

Pharmacological effects (gastrointestinal, respiratory and vascular) of *Calligonum polygonoides*: Animal model based studies

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Abstract: The aim of this study was to analyze gastrointestinal, respiratory and vascular pharmacological effects of 70% hydro-alcoholic extract of *Calligonum polygonoides* (*Cp. Cr*) in animal models. All the procedures were carried-out as per previous literature with slight modification where necessary. It was found that *Cp. Cr* affected significant relaxation of spontaneous and K⁺ (80 mM) induced contractions. The results showed a corresponding shift of calcium concentration response curves. Similarly *Cp. Cr* showed relaxant effect on trachea in carbachol (Cch) induced tracheal contractions. Moreover, contractions induced by phenylephrine (1μM) in quarantine rabbit aortic preparations causes *Cp. Cr* induced relaxation of aortal contractions. Verapamil was used as a standard calcium channel blocker. The findings of this study suggested vasodilator, bronchodilator and spasmolytic effects of *Cp. Cr*.

Keywords: *Calligonum polygonoides*, spasmolytic effect, vasodilation effect, bronchodilation effect, Aorta

INTRODUCTION

Calligonum polygonoides belongs to *Polygonaceae* family. *Polygonaceae* is one of the biggest family of 30-50 genus and more than thousand species that are distributed throughout the world (Abd-ElG awad *et al.*, 2020). It is a small Psammophytic shrub found in profusion in Thar, Thal and Rohi Desert zones of Pakistan (Vyas, 2012). *Calligonum polygonoides* can be geographically regarded as cosmopolitan as it can be found distributed from tropics to arctic. Concentrated in northern temperate zone, it has significant distribution in desert and semi-desert zones of western and south-east Asia (Rathore *et al.*, 2015). Widespread distribution of *Calligonum polygonoides* species owes to its diverse morphological characteristic and adaptation to harsh and unbearable temperature changes of the desert ecosystem (Nasrullah *et al.*, 2015). Plant species that bears the test of time and climatic changes have survived millions of years and *Calligonum polygonoides* species are among one of those exceptions (Samejo *et al.*, 2013).

Samejo et-al. reported the presence of Flavonoids (buds, seeds, flowers, and stem), Alkaloids (roots, buds and seeds), Proteins (flowers and seeds), tannins, phenols, steroids, terpenoids and carbohydrates (roots, stem, buds, flower, and seeds) in *Calligonum polygonoides* Linn (Samejo *et al.*, 2011; Ahmed *et al.*, 2016).

The paste formed by the roots of *Cp. Cr* is used for the treatment of prickly heat and sores. Flowers are used for the treatment of asthma, eczema, cough and cold. Plant is traditionally used for treatment of stomach and gastric ailments (Ahmad *et al.*, 2016).

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The present study aimed to investigate therapeutic effects of 70% hydro-alcoholic extract of *Calligonum polygonoides* (*Cp. Cr*) in gastrointestinal, respiratory and vascular ailments.

MATERIALS AND METHODS

Identification of plant and extraction of crude extract (*Cp. Cr*)

Fresh entire shrub of *Calligonum polygonoides* is a plant which was dried and collected locally from Bahawalpur, Pakistan. The plant was recognized by a taxonomist in the Department of Botany, The Islamia University of Bahawalpur. A receipt sampling was placed in the Herbarium of same department (receipt No. 96/Botany).

An entire shrub of *Calligonum polygonoides* was shade dried and was crushed to coarse powder using electrical grinder. 70% hydro-alcohol was for soaking 1kg crushed plant material. Cold maceration was used for extraction for 7 days at room temperature with random shaking. A doubled layered muslin cloth was used to conduct coarse filtration. A coarse filtration was carried out by a double layered muslin cloth and subsequently a fine filtration was carried-out through a whatmann filter paper. The obtained filtrate was concentrated in rotary evaporator at 40°C under reduced pressure to a thick, semi-solid mass (Rahman *et al.*, 2019). The obtained percentage of yield was approximated 6.25%. The dried mass was shifted to a beaker for further use. The typical solutions of the *Calligonum polygonoides* were prepared and diluted by adding distilled water. Fractionation is applied to ethanolic extract of *Cp. Cr*. Definite proportions of DCM and water was mixed to prepare DCM and aqueous

divisions in a separating funnel having *Cp. Cr* by shaking it for approximately 15 minutes. The upper aqueous film was collected and concentrated by rotary evaporator while the lower DCM layer was collected and concentrated into DCM fraction both in triplicate.

Chemicals and reagents

Carbachol (Cch.), Calcium chloride, Potassium chloride, Acetylcholine chloride (Sigma USA), Glucose, Magnesium chloride, Sodium bicarbonate, Sodium dihydrogen sulphate, Potassium dihydrogen phosphate, Sodium dihydrogen phosphate, Magnesium sulphate, Sodium chloride, Ethanol (Merck, Germany), Ammonium hydroxide (BDH, England), Sodium hydroxide, all the chemicals and reagents were of analytical grade.

Experimental animals

Rabbits having weight of 2.0 to 3.0 Kg and BALB/c mice of 20 to 40 g of either gender were accommodated at 23-25°C of temperature and 45%-50% of RH at the *In-vivo* Research Animal House of Faculty of Pharmacy, Bahauddin Zakariya University Multan, Pakistan.

Preparation of animals

Experimental animals were fed with typical diet and water. Prior to conduct the experiment, the animals were allowed to easily access water while preserved at fasting 24 hrs. *In-Vitro* study was conducted by scarifying the rabbits on the back of their head and cervical displacement. The experiments were conducted as per NIH guidelines set for Lab animals and protocols were established by the Institute of Laboratory Animal Resources, Commission on Life Sciences. The Institutional Ethical Review Board (IERB) of the Bahauddin Zakariya University, Multan approved study by official letter vide reference No 10/PEC/2015.

In vitro studies

Experiments on isolated tissue preparation of rabbit were accomplished as described by Gilani *et al* with slight modification. Briefly, freshly prepared jejunum, tracheal and aortic tissue segments from the rabbit were used. These were preserved effectively well in the respective buffer solutions (Gilani *et al.*, 2005).

Preparations of Isolated rabbit jejunum

Cp. Cr was vetted for the possible presence of either spasmolytic or spasmogenic activity. Approximately 2 cm long segments were prepared from isolated jejunum tissue. Respective segments were mounted in a 15ml Tyrode's solution in tissue organ bath. The temperature of the bath was maintained at 37°C and tissue was constantly aerated with carbogen. Intestinal response was recorded by using the isotonic bioscience transducers and Power Lab data acquisition system with lab chart software (V-6). Every fleshy tissue was permitted to equilibrate for at least 30 min. prior to the addition of any chemical substance. The sub-maximal response was achieved by

stabilizing isolated jejunum with subsequent exposure to 0.3 μM acetylcholine followed by washing with Tyrode's solution. The rabbit jejunum presented with impulsive rhythmic contractions under these circumstances. *Cp. Cr* was vetted and presence of either spasmolytic or spasmogenic action was screened by applying plant dose. The spasmolytic/spasmogenic activity of *Cp. Cr* was screened by the application of plant dose in collective manner to pre-stabilized rabbit's jejunum (Rahman *et al.*, 2019). The spontaneous rhythmic contractions of jejunum tissue were measured as percent change for inhibitory effect created by *Cp. Cr*. The test To see the possible mechanism of spasmolytic effect, the experimental material was investigated in contradiction to induced contractions in order to understand the spasmolytic effects.

The depolarization of substance produced by extraordinary K⁺ (80 mM) was used to assess calcium channel blocking (CCB) activity as per literature. High K⁺ is acknowledged for mediation of voltage dependent calcium channel as the inward movement of extracellular Ca²⁺ lead to contraction of smooth muscles (Janbaz *et al.*, 2015). The substance was considered as inhibitory of calcium channel that bases for the relaxation of contractions induced by huge K⁺ influx (Imran, 2011; Al-Shboul, 2018, Sweeney and Hammers, 2018, Touyz *et al.*, 2018; Rahman *et al.*, 2019).

Rahman *et al.*, 2019 described the method to establish blocking activity of calcium channel of the investigated substance. In order to stabilize the isolated jejunum preparations, they were allowed to kept in normal Tyrode's solution which was then substituted with EDTA (0.1 Mm) retained in bath for 30 minutes so as to efflux Ca²⁺ from the tissue. Furthermore, the solution was replaced with Ca²⁺ free and K⁺ rich Tyrode's solution having composition of (in Mm): Glucose (5.55), KCl (50), NaH₂PO₄ (0.42), NaCl (91.04), MgCl₂ (1.05), NaHCO₃ (11.90), and EDTA (0.1).

The control of Ca²⁺ concentration-response curve (CRC) was established after preliminary incubation period of 30 minutes. A super imposable Ca²⁺ CRC was achieved (usually after 2 cycles) when jejunum tissue was incubated with plant dose for 1 hr. The Ca²⁺ CRC was reconstructed and detected for calcium channel blocking activity after incubation of different dosages of test substance. The interpretation of K⁺ channel opening significance was confirmed against contractions produced by low K⁺ (25 Mm) in order to check the test material incapable of relaxation of high K⁺ (80 Mm) induced contractions.

Isolated rabbit tracheal preparations

The rabbit's trachea was dissected and preserved in Krebs's solution whose composition was (Mm): glucose

(11.7), CaCl_2 (2.5), KH_2PO_4 (1.3), NaCl (118.2), KCl (4.7), NaHCO_3 (25.0), and MgSO_4 . The adjacent tissues were removed from isolated trachea and then it was cut into segment having diameter of 2-3 mm. Each segment was then cut longitudinally so that it forms a strip in which smooth muscles were located on either sides of C-shaped cartilaginous part. A thermo-regulated bath of capacity 15 ml containing Krebs's solution maintained at temperature of 37°C and having pH 7.4, aerated with carbogen was maintained by mounting tracheal tissue organ. An appropriate pre-load of 1 g was applied and the mounted tissue was allowed to equilibrate for roughly 1 hr. prior to addition of any substance. The vasorelaxant effect of investigated substance was evaluated by adding dose of the test substance in collective way by inducing sustained contractions as a result of High K (80 mM) and carbachol (1 μM). The responses were recorded by using bioscience transducers which were attached to power lab data acquisition system linked to lab chart software (Gilani et al., 2005; Rahman et al., 2019).

Isolated rabbit aorta preparation

Wide segments of 2-3 in numbers are prepared by dissecting and cutting aorta. A bath having capacity of 15 ml containing Krebs's solution maintained at 37°C having pH 7.4 aerated with carbogen was maintained by mounting these segments.

An appropriate pre-load of 2 g applied and the mounted tissue was allowed to equilibrate for 1 hr. Repeated exposure to phenylephrine (1 μM) leads to stabilization of the tissues after accomplishment of equilibrium. The vasorelaxant and vasoconstrictive effect of test material was assessed in tissue bath by adding test substance in which sustained contractions were induced in cumulative way. The responses were recorded by using bioscience transducers. These transducers were attached to power lab data acquisition system by computer linked to lab chart software (V6).

STATISTICAL ANALYSIS

The statistical significance among experiments was calculated using Student's t-test. The collected data was evaluated as Mean+SEM and medium effective concentration (EC_{50}) with probability level of 0.05. Concentration response curves were obtained by applying Graph pad prism (V 8.0, USA). Two-way ANOVA test was applied on several models followed by Turkey's test. 5 % P value ($p < 0.05$) was considered to be statistically significant.

RESULTS

Effect on rabbit jejunum preparations

When the unprocessed extract of Cp-Cr was applied on the jejunal preparation of rabbit, restrictive effect was seen against the spontaneous and High K (K^+ -80mmol/L)

induced contractions. Cp. Cr was applied in different concentration range from 0.01 to 10mg/mL. Calculated EC_{50} values were 0.212 mg/mL (95% CI: 0.0014-31.62 mg/mL) for the spontaneous and 5.140 mg/mL (95% CI: 4.20-6.27 mg/mL) for high potassium respectively. Moreover Ca^{2+} mediated contraction of the tissues was stopped by the Cp. Cr at the dose of 3.0mg/mL, 5.0mg/mL and 10.00mg/mL. Comparably a calcium channel blocker drug verapamil also inhibits automatic and K^+ -80 mmol/L induced contractions. The values of verapamil for respective EC_{50} response were 0.07 ± 0.05 (95% CI: 0.05-0.10 mg/mL) and 0.25 ± 0.2 (95% CI: 0.21-0.33 mg/mL) and shifted the calcium response curves toward right by inhibiting the contractile response of Ca^{2+} . Cp. DCM exhibited relaxant result at same dose with EC_{50} value of 2.90 ± 4.2 (95% CI: 0.85-10.17 mg/ml) for extemporaneous, 0.176 ± 0.1 (95% CI: 0.005-5.96 mg/mL) and high- K^+ -produced contractions respectively. Complete relaxation of extemporaneous contractions was attained with the application of EC_{50} value of 0.89 ± 0.2 (95% CI: 0.4-1.23 mg/mL) and for high K produced EC 50 values are 0.010 ± 0.03 (95% CI: 0.9-5.23mg/mL).

It was observed that the Cp. Cr extract was applied to the tissue which results in shifting of the CRCs towards right, supports the blocking activity of calcium channel by Cp. Cr. The data has been presented in fig. 1 and 2.

Effect on the rabbit tracheal preparations

Carbachol (Cch) was used to induce the contractions in separated rabbit trachea. Relaxant effect observed was dose dependent against the pre-contracted tissues treated with Cp-Cr. Cp-Cr in amount of 0.01mg/mL to 10.00 mg/mL caused thorough relaxation of the preliminary contracted trachea with carbachol (CCh) 1 $\mu\text{mol/L}$ with particular EC_{50} standards of 1.412 ± 2.8 (95% CI: 0.20-7.9 mg/mL) and 2.42 ± 1.6 (95% CI: 1.88 to 3.14 mg/mL) similar to verapamil.

Furthermore, the application of Cp extract in DCM with EC_{50} values of 0.3 ± 0.6 (95% CI: 0.05-1.87 mg/mL), causes C-Ch induced contractions in which a marked variation in relaxation was detected while application of Cp. Aq in DCM at amount of 10.0 mg/mL with EC_{50} values of 0.121 ± 2.8 (95% CI: 0.8-7.64mg/ml).in DCM results in complete relaxation of contractions induced by CCh. Similar results were obtained with the standard Verapamil and presented in fig. 3 & fig. 4.

Effect on rabbit aortal preparations

Complete relaxation of pre-contracted aorta with phenylephrine (PE) 1 $\mu\text{mol/L}$ with respective EC_{50} values of 5.4 ± 3.9 (95% CI: 2.09-13.7mg/mL) and 2.42 ± 1.1 (95% CI: 1.88-3.14 mg/mL) is due to Cp-Cr in a concentration of 0.01 to 10.00 mg/mL as same as verapamil. Furthermore, the application of Cp. DCM exhibit thorough relaxation of PE-induced contractions at

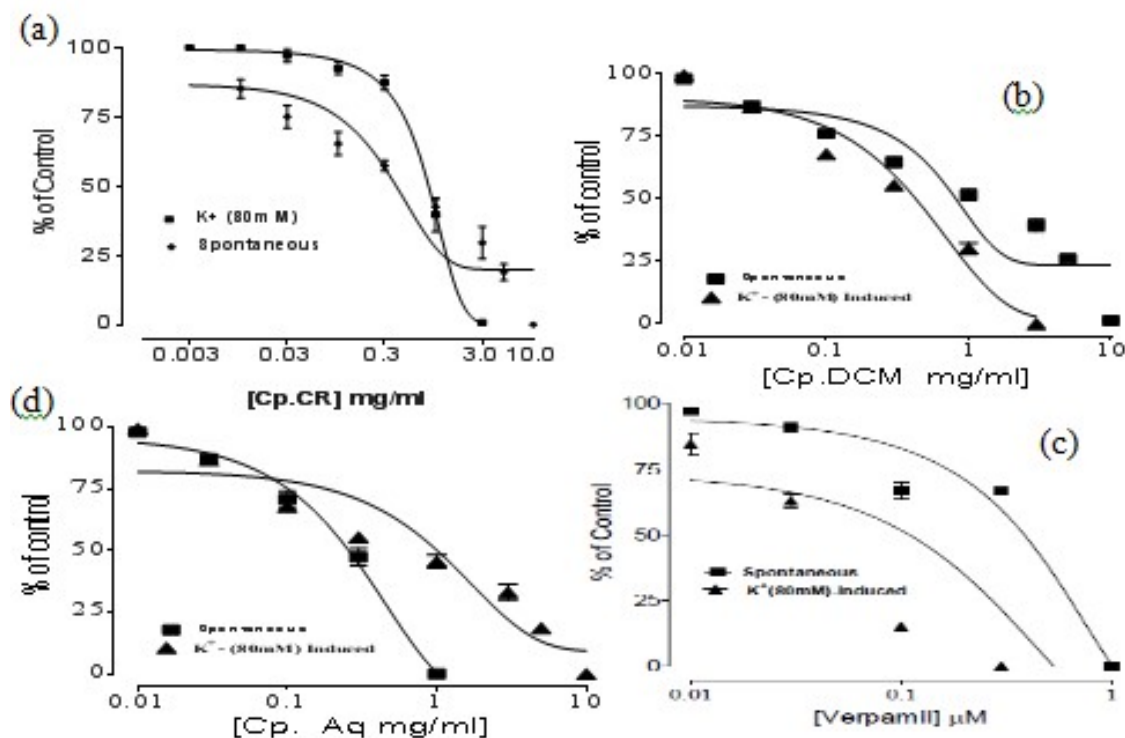


Fig. 1: Concentration dependent inhibitory response of (a) effects of crude extract of *Calligonum polygonoides* (Cp. Cr) (b) effects of *Calligonum polygonoides* DCM (Cp. DCM) (c) effects of *Calligonum polygonoides* Aqueous (Cp. Aq) and (d) effects of Verapamil on CCh.-induced contractions on rabbit tracheal preparations.

Typical values are expressed in the form of Mean \pm S.E.M, n=5. Two-way ANOVA was adopted for statistical analysis of data which was followed by Tukey's Test (Probability values of *p<0.05, **p<0.01, ***p<0.001 were considered significant).

the dose of 3.00 mg/mL with EC₅₀ values of 3.427 \pm 1.6 (95% CI: 1.84-6.33mg/ml) and Cp. Aq fraction causes relaxation at dose of 5.0 mg/ml with EC₅₀ values of 1.90 \pm 1.3 (95% CI: 0.62-5.92mg/ml) as shown in fig. 5.

DISCUSSION

Results in this study demonstrated that herbal medications are constantly used for mitigation and treatment of different diseases throughout the world. The phytochemicals have major role in pharmaceutical industry as basis of potent medicinal agent (Abd-EIG awad *et al.*, 2020). Herbal medications have pharmacological potential to facilitate management of cardiovascular, respiratory and gastrointestinal diseases (Ayrle *et al.*, 2016). *Calligonum polygonoides* has a traditional reputation to offer relief in diarrhea and dysentery (Ahmad *et al.*, 2016).

Diarrhea is mainly caused when the intestinal motility of GIT is boosted (Melese *et al.*, 2019; Ciobanu and Dumitrascu, 2011). The blockage of calcium channel as a result of inhibition of calcium influx results in is due to relaxation of smooth muscles. It is advantageous for the management of smooth muscles ailments as voltage gated channels are blocked. For examples, hypertension,

asthmatic cough and diarrhea (Janbaz *et al.*, 2015; Lee *et al.*, 2012). Voltage dependent calcium channel of tissues are opened causing contraction of these smooth muscles when smooth muscle tissues K⁺ are exposed to the extraordinary concentration of K⁺.

Inhibitory response was detected when *Calligonum polygonoides* was applied to the high K⁺ treated isolated tissues of rabbit's jejunum which is almost similar to the Verapamil induced contraction. The effect of the plant is due to the Calcium channel blocking activity. The movement of calcium curve towards right is due to inhibition of calcium contractile effect (Rahman *et al.*, 2019).

Calligonum polygonoides may have traditional application as novel drug design in the management of asthma and cough. The crude extract of these medicinal plants was test on isolated and obtained piece of rabbit's trachea for validation of therapeutic uses of Cp. Cr. Carbachol induced narrowing of trachea was relaxed by using Cp. Cr. The tool for producing contraction and consequent stimulation of M-3 receptors was accomplished by application of carbachol on isolated trachea. The triggering of an enzyme Phospho lipase was achieved as a result of stimulation of M-3 muscarinic receptors.

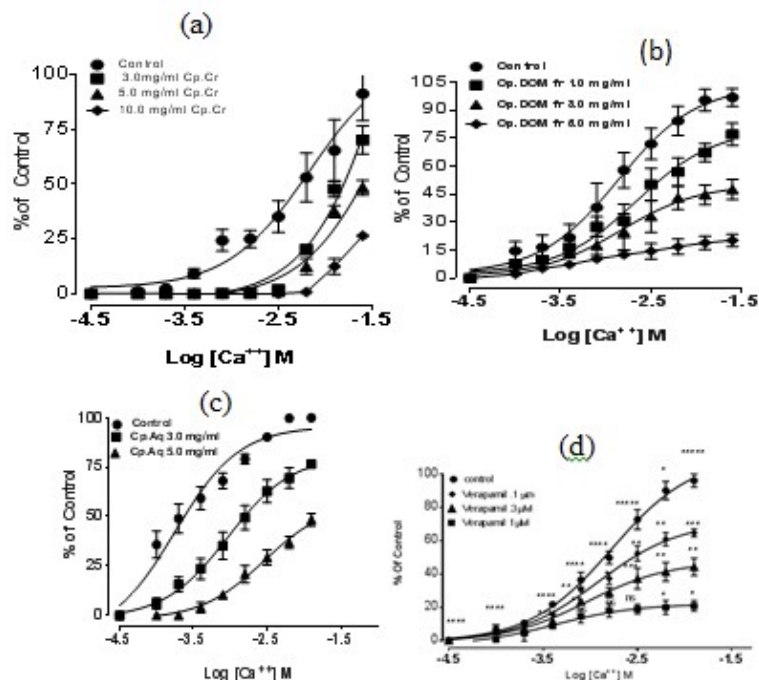


Fig. 2: Concentration vs response curves of calcium (Ca²⁺) in presence or absence of growing concentrations of (a) Effects of crude extract of *Calligonium polygonoides* (Cp. Cr) (b) Effects of *Calligonium polygonoides* DCM (Cp. DCM) (c) Effects of *Calligonium polygonoides* Aqueous (Cp. Aq) and (d) Verapamil in isolated rabbit tracheal preparations.

Typical values are expressed in the form of Mean + S.E.M, n=5. Student's t-test was used for numerical statistical investigation of data followed by Turkey's Test (Probability values of *p<0.05, **p<0.01, ***p<0.001 were considered to be statistically significant).

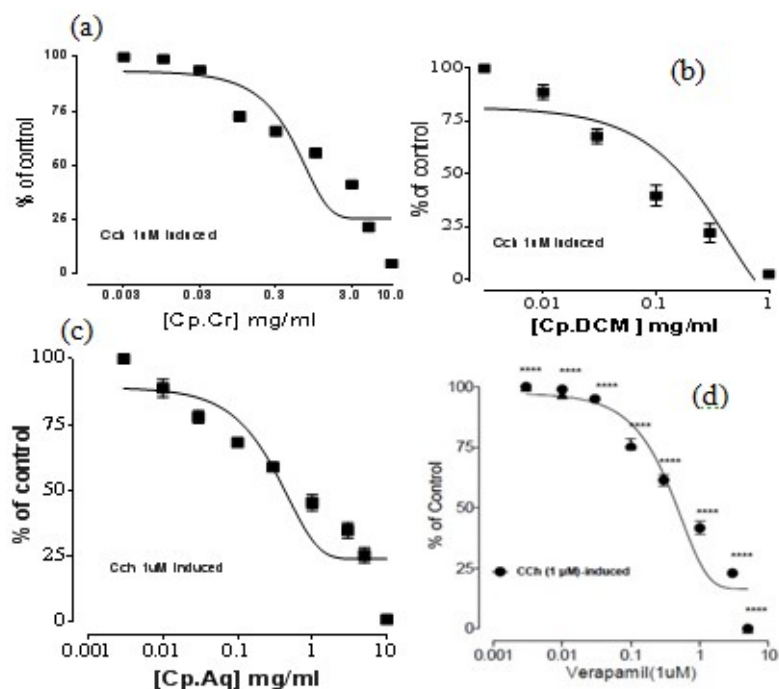


Fig. 3: Concentration dependent inhibitory response of (a) effects of crude extract of *Calligonium polygonoides* (Cp. Cr) (b) effects of *Calligonium polygonoides* DCM (Cp. DCM) (c) effects of *Calligonium polygonoides* Aqueous (Cp. Aq) and (d) effects of Verapamil on Cch-induced contractions on rabbit tracheal tissues.

Typical values are expressed in the form of Mean + S.E.M, n=5. Student's t-test was used for numerical statistical investigation of data followed by Turkey's Test (Probability values of *p<0.05, **p<0.01, ***p<0.001 were considered to be statistically significant).

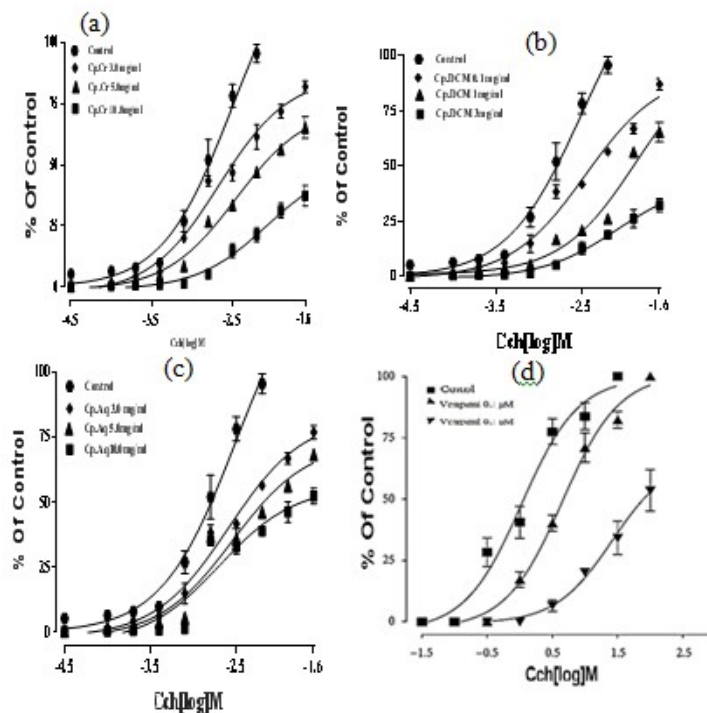


Fig. 4: Concentration vs response curves of Carbachol in presence or absence of growing concentrations of (a) Effects of crude extract of *Calligonum polygonoides* (Cp. Cr) (b) Effects of *Calligonum polygonoides* DCM (Cp. DCM) (c) Effects of *Calligonum polygonoides* Aqueous (Cp. Aq) and (d) Verapamil in isolated rabbit tracheal preparations. Typical values are expressed in the form of Mean + S.E.M, n=5. Student's t-test was used for numerical statistical investigation of data followed by Turkey's Test (Probability values of *p<0.05, **p<0.01, ***p<0.001 were considered to be statistically significant).

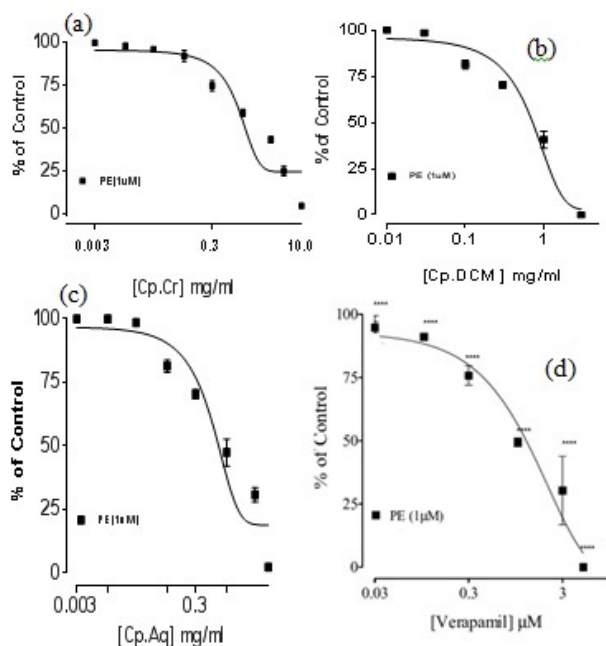


Fig. 5: (a) Phenylephrine mediated contractions of rabbit's trachea relaxed by Cp. Cr. (b) Phenylephrine mediated contractions of rabbit's trachea relaxed by Cp. DCM (c) Phenylephrine mediated contractions of rabbit's trachea relaxed by Cp. Aqueous (d) Phenylephrine mediated contractions of rabbit's trachea vasodilation by verapamil. Standard values are expressed in the form of Mean + S.E.M, n=5. Student's t-test was used for numerical statistical investigation of data followed by Turkey's Test (Probability values of *p<0.05, **p<0.01, ***p<0.001 were considered to be statistically significant).

The stimulation of phospholipase-C leads to the formation of phosphoinositol 4, 5 biphosphate, inositol triphosphate and diacyl glycerol (Katzung and Chatterjee, 2012). The phosphokinases, proteins and enzymes are stimulated by diacyl glycerol while voltage mediated calcium channel of cell membrane are activated and opened by IP-3. Consequently, bronchoconstriction is achieved (Townsend *et al.*, 2013). The resulted Carbachol induced bronchoconstriction is due to bronchodilation caused by Cp. Cr. The similar results were detected when Cp. DCM was used in the trachea of rabbit. Likewise, same results were observed when Cp. Aqueous was applied to tracheal contractions mediated by C.Ch. The blocking activity of calcium channels caused by *Calligonum polygonoids* was additionally confirmed by establishing C.Ch-response curve, in which the established curve move towards right in parallel manner. The findings of the study suggested that voltage mediated calcium channel blocking activity seen in rabbit's jejunal studies is because of the relaxation of trachea. Therefore, the aforementioned reasonable proofs demonstrate traditional application of *Calligonum polygonoids* for the management of asthma.

Hence, the findings of the present study demonstrated that vaso-relaxant, bronchodilator and spasmolytic potential of *Calligonum polygonoides* is because of the presence of flavonoids, alkaloids, tannins, phenols, polyphenols, steroids, terpenoids, and carbohydrates that have the calcium channel blocking ability (Samejo *et al.*, 2011; Ahmed *et al.*, 2016).

CONCLUSION

The present phytochemical investigation illustrate shows an antihypertensive, anti-asthmatic and anti-diarrheal effects of *Calligonum polygonoides*. The findings and discussion revealed that *Calligonum polygonoides* have the capacity to block M-3 muscarinic receptors, α -receptors and voltage intermediated calcium channels due to which the shrub has pharmacological effects pertaining to spasm, bronchodilation and vasodilation. The isolated, purified photochemical ingredients of medicinal herbs could be suitable and strong drug candidate for management of diseases. Furthermore, novel drug design of these medicinal plants may be achieved by isolation/purification and characterization.

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REFERENCES

Abd-ElGawad AM, Rashad YM, Abdel-Azeem AM, Al-Barati SA, Assaeed AM and Mowafy AM (2020).

Calligonum polygonoides L. shrubs provide species-specific facilitation for the understory plants in coastal ecosystem. *Biol.*, **9**(8): 232-242.

Ahmed H, Moawad A, Owis A, AbouZid S and Ahmed O (2016). Flavonoids of *Calligonum polygonoides* and their cytotoxicity. *Pharm Biol.*, **54**(10): 2119-26.

Al-Shboul O (2018). Contraction and relaxation signaling in gastrointestinal smooth muscle. *Gastroenterol.*, **5**(5): 315-321.

Ayrle H, Mevissen M, Kaske M, Nathues H, Gruetzner N, Melzig M and Walkenhorst M (2016). Medicinal plants prophylactic and therapeutic options for gastrointestinal and respiratory diseases in calves and piglets? A systematic review. *BMC. Vet. Res.*, **12**(8): 2242-2253.

Ciobanu L and Dumitrascu DL (2011). Gastrointestinal motility disorders in endocrine diseases. *Pol. Arch. Med. Wewn.*, **121**(4): 129-136.

Gilani AH, Bashir S, Janbaz KH and Shah AJ (2005). Presence of cholinergic and calcium channel blocking activities explains the traditional use of *Hibiscus rosasinensis* in constipation and diarrhoea. *J. Ethnopharmacol.*, **102**(2): 289-294.

Imran I, Hussain L, Zia-Ul-Haq M, Janbaz KH, Gilani AH and De Feo V (2011). Gastrointestinal and respiratory activities of *Acacia leucophloea*. *J. Ethnopharmacol.*, **138**(3): 676-682.

Janbaz KH, Akhtar T, Saqib F, Imran I, Zia-Ul-Haq M, Jansakul C, De Feo V and Moga M (2015). Pharmacological justification of use of *Solena heterophylla Lour* in gastrointestinal, respiratory and vascular disorders. *J. Trans. Med.*, **13**(1): 134.

Katzung BG and Chatterjee K (2012). Vasodilators and the treatment of angina pectoris. *Basic. Clin. Pharmacol.*, **7**: 20-25.

Lee G, Yori P, Olortegui MP, Pan W, Caulfield L, Gilman RH, Sanders JW, Delgado HS and Kosek M (2012). Comparative effects of vivax malaria, fever and diarrhoea on child growth. *Int. J. Epidemiol.*, **41**(2): 531-539.

Melese DA, Liknaw BZ, Yitayal AG, Yonas L and Yohannes MA (2019). Diarrhea prevention practice and associated factors among caregivers of under-five children in enemay district, northwest Ethiopia. *J. Environ. Public. Health.*, pp.1-8.

Rahman HA, Rasool MF and Imran I (2019). Pharmacological studies pertaining to smooth muscle relaxant, platelet aggregation inhibitory and hypotensive effects of *Ailanthus altissima*. *Evid. Based. Complement. Alternat. Med.*, pp.1-9.

Rahman KA, Rasool MF and Imran I (2017). Pharmacological evaluation of smooth muscle relaxant and cardiac modulation potential of *Phylla nodiflora in ex-vivo* and *in-vivo* experiments. *Asian. Pac. J. Trop. Med.*, **10**(8): 121-145.

Rathore VS, Singh JP, Bhardwaj S, Nathawat NS, Kumar M and Roy MM (2015). Potential of native shrubs

- Haloxylon salicornicum* and *Calligonum Polygonoides* for restoration of degraded lands in Arid Western Rajasthan. *India. Environ. Man.*, **55**(1): 205-216.
- Samejo MQ, Memon S, Bhanger MI and Khan KM (2013). Isolation and characterization of steroids from *Calligonum polygonoides*. *J. Pharm. Res.*, **6**(3): 346-349.
- Sweeney HL and Hammers DW (2018). Muscle contraction. *Cold Spring Harbor perspectives in biology. J. Biol.*, **10**(2): 1-13.
- Touyz RM, Alves-Lopes R, Rios FJ, Camargo LL, Anagnostopoulou A and Arner A (2018). Vascular smooth muscle contraction in hypertension. *Cardiovas. Res.*, **114**(4): 529-539.
- Townsend EA, Siviski ME, Zhang Y, Xu C, Hoonjan B and Emala CW (2013). Effects of ginger and its constituents on airway smooth muscle relaxation and calcium regulation. *American J. Resp. Mol. Biol.*, **48**(2): 157-163.