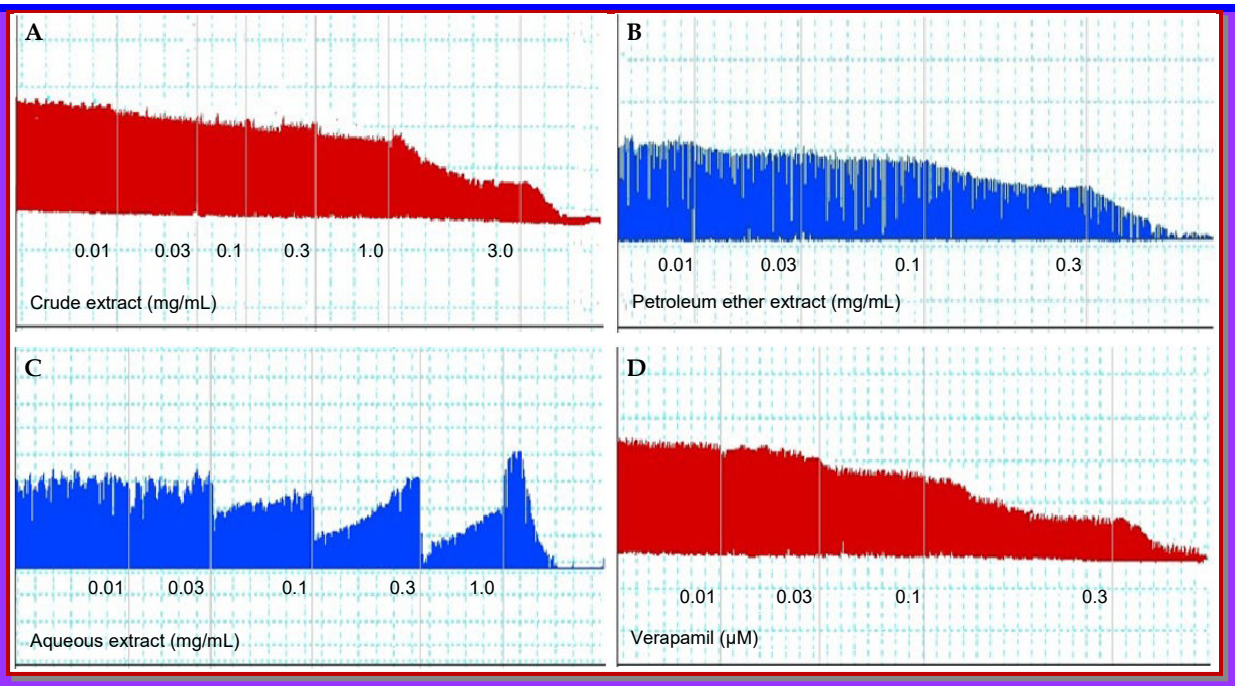

BJP

Bangladesh Journal of Pharmacology

Research Article

Pharmacological effects of *Cnicus arvensis* on the gastrointestinal system

A Journal of the Bangladesh Pharmacological Society (BDPS)
Journal homepage: www.banglajol.info
Abstracted/indexed in Academic Search Complete, Asia Journals Online, Bangladesh Journals Online, Biological Abstracts, BIOSIS Previews, CAB Abstracts, Current Abstracts, Directory of Open Access Journals, EMBASE/Excerpta Medica, Global Health, Google Scholar, HINARI (WHO), International Pharmaceutical Abstracts, Open J-gate, Science Citation Index Expanded, SCOPUS and Social Sciences Citation Index;
Bangladesh J Pharmacol 2018; 13: 16-22
ISSN: 1991-0088

Pharmacological effects of *Cnicus arvensis* on the gastrointestinal system

Fatima Saqib, Sakeena Hassan, Imran Imran, Ambreen Aleem and Khalid Hussain Janbaz

Faculty of Pharmacy, Bahauddin Zakariya University, Multan, Pakistan.

Article Info

Received: 12 June 2017
Accepted: 25 December 2017
Available Online: 20 January 2018

DOI: 10.3329/bjp.v13i1.32905

Cite this article:
Saqib F, Hasan S, Imran I, Aleem A, Janbaz KH. Pharmacological effects of *Cnicus arvensis* on the gastrointestinal system. Bangladesh J Pharmacol. 2018; 13: 16-22.

Abstract

The current study was conducted to screen the possible pharmacological activity of *Cnicus arvensis* on the gastrointestinal system. The methanolic extract exerted concentration-dependent (0.01–3.0 mg/mL) spasmolytic effect using isolated tissue preparations of rabbit jejunum and caused relaxation of K^+(80 mM)-induced spastic contractions. There was non-parallel shift in Ca^{++} concentration response curves towards right at tissue bath concentrations of 0.3 and 1.0 mg/mL. The solvent-solvents fractionation revealed domination of spasmolytic effects in petroleum ether fraction as compared to aqueous fraction. The methanolic extract also found to reduce the retching in chicks at 150 mg/kg as compared to chlorpromazine. The methanolic crude extract also found to reduce the number of wet spots using castor oil-induced diarrhea in rat as compare to loperamide. Our results reflected the presence of Ca^{++} channel blocking activity in methanolic extract, thus rationalizing the medicinal use of *C. arvensis* in diarrhea and vomiting.

Introduction

Cnicus arvensis (L.) Roth. (Hoffm.), synonym: *Cirsium arvense* (L.) Scop., is a rigid aromatic plant which is well -known for its deep, creeping root system and colony-forming capabilities that make invasion hard to control and easy to spread (Moore, 1975).

Plant also contain some bitter ethereal components such as volatile oils and tannins in addition with alkaloids, phenols, saponins and flavonoids (Ahmad et al., 2011; Hill, 1983). It also contains the vanillic acid and epicatechin. (Rahman et al., 2015).

The plant recognized traditionally for its therapeutic efficiency in the cirrhosis, diabetes, excessive menstruation, gout, gastritis, liver cancer, jaundice, lipoma (tumor) and scabies. The rhizome is popularly used for its astringent properties. It is used as cholagogic and diuretic. Thistle teas are useful for the treatment of appendicitis, internal bleeding and inflammations. The whole plant is tonic, anthelmintic, diaphoretic, verminfuge, and emmenagogue and is used for epistaxis, pulmonary disease, tuberculosis and pyogenic infections. Leaf juice is used on wounds. Roots are used in urinary complaints. The aqueous extract of root is given for the treatment of liver disorders (Uddin, 2006).

The crude extracts of plant is reported to exert the depressant effects on central nervous system and also have the anti-nociceptive properties (Rahman et al., 2015; Hasan et al., 2010).

Although *C. arvensis* has traditionally been used to manage various human ailments but it was not previously pharmacologically screened for its possible use in gastrointestinal ailments. The present study was undertaken to explore the pharmacological potential of the crude ethanolic extract and its solvent fractions to provide mechanistic basis for its medicinal use in the gastrointestinal ailments.

Materials and Methods

Plant material

Complete plants of *C. arvensis* were collected in March 2016 from Multan, Pakistan. The plant material was identified by Dr. Zafar Ullah Zafar, Department of Botany, Bahauddin Zakariya University, Multan [voucher number (Stewart 733(4)]. The plants material was rendered free from extraneous matter, chopped into small pieces and dried in shade for two weeks.

Preparation of the crude extract

The dried material of the plant was subjected to the electrically driven special herbal grinder to get a coarse powder. The powdered material of the plant was extracted through triple maceration and about 1 kg of coarse powder was dipped in 70% aqueous methanol in an amber glass bottle for 7 days with occasional shaking, laterally soaked plant material was pass through muslin cloth to remove the vegetative debris and fluid obtained was filtered by using Whatman filter paper No. 1. The filtrate was stored in an amber glass air tight container and previously mentioned extraction process was repeated twice on the mark by using fresh solvent. The filtrates of three successive soaking were mixed and evaporated under reduced pressure at room temperature on a rotary evaporator (Buchi R-200, Switzerland) attached to a vacuum pump, recirculation chillier and water bath at 30-40°C to a thick, semisolid paste. The extract was stored at -20°C in air tight jars. The approximate yield of the extract was calculated to be 15%.

Solubility-directed fractionation of the crude extract was done by standard phytochemical procedures using different organic solvents. A known quantity of the extract (20 g) was mixed in distilled water. This was, then, introduced in a separating funnel and petroleum ether (90–100 mL) was then added. This mixture was shaken vigorously, regularly allowing the air to escape out, and allowed the two layers to separate. The upper layer of petroleum ether was separated and air dried to obtain the petroleum ether fraction. The remaining layer was freeze dried and the resultant fraction was measured as the aqueous fraction.

The crude extract of *C. arvensis* was completely soluble in 10% DMSO and 90% distilled water. The stock solution (300 mg/mL) was prepared by dissolving 300 mg in 0.9 mL of distilled water and 0.1 mL of DMSO, then it was diluted in succession to 30 and 3 mg/mL on the days of experiment. The vehicle had no effect on tissue in the control experiment.

Animals

Animals (♂/♀) utilized in this study were local strain rabbit (1.0-1.8 kg), rat (Swiss albino 28-30 g) and chick (white leghorn meant for laying eggs). Animals were housed in stainless steel cages under controlled environmental condition (23-25°C) at the animal house of the Faculty of Pharmacy, Bahauddin Zakariya University, Multan. Standard food and tap water were provided to the animals. They were deprived of food 24 hours prior to the experiments but were given free access to water. Rabbits were sacrificed following a blow on back of head to be used for *in vitro* studies.

Chemicals and reagents

Acetylcholine chloride, carbachol, isoprenaline, potassium chloride, verapamil hydrochloride, phenylephrine, ethylene tetraacetic acid (EDTA) were purchased from the Sigma Chemicals Co. (USA). Calcium chloride, glucose, magnesium sulfate, potassium dihydrogen phosphate, sodium bicarbonate, sodium dihydrogen phosphate and methanol were obtained from the Merck (Germany). Ammonium hydroxide, sodium chloride, and sodium hydroxide were bought from the BDH Laboratory Supplies (England). The above-mentioned chemicals were of highest purity and reagent analytical research grade.

Isolated rabbit jejunum preparation

The crude extract of *C. arvensis* was tested on the jejunum of the rabbit to check the possible presence of spasmogenic or spasmolytic activity. For this purpose, the rabbit jejunum was dissected out and placed in the Tyrode's solution (Gilani et al., 2005a,b; Aleem and Janbaz, 2017). Segments of 2 cm length were cut and rendered free from adhering mesenteries and mounted in the isolated tissue organ bath of 20 mL containing Tyrode's solution maintained at 37°C and aerated with carbogen (mixture of 95% O_2 and 5% CO_2). The contractions of intestine were recorded through a Power Lab Data Acquisition System (AD Instruments, Australia) attach-ed to a computer installed lab chart software (version 7). Each tissue was allowed to get stabilized for at least 30 min and 1 g preload tension was given. This was all done before the addition of any drug and the responses were recorded. The doses of tested material were added in the organ bath in cumulative style to observe its effect on isolated tissues of rabbit jejunum. Spontaneous rhythmic contractions exhibited by rabbit jejunum tissues were allowed for testing of effect of extract in the absence of agonist. The K^+ (80 mM) was added to the tissue organ bath to induce the sustained contractions. The K^+ (80 mM)-induced contractions were relaxed by the testing substance to seek the possible activity of testing substance (Saqib et al., 2015; Saqib and Janbaz, 2016).

For the confirmation of calcium channel antagonistic activity of the test substance, the tissue was allowed to get stabilized for 30 min in the normal Tyrode's solution. Then, it was exchanged with the calcium free Tyrode's solution containing 0.1 mM EDTA to remove the calcium from the tissue. The solution of isolated

tissue bath was further replaced with the Tyrode's rich or calcium free Tyrode's solution that had a composition as follows (mM): KCl (50), NaCl (91.0), $MgCl_2$ (1.1), $NaHCO_3$ (11.9), NaH_2PO_4 (0.4), glucose (5.6) and EDTA (0.1). After the incubation period of 30 min, the control concentration response curves of Ca^{2+} ($CaCl_2$) were generated by putting the Ca^{++} in the organ bath in cumulative fashion after equal intervals of time. Two cycles of Ca^{2+} concentration response curves were prepared. Then, washed the tissue with Tyrode's rich solution until the calcium was completely removed from the tissue. The tissue was allowed to be equilibrated in the presence of plant extract for 1 hour prior to recording of the concentration response curves of Ca^{2+} for comparison to the control concentration response curves. The concentration response curves of Ca^{2+} were recorded in the presence of different concentrations of the plant extract in the tissue bath. Standard drug such as verapamil hydrochloride was employed as the positive control.

Antiemetic activity

The antiemetic activity of the methanolic extract was evaluated by using chick emesis model (Khan et al., 2013). The chicks were placed under large beakers and left to settle for 30 min. The animals of Group I (n = 5) were given an oral dose of normal saline and the copper sulfate was given orally (150 mg/kg) about 10 min after the last treatment. The Group II animals (n = 5) were treated similar to Group I except the normal saline was replaced by the methanolic extract (150 mg/kg; dissolved in normal saline). Whereas, the Group III of animals (n = 5) were treated similar to Group II of animals except chlorpromazine (150 mg/kg) was substituted for methanolic extract. The numbers of retches (an emetic action without emitting gastric material) were counted during the next 10 min and the percent inhibition was evaluated by the formula as given below (Ahmad et al., 2011; Kanwal et al., 2013):

$$\%\text{Inhibition} = [(A - B) / A] \times 100$$

Where, A= frequency of retching in control group; B= frequency of retching in test group

Antidiarrheal activity

Castor oil-induced diarrhea method is employed by the method described earlier (Abdullahi et al., 2011) with minor modifications. Albino rats of either sex (200-250 g) were divided into five groups, five animals in each. They were fasted for 24 hours prior to the test, but allowed free access to water. Group I was treated with 1 mL/kg of normal saline, which served as control; Group II received standard drug (loperamide 3 mg/kg). Groups III, IV and V received different doses of the extract (100, 200 and 400 mg/kg) respectively. All doses were administered orally. The animals were then housed singly in cages lined with filter paper. One hour after pre-treatment with the extract, the animals were challenged with 1 mL of castor oil orally. Thereafter, they were observed for 4 hours for the presence of diarrhea defined as watery (wet), unformed stool. (Akuodor et al., 2011).

Following formula applied to calculate the percentage of inhibition.

$$\%\text{Inhibition} = [(A-B)/A] \times 100$$

Where, A = Number of spots of stool in the control group; B = Number of spots of stool in the test group

Statistical analysis

The data are expressed as the mean ± standard error of mean (SEM) and/or median effective concentrations (EC_{50})with 95% confidence interval (CI). GraphPad Prism software (GraphPad, USA) was used to analyze data and construct the graphs. One-way analysis of variance (ANOVA) followed by Dunnett's test were used to compare the experimental groups with the control group. The values of $p<0.05$ were regarded as statistically significant.

Results

The application of the methanol extract of *C. arvensis* to the isolated rabbit jejunum preparations exerted the concentration-dependent effect at a tissue bath concentration range of 0.01-3.0 mg/mL, with an EC_{50} value of 2.8 mg/mL (95% CI: 2.3-3.3; n = 5) (Figure 1A). The *C. arvensis* also caused relaxation of K^+ (80 mM)-induced spastic contractions on isolated rabbit jejunum at a tissue bath concentration range of 3.0 mg/mL with EC_{50} value of 0.9 mg/mL (95% CI: 0.5-1.6; n = 5) (Figure 2A). The *C. arvensis*-induced spasmolytic effect was found to be comparable to the effect of verapamil, which relaxed the spontaneous and K^+ (80 mM)-induced contractions on isolated rabbit jejunum with respective EC_{50} value of 0.7 µM (95% CI: 0.7-0.7; n = 5) and 0.2 µM (95% CI: 0.2 - 0.2; n = 5) (Figure 1; Figure 2D). Furthermore, the petroleum ether fraction of crude methanolic extract of *C. arvensis* inhibited the spontaneous and K^+ (80 mM)-induced contraction on isolated rabbit jejunum at a lower tissue bath concentration range as compared to the crude extract in a manner similar to verapamil with EC_{50} value of 0.6 mg/mL (95% CI: 0.2-1.4; n = 5) (Figure 2B), EC_{50} value of 0.1 mg/mL (95% CI: 0.06-0.1; n = 5) (Figure 1B; Figure 2B).

While the aqueous fraction of *C. arvensis* exhibited the spasmolytic effect followed by the mild contractile response on spontaneous contractions of isolated rabbit jejunum preparations with EC_{50} value of 0.6 mg/mL (95% CI: 0.4-0.9; n = 5) (Figure 1C) and produced the mild relaxation of K^+ (80 mM)-induced contractions at higher bath concentration range as compared to the petroleum ether fraction with EC_{50} value of 32.7 mg/mL (95% CI: 1.9-57.3; n = 5) (Figure 2C). Additionally,

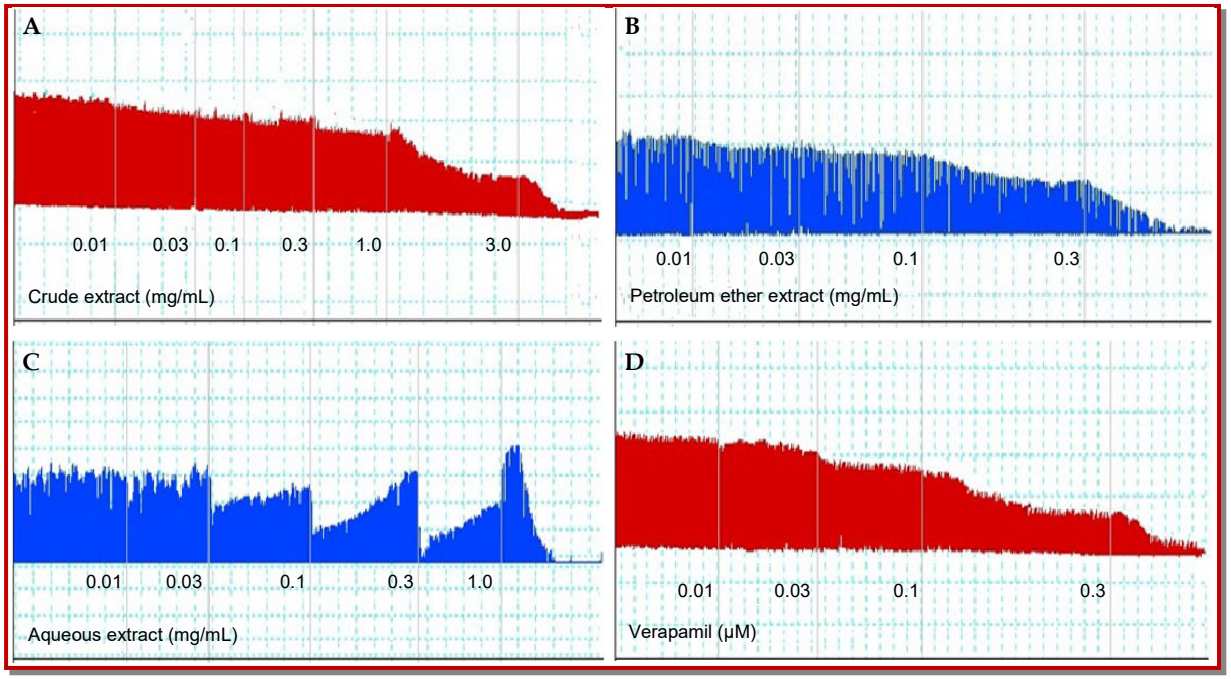

Figure 1: Effect of crude extract (A), petroleum ether extract (B), aqueous extract (C) and verapamil on isolated rabbit jejunum preparations

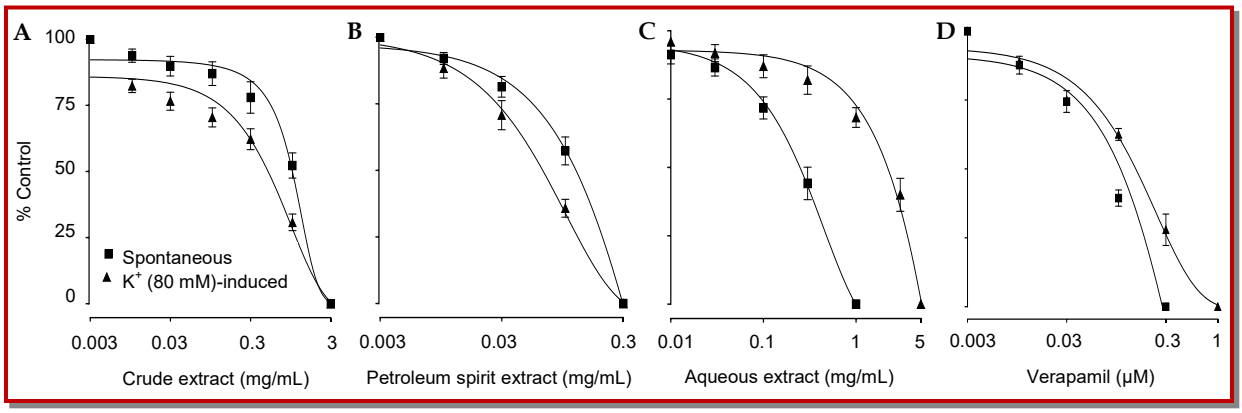

Figure 2: Concentration dependent effect of crude extract (A), petroleum ether extract (B), aqueous extract (C) and verapamil (D) on spontaneous and K^+ (80 mM)-induced contractions on isolated rabbit jejunum preparation. Values are expressed as the mean ± SEM, n = 5

pretreatment of the isolated rabbit jejunum preparation with crude extract (0.3-1.0 mg/mL) caused rightward shifting of concentration response curves for Ca^{2+} (Figure 3A, n = 5). These effects were found to be similar to those produced by verapamil (0.1–0.3 μM), (Figure 3B, n = 5).

The crude extract demonstrated antiemetic properties *in vivo* in chicks after administration of copper sulfate. The crude extract (150 mg/kg) decreased the retching by 60.0% as compared to the chlorpromazine (150 mg/kg) decreased retching by 77.7%, a standard antiemetic drug (Figure 4).

The methanolic extract of crude extract produced marked antidiarrheal effect in rats (Figure 5). All the tested doses of extract significantly decrease the total number of wet feces produced upon the administration of castor oil. The crude extract (100 mg/kg) reduced the castor oil-induced diarrhea by 35%. The crude extract (200 mg/kg) reduced the diarrhea induced by castor oil by 50% while the third tested dose of crude extract (400 mg/kg) shown the maximum response, reduced the wet spots by 63.5% as compared to the standard anti-diarrheal drug loperamide (3 mg/kg) reduced the number of wet spots by 74.1%, induced by castor oil.

Discussion

The methanol extract of *C. arvensis* was applied to the

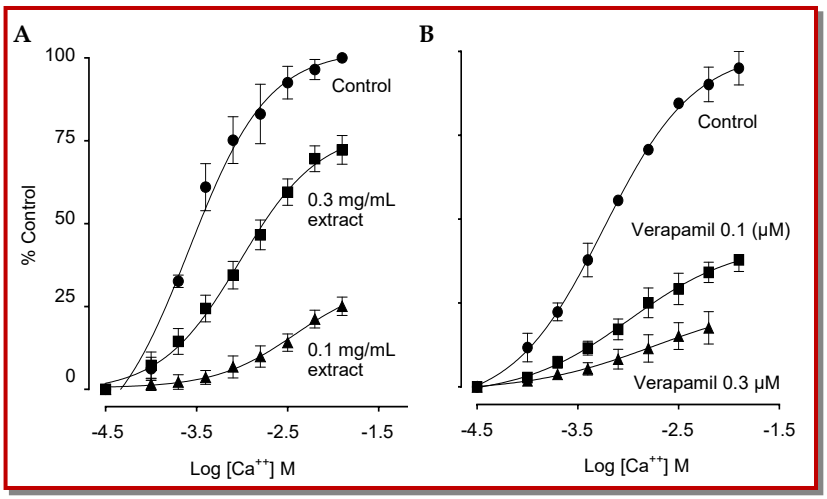

Figure 3: Concentration response curves of Ca^{++} in the presence and absence of increasing concentration of crude extract (A) and verapamil (B) in the isolated rabbit jejunum preparations. Values are expressed as the mean ± SEM, n = 5

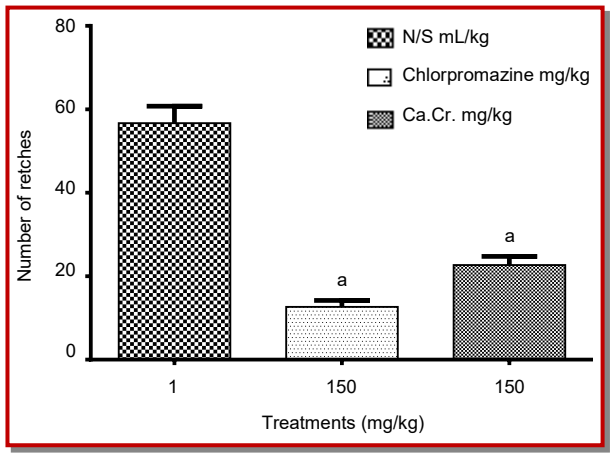

Figure 4: Bar graph showing the response of Ca.Cr. 150 mg/kg on chick emesis model. Values are shown as the mean ± SEM. Data were analyzed by unpaired t-test considering $p<0.05$ as significant. When data were compared with control it showed significant antiemetic activity($^{a}p<0.01$)

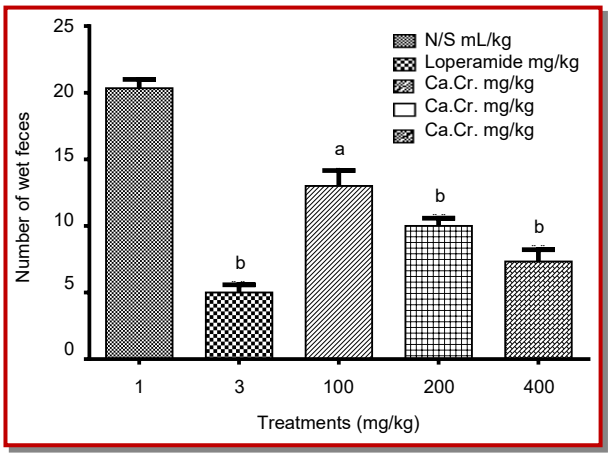

Figure 5: Antidiarrheal effect of different doses of Ca.Cr.(100, 200, 400 mg/kg) using castor oil induced-diarrhoea model in rats, values are expressed as mean ± SEM. Data were analyzed by using unpaired t-test considering $p<0.05$ as significant. When data were compared with control it showed significant antidiarrheal activity ($^{a}p<0.5$, $^{b}p<0.01$)

spontaneously contracting isolated rabbit jejunum preparation. It decreased both the amplitude and frequency of the spontaneous contractions of the jejunum, i.e., exhibiting antispasmodic activity without using an agonist. The rhythmic spontaneous contractions of intestine are synchronized by action potential which is due to continuous depolarization and repolarization. Depolarization demonstrates that the action potential produced due to inward movement of Ca^{++} by L-type voltage dependent Ca^{++} channels (VDCS) (Brading, 1981) or Ca^{++} released from intracellular stores of sarcoplasmic reticulum, which leads to contraction in smooth muscle (Karaki and Weiss, 1983). The resultant inhibition of rhythmic spontaneous contractions in isolated rabbit jejunum tissue by crude extract might be due to difficulty either with entry of Ca^{++} at maximal depolarization through VDCs or obstruction of evoked depolarization due to Ca^{++} discharged from intracellular supplies. As a result, the action potential falls and repolarization occurred (Janbaz et al, 2011; Janbaz et al, 2013a,b) as evident by the relaxation of jejunum.

It is acknowledged that K^{+} (>30 mM) causes the opening of the VDCs (Bolton, 1979), adjusting the Ca^{++} present at outside the cells is the principle reason of contraction of the smooth muscles (Godfraind et al., 1986). Therefore, the substances that are capable to relax the K^{+} (80 mM)-induced contractions are predictable as Calcium channel blocker. The K^{+} (80 mM) evoked the long lasting sustained depolarization with highly conductance of Ca^{++} current into cell, elevated intracellular Ca^{++} ions and highly depolarized cell membrane cause spasmogenic contractions in jejunum

preparations of rabbit (Foster et al., 1984). Addition of crude extract to the tissue organ bath in a cumulative pattern (Van Rossum, 1963) leads to the relaxation of high K^+ (80 mM)-induced contractions in the isolated rabbit jejunum via blockade of influx of Ca^{++} current which results in the repolarization of membrane potential (Godfraind et al., 1986).

The above-mentioned findings were confirmed further as *C. arvensis* treatment on isolated rabbit jejunum preparation caused decreased response to Ca^{++} and rightward shift of the concentration response curves for Ca^{2+} in a manner similar to verapamil as standard Ca^{++} channel blocker (Fleckenstein, 1977). The Ca^{2+} channel blocker is an established class of therapeutic agent and is known to be effective in hyperactive disease of the gut (Van Rossum, 1963).

The liquid-liquid extraction was performed on *C. arvensis* by using petroleum ether and water. The fractions of *C. arvensis* checked for Ca^{2+} channel bloc-king activity, petroleum ether relaxed the spontaneous and K^+ (80 mM)-induced contractions on the isolated rabbit jejunum preparation at a dose of 0.3 mg/mL while aqueous extract relaxed the spontaneous and K^+ (80 mM)-induced contractions in the isolated rabbit jejunum preparation at the dose of 1 and 5 mg/mL respectively. The study resulted in manifestation that Ca^{2+} channel blocking activity is dominant among the petroleum ether fraction.

The vomiting center can be activated directly by irritants or indirectly following input from 4 principal areas, i.e., gastrointestinal tract, cerebral cortex and thalamus, vestibular region, and chemoreceptor trigger zone (CTZ). The CTZ is in vicinity to medulla and contrary to other brain centers it is not protected by the blood-brain barrier (Becker, 2010). The observed anti-emetic effect of crude extract can be likely mediated through inhibition of CTZ (Khan et al., 2013).

The crude extract of *C. arvensis*, like loperamide, a standard antidiarrheal agent subdued significantly the frequency of defecation as well as wetting of feces compared with the untreated group (i.e. rat received neither crude extract, nor loperamide, but saline only). Crude extract 400 mg/kg was most effectual dose rather than 100 and 200 mg/kg tested doses, reduced the number of wet masses by 63.5% as compared to the loperamide (3 mg/kg) reduced the number of wet masses by 74.1%.

The induction of diarrhea by castor oil results from the action of ricinoleic acid produced in the hydrolysis of the oil, which produces alteration in the transport of water and electrolytes (reduce active Na^+ and K^+ absorption and decrease Na^+, K^+ ATPase activity in the small intestine and colon), resulting in a hyper-secretory response and creation of massive contraction of the intestine (Shoba and Thomas, 2001; Chitme et al., 2004). Thus, a potential antidiarrheal agent may exhibit its antidiarrheal effect by inhibiting gut motility and/or electrolyte outflux (diarrheal droppings). The protective effect of the crude extract of *C. arvensis* against the castor oil-induced diarrhea in rats, similar to loperamide and verapamil, suggests that it has either an inhibitory effect on contraction or on electrolyte outflux.

Conclusion

This study clearly indicate the presence of calcium antagonistic components in the crude extract of *C. arvensis* which provide sound mechanistic basis for its use in gastrointestinal disorders e.g. diarrhea and vomiting.

Ethical Issue

All the experiments performed complied with the rulings of Institute of Laboratory Animal Resources, Commission on Life Sciences and approved by ethical committee of Bahauddin Zakriya University, Multan.

Conflict of Interest

The author(s) declare that there is no conflict of interests regarding the publication of this article.

Acknowledgement

The author(s) are thankful to the laboratory staff of the Faculty of Pharmacy, Bahauddin Zakariya University, Multan for the provision of research related facilities.

References

Ahmed S, Sultana M, Hasan MMU, AzharI. Analgesic and antiemetic activity of Cleome viscosa L. Pakistan J Bot. 2011; 43: 119-22.

Akuodor GC, Muazzam I, Usman-Idris M, Megwas UA, Akpan JL, Chilaka KC, Osunkwo UA. Evaluation of the anti-diarrheal activity of methanol leaf extract of *Bombax buonopozense* in rats. Pakistan J Bot. 2011; 3: 15-20.

Aleem A, Janbaz KH. Ethnopharmacological evaluation of *Cen-chrus ciliaris* for multiple gastrointestinal disorders. Bangladesh J Pharmacol. 2017; 12: 125-32.

Abdullahi AL, Agho MO, Amos S, Gamaniel KS, Wambebe C. Anti-diarrhoeal activity of the aqueous extract of *Terminalia avicennoides* roots. Phytother Res. 2011; 15: 431-34.

Becker DE. Nausea, vomiting, and hiccups: A review of mechanisms and treatment. Anesthe Prog. 2010; 57: 150-57.

Brading AF. How do drugs initiate contraction in smooth

muscles? Trend Pharm Sci. 1981; 2: 261–65.

Bolton TB. Mechanism of action of transmitters and other substances on smooth muscles. Physiol Rev. 1979; 59: 606–718.

Chitme HR, Chandra R, Kaushik S. Studies on antidiarrheal activity of *Calotropis gigantea* R. Br. in experimental animals. Pharm Sci. 2004; 7: 70-75.

Foster RW, Small RC, Weston AH. The spasmogenic action of potassium chloride in guinea-pig trachealis. Br J Pharmacol. 1984; 80: 553-59.

Fleckenstein A. Specific pharmacology of calcium in myocardium, cardiac pacemakers, and vascular smooth muscle. Ann Rev Pharmacol Toxicol. 1977; 17: 149-66.

Gilani AH, Jabeen Q, Ghayur MN, Janbaz KH, Akhtar MS. Studies on the anti-hypertensive, anti-spasmodic, bronchodilator and hepatoprotective activities of the *Carum copticum* seed extract. J Ethnopharmacol. 2005a; 98: 127-35.

Gilani AH, Bashir S, Janbaz KH, Shah AJ. Presence of cholinergic and calcium channel blocking activities explains the traditional use of *Hibiscus rosasinensis* in constipation and diarrhoea. J Ethnopharmacol. 2005b; 102: 289-94.

Godfraind T, Miller R, Wibo M. Calcium antagonism and calcium entry blockade. Pharmacol Rev. 1986; 38: 321-416.

Hasan SR, Hossain MM, Akter R, Jamila M, Mazumder MEH, Alam MA, Rahman S. Analgesic activity of the different fractions of the aerial parts of *Commelina benghalensis* Linn. Int J Pharmacol. 2010; 6: 63-67.

Hill RJ. Canada thistle, *Cirsium arvense* (L.) Scop. Reg. Horticulture, 1983; 9: 27-34.

Janbaz KH, Farhaj M, Saqib F, Imran I, Zia-Ul-Haq M, De Feo V. Pharmacological effects of *Lactuca serriola* L. in experimental model of gastrointestinal, respiratory, and vascular ailments. Evid Based Complement Alternat Med. 2013a; 2013.

Janbaz KH, Nisa M, Saqib F, Imran I, Zia-Ul-Haq M, De Feo V. Bronchodilator, vasodilator and spasmolytic activities of methanolic extract of *Myrtus communis* L. J Physiol Pharmacol. 2013b; 64: 479-84.

Janbaz KH, Hamid I, Mahmood MH, Gilani AH. Broncho-dilator, cardiotonic and spasmolytic activities of the stem barks of *Terminalia arjuna*. J Appl Sci. 2011; 3: 104-20.

Kanwal W, Syed AW, Salman A, Mohtasheem HM. Anti-emetic and anti-inflammaotry activity of fruit peel of *Luffa cylindrica* (L.) Roem. J Ethno Trad Med Photon. 2013; 118: 258 -63.

Khan IA, Aziz A, Munawar SH, Munzoor Z. Antiemetic activity of methanolic leaf extract of *Rumex vesicarius* Linn. Int J Pharm All Sci. 2013; 2: 33-37.

Karaki H, Weiss B. Calcium release in smooth muscle. Life Sci. 1983; 42: 111-22.

Moore RJ. The biology of Canadian weeds. Can J App Sci. 1975; 55: 1033-48.

Rahman M, Khatun A, Nesa ML, Hussain H, Jahan IA. Bioactive polyphenols from the methanol extract of *Cnicus arvensis* (L.) Roth demonstrated antinociceptive and central nervous system depressant activities in mice. Evid Based Complement Alternat Med. 2015, 2015.

Saqib F, Janbaz KH. Rationalizing ethnopharmacological uses of *Alternanthera sessilis*: A folk medicinal plant of Pakistan to manage diarrhea, asthma and hypertension. J Ethnopharmacol. 2016; 182: 110-21.

Saqib F, Ahmed MG, Janbaz KH, Dewanjee S, Jaafar HZ, Zia-Ul-Haq M. Validation of ethnopharmacological uses of *Murraya paniculata* in disorders of diarrhea, asthma and hypertension. BMC Complement Altern Med. 2015; 15: 319.

Shoba FG, Thomas M. Study of anti-diarrhoeal activity of four medicinal plants in castor-oil induced diarrhoea. J Ethnopharmacol. 2001; 76: 73-76.

Uddin NS. Traditional uses of ethnomedicinal plants of the Chittagong Hill Tracts. Bangladesh National Herbarium. Bangladesh, 2006.

Van Rossum JM. Technique for the making of dose-response curves in isolated organs and the evaluation of drug parameters. Arch Intern Pharmacodyn Ther. 1963; 143: 299-330.

Author Info
Fatima Saqib (Principal contact)
e-mail: Fatima_pharmacology@hotmail.com