Pharmacological studies pertaining to spasmolytic, bronchodilator and vasodilating effect of *Typha domingensis*: An evidence-based approach

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Abstract: This study was designed to analyze the pharmacological effects of *Typha domingensis* crude 70% aqueous-ethanol extract of *Typha domingensis* (Td. Cr) in gastrointestinal, respiratory and vascular diseases. Rabbits (2.0-3.0 kg) and BALB/c mice (20-40 g) of local breed have been used as experimental animals using the established methodologies from literature with slight modification. The findings suggested that *Typha domingensis* caused complete relaxation of spontaneous and K+ (80mM)-induced contractions in isolated rabbit jejunum. Rightward parallel shift of calcium concentration response curves was observed. *Typha domingensis* exhibited relaxant effect on Carbachol (Cch)-induced contractions in isolated rabbit tracheal preparations. Furthermore, *Typha domingensis* caused relaxation of phenylephrine (1μM)-induced contractions in isolated rabbit aorta preparations. These effects were similar to verapamil, a standard calcium channel blocker. These findings could be the basis for explaining the spasmolytic, bronchodilator and vasodilator activities of the extract, through a possible calcium channel blocking activity.

Keywords: Spasmolytic effect, *Typha domingensis*, broncho-dilation effect, vasodilation effect.

INTRODUCTION

*Typha domingensis* Pers also known as Cat-tail (Rao et al., 2016) or Cumbungi, is a fast-growing herbaceous perennial plant with unbranched leafy stem (Chai et al., 2014). This plant has tall size that can reach up to 1.5 to 3.0 meters. With stout stem their leaf sheath opens at throat with free margins, 5-12 millimeter broad lamina. They are bisexual i.e. male and female flowers are separate with linear or pointed hairs that are covered with bracts (Bernd et al., 2015). *Typha domingensis* Pers is a vigorously invasive plant specie that spreads freely in favorable conditions (Vines, 2004). In moderate temperate climate, the plant grows all year long. Therefore, its excessive growth may pose threat to other plant species sharing habitat with *Typha domingensis* (Akkol et al., 2011). *Typha domingensis* Pers is distributed throughout tropical and temperate regions. Owing to its resilient morphology, the plant has its presence in many countries of the world. Having pantropical distribution, *Typha domingensis* Pers., covers both hemispheres. They are found in abundance in mangrove ecosystem i.e. tropical deltas and lagoons (Gupta et al., 2007).

Phytochemical analysis performed by Sarin, 2014 revealed the presence of total phenols, flavonoids, proanthocyanides and hydroxycinnamic acids in *Typha domingensis*. Similarly presence of antioxidant constituents in the fruits and flowers have also been reported (Gallardo et al., 2002). Furthermore, presence of iron chelators and anti-glucosidase inhibitors are also reported in the aqueous extract of *Typha domingensis* (He et al., 2015). Traditionally, this plant has been used in the form of paste for topical application for wound healing. Moreover, 5% ointment of *Typha domingensis* in rat and mice model has shown highly significant wound healing activity (Akkol et al., 2011). To explore biological activities of medicinal plants, the present study was performed to evaluate the folkloric uses of “*Typha domingensis*” related to its effects in the treatment of gastrointestinal, bronchial and cardiovascular diseases.

MATERIALS AND METHODS

Plant material and preparation of extract

Fresh whole plant of *Typha domingensis* Pers was collected from the native fields of Bahawalpur, Pakistan, and was identified by an expert taxonomist in the Department of Botany, Islamia University Bahawalpur. A specimen is deposited in Herbarium of the Institute (voucher # 95/Botany). Following shade drying, the plant material was manually made free from soil and other adulterants and was ground to coarse powder by an electrically driven mill. Approximately 1 kilo gram powdered plant material was soaked in 70% aqueous-ethanol by cold maceration at room temperature for 7 days with occasional shaking. It was filtered through a double layered muslin cloth and subsequently through a filter paper. The residue was re-soaked in the fresh solvent and the process was repeated thrice. The combined filtrate was concentrated in rotary evaporator (Rotavapor-BUCHI Laboratories/ Model of 9230) (Rahman et al., 2017) at 40°C under reduced pressure to a thick, semi-solid mass, with approximated yield of 6.25%. *Typha domingensis* extract was transferred to a glass bottle. The stock solutions of *Typha domingensis* were prepared in distilled water and the dilutions were made fresh in distilled water on the day of experiment.
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The ethanolic extract of Td. Cr was further subjected to fractionation. Dichloromethane (DCM) and aqueous (Aq.) fractions were prepared by mixing the defined proportion of water and DCM in a separating funnel already having Td. Cr and smoothly shaken for about 15 minutes. The lower DCM layer and upper aqueous layer were collected three times and subsequently concentrated into DCM fraction and aqueous fraction by rotary evaporator.

**In vitro experiments**

Experiments on isolated tissue preparation were performed as described by Gilani *et al.*, 2005. Briefly, we used freshly prepared jejunum, tracheal and aortic tissue segments from the rabbit. These were maintained adequately well in the respective buffer solutions. Extraction procedure of each tissue is described below under respective heading with detailed elaboration.

**Isolated rabbit jejunum preparations**

The crude aqueous-ethanol extract of *Typha domingensis* was screened for the possible presence of either spasmytic or spasmygenic activity. Isolated jejunum tissue was cut into segments of about 2 cm in length. Respective segments were mounted in a 15 ml tissue organ bath containing Tyrode’s solution. The composition of Tyrode’s solution in mM was: NaCl (136.9), KCl (2.68), MgCl₂ (1.05), NaHCO₃ (11.90), NaH₂PO₄ (0.42), CaCl₂ (1.8) and C₆H₁₂O₆ (5.55). The temperature of the bath was controlled at 37°C by using thermo-regulator and tissue was continuously aerated with carbogen (95% O₂ and 5% CO₂) during the experiment. Intestinal response was recorded by using the isotonic Bioscience transducer and Power Lab data acquisition system (AD Instruments, Sydney, Australia) attached to a computer installed with lab chart software (Version 6). Each tissue was allowed to equilibrate for at least 30 min. prior to the addition of any chemical entity. The isolated jejunum was stabilized with subsequent exposure to 0.3 µM acetylcholine and then washed with the Tyrode’s solution until the sub maximal response was achieved. Under these conditions, rabbit jejunum exhibits the spontaneous rhythmic contractions. The spasmytic/spasmogenic activity of plant extract was screened by the application of plant dose in cumulative manner to pre-stabilized isolated jejunum tissue (Rahman *et al*., 2019). The inhibitory effect produced by the plant extract was measured as percent change in spontaneous rhythmic contractions of isolated rabbit jejunum preparations. To find the possible mechanism of spasmytic effect, the test substance was studied against the induced contractions. For the elucidation of the Ca²⁺ channel blocking (CCB) activity, the depolarization of preparation induced by high K⁺ (80 mM) was used as described by Janbaz *et al*., 2015. High K⁺ (>30mM) is known to cause the opening of the voltage dependent Ca²⁺ channels resulting in contraction of smooth muscles due to inward movement of extracellular Ca²⁺ (Janbaz *et al*., 2015). Substance that causes the relaxation of high K⁺ (80mM)- induced contractions were considered as inhibitor of Ca²⁺ influx through L-type Ca²⁺ channels (Al-Shboul, 2018, Sweeney and Hammers, 2018, Touyz *et al*., 2018, H.M. A Rahman, 2017, Imran I, 2011, Panda and Misra, 2011).

Calcium channel blocking activity of the test substance was further confirmed by the method described earlier by Rahman *et al*., 2019. Isolated jejunum preparations were...
allowed to stabilized in normal Tyrode’s solution, which was then subsequently substituted with Tyrode’s solution in which instead of Ca\(^{2+}\), EDTA (0.1mM) was present which was remained in bath for a period of 30 min so as to flush out Ca\(^{2+}\) from the tissue. The solution was further substituted with Ca\(^{2+}\)-free and potassium rich Tyrode’s solution, the composition of which was (mM): KCl (50), NaCl (91.04), MgCl\(_2\) (1.05), NaHCO\(_3\) (11.90), NaH2PO4 (0.42), glucose (5.55) and EDTA (0.1). After an initial incubation period of 30 min, control of Ca\(^{2+}\) concentration-response curves (CRC’s) were obtained and when super-imposable Ca\(^{2+}\) CRC’s were obtained (usually after 2 cycles), the tissue is incubated with plant dose for 1 hr. After the incubation of different doses of test material, the Ca\(^{2+}\) CRC’s were reconstructed and observed for the calcium channel blocking activity. The test substances which were unable to relax the high K\(^{+}\) (80 mM)-induced contractions were tested against the contractions induced by low K\(^{+}\) (25mM) for the elucidation of K\(^{+}\) channel opening effect (Gilani et al., 2015).

**Isolated rabbit tracheal preparations**

The trachea was dissected out from the rabbit and kept in Kreb’s solution, whose composition was (mM): NaCl (118.2), MgSO\(_4\) (1.2), NaHCO\(_3\) (25.0), KH\(_2\)PO\(_4\) (1.3), CaCl\(_2\) (2.5), KCl (4.7) and glucose (11.7). The surrounding fatty tissues were removed from the isolated trachea and the tracheal tube was cut into 2-3 mm wide rings, containing at least two cartilages. Each ring was then cut longitudinally opposite to the smooth muscle layer, forming a strip in which the smooth muscle was in the center of the C-shaped cartilaginous part on both sides. Respective isolated tracheal tissues were mounted in a thermo-regulated 15 ml tissue organ bath, containing Kreb’s solution (pH 7.4) maintained at 37°C and aerated with carbogen (95% oxygen and 5% carbon dioxide). A suitable resting tension of 1 g was applied to mounted tissue and allowed to equilibrate for about 1 hr. prior to the addition of any chemical substance. To stabilize the tissue carbacol (Cch) (1µM) was used. High K\(^{+}\) (80mM) and Cch (1µM) - induced sustained contractions were obtained in respective tissues and the relaxant effect of the test substance was assessed by adding the dose of the test substance in cumulative fashion. Isometric Bioscience transducers were used to record the responses which were coupled with Power lab data acquisition system (AD Instrument, Sydney, Australia) connected to a computer running lab chart software (Version 6) (Rahman et al., 2017; Gilani et al., 2005).

**Isolated rabbit aorta preparation**

The aorta was dissected out and cut into 2-3 mm wide segments. These segments were mounted in a 15 ml tissue organ bath containing Kreb’s solution maintained at 37°C and aerated with carbogen. A suitable pre-load of 2 g was applied and tissues were allowed to equilibrate for about 1 hr. After the equilibrium period, tissues were stabilized by repeated exposure to phenylephrine (1µM). The vasorelaxant / vasoconstrictive effect of the substance being tested were assessed by adding the dose of the test substance in tissue bath containing pre-stabilized tissue in cumulative fashion. Change in tension was recorded with the help of Bioscience transducers coupled with Power lab data acquisition system (AD Instrument, Sydney, Australia) attached to a computer running lab chart software (Version 6) (Nisa et al., 2013).

**STATISTICAL ANALYSIS**

Data was presented as Mean ±SEM (n= No of individual experiments) and median effective concentrations (EC\(_{50}\)) with 95% confidence intervals (CI). Concentration response curves were assessed by statistical software (Graphpad Prism, USA version 6.01) by means of nonlinear regression. Two-way ANOVA test was also applied on various models which was followed by Tukey’s Test. Probability values of *p< 0.05, **p<0.01, ***p<0.001 were considered significant.

**RESULTS**

**Effect on rabbit jejunal preparations**

The inhibitory effect was observed on rabbit jejunal preparation against the spontaneous as well as contractions induced by High K\(^{+}\) by the use of the extract of *Typha domingensis*. Significant relaxation of spontaneous and high potassium (K\(^{+}\)-80 mmol/L)-induced contractions of jejunum was observed after the application of Td.Cr extract solution in concentration range from 0.01 mg/mL to 10.00mg/mL.

Calculated EC\(_{50}\) values were 3.64±1.5 (95% CI: 2.25-2.54 mg/mL) for the spontaneous and 1.82±0.6 (95% CI: 0.70–2.9 mg/mL) for high potassium respectively. For verapamil the respective EC\(_{50}\) values response was 0.25±0.18 (95% CI: 0.21–0.33mg/mL) and 0.07±0.05 (95% CI: 0.05–0.10mg/mL).

*Typha domingensis* in DCM showed relaxant effect at lower doses with EC\(_{50}\)value of 0.3±0.1 (95% CI: 0.014-0.58mg/ml) for spontaneous, 0.3±0.3 (95% CI: 0.2–0.9 mg/mL) contractions induced by High-K\(^{+}\) respectively. With the application of *Typha domingensis* in water, complete relaxation of spontaneous contractions was achieved with EC\(_{50}\) value of 0.89 ± 0.5 (95% CI: 0.4–1.23 mg/mL) fig. 1.

These effects showed the calcium channel blocking activity which was further assured by the application of extracts at doses of 3.0, 5.0 and 10.0 mg/mL and calcium response curves (CRC’s) were formed and compared with the standard calcium channel blocker verapamil CRC’s.
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Fig. 1: Concentration dependent inhibitory effects of (a) crude extract of Typha domingensis (Td.Cr) (b) Typha domingensis DCM (Td.DCM), (c) Typha domingensis Aqueous (Td.Aq) and (d) Verapamil on spontaneous and high K+-induced contractions on rabbit jejunum preparations. Values are expressed in the form of Mean ± 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).

Fig. 2: Concentration response curves of Ca++ in the absence and presence of increasing concentrations of (a) crude extract of Typha domingensis (Td.Cr) (b) Typha domingensis DCM (Td.DCM) (c) Typha domingensis Aqueous (Td.Aq) and (d) Verapamil in isolated rabbit jejunum preparations. Values are expressed in the form of Mean ± 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).
Fig. 3: Concentration dependent inhibitory effects of (a) crude extract of Typha domingensis (Td.Cr) (b) Typha domingensis DCM (Td.DCM) (c) Typha domingensis Aqueous (Td.Aq) and (d) Verapamil on Cch-induced contractions on rabbit tracheal preparations. Values are expressed in the form of Mean ± 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).

Fig. 4: Concentration response curves of Carbachol in the absence and presence of increasing concentrations of (a) crude extract of Typha domingensis (Td.Cr) (b) Typha domingensis DCM (Td.DCM) (c) Typha domingensis Aqueous (Td.Aq) and (d) Verapamil in isolated rabbit tracheal preparations. Values are expressed in the form of Mean ± 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).
Tyrod's solution used for CRCs does not contain calcium but contain high amount of potassium. It is noted that the pre application of the *Typha domingensis* extracts to tissues resulted in shifting of the CRCs towards right, which is the confirmation of the calcium channel blocking activity of *Typha domingensis* fig. 2.

**Effect on the rabbit tracheal preparations**

Td.Cr in a concentration from 0.01 to 3.00 mg/mL resulted in complete relaxation of pre-contracted trachea with carbachol (Cch) 1μmol/L with respective EC$_{50}$ values of 2.284 mg/mL (95% CI: 0.90–3.86 mg/mL) and 2.42 mg/mL (95% CI: 1.88–3.14 mg/mL) same as verapamil. Moreover, more pronounced pattern of relaxation was observed Cch-induced contractions by the application of Td. DCM and Td.Aq fraction showed complete relaxation of Cch-induced contractions at the dose of 5.00 mg/mL with EC50 values of 1.927mg/ml (95%CI: 0.6-5.92mg/ml) fig. 5.

**Effect on rabbit aortal preparations**

Td.Cr in a concentration from 0.01 to 3.00 mg/mL resulted in complete relaxation of pre-contracted aorta with phenylephrine (PE) 1μmol/L with respective EC$_{50}$ values of 3.04 mg/mL (95% CI: 2.02–4.6 mg/mL) and 2.42 mg/mL (95% CI: 1.88–3.14 mg/mL) same as verapamil. Moreover, more pronounced pattern of relaxation was observed by the application of Td. DCM and Td.Aq fraction showed complete relaxation of PE-induced contractions at the dose of 5.00 mg/mL with EC50 values of 1.927mg/ml (95%CI: 0.6-5.92mg/ml) fig. 5.

**DISCUSSION**

The herbal medicines are continuously used throughout world for prevention and treatment of different ailments and the natural products have significant contribution toward pharmaceutical industry as a source of potent medicinal agent (Gillani *et al*., 2012). Plants have shown potential to facilitate management of gastrointestinal, respiratory and cardiovascular ailments (Kenner *et al*., 2001). *Typha domingensis* has a folkloric repute to provide relief in diarrhea and dysentery (Panda & Misra., 2011; Edible Medicinal and Non-Medicinal plants., 2012).
Relaxation of smooth muscles is caused by blocking the Calcium channels as a result of inhibition of calcium influx. When these Voltage mediated channels are blocked, it is useful for the treatment of smooth muscle disorders for example hypertension, asthmatic cough and diarrhea (Aslam et al., 2016; Janbaz et al., 2015; Sanders et al., 2012; Somlyo and Somlyo, 2000). When smooth muscle tissues are exposed to the high concentration of K⁺, Voltage dependent calcium channels of these tissues are opened result in contraction of these smooth muscles. Agents having Calcium channel blocking activity plays an inhibitory action not only on impulsively contracting smooth muscles but also to the muscles pre-exposed to high K⁺ (Aslam et al., 2016; Rahman et al., 2017 Al-Shboul et al., 2018; Sweeney and Hammers, 2018; Touyz et al., 2018)

Extract of Typha domingensis when applied to the normally contracting as well as against the High K⁺ induced contraction in isolated jejunum showed inhibitory response by inhibition of both contractions. The inhibitory response was also shown by standard calcium channel blocker Verapamil against high K⁺ mediated contraction. When the Td DCM is applied to the isolated piece of rabbit’s jejunum the same inhibitory response can be seen but this response is evident at lower dose than Td.Cr. The same effect was produced by the application of Td.Aq. This calcium channel blocking activity confirmed further by making calcium response curves. The shifting of calcium curve towards right is a result of inhibition of Calcium contractile effect (Aslam et al., 2016; Rahman at al., 2017).

The folkloric use of Typha domingensis in asthma and cough also opens the new fields for investigation in pharmacology. For the validation of these medicinal uses of Td. Cr, its crude extract was tested on isolated piece of trachea obtained from rabbit. It causes the relaxation of this tracheal piece which was contracted by the application of carbachol. This effect was prevailing against contractions induced by carbachol. The mechanism for producing contractions by carbachol on isolated trachea is the binding with M-3 receptors leading to its stimulation. These M-3 are muscarinic receptors causes the activation of an enzyme Phospho lipase –C after getting stimulated (Yaaq et al., 2012). This activation of phospholipase-C results in the formation of diacetyl glycerol and inositol triphosphate and phosphoinositol 4,5 bisphosphate (Katzung and Chatterjee, 2012; Widmair et al., 2011). The Voltage mediated channels of cell membrane (calcium-channel) are opened by this IP-3, while the enzymes, proteins and phosphokinases are activated due to the diacetyl glycerol. Thus, hence all these procedure leads to broncho-constriction (Townsend et al., 2013). Td. Cr cause broncho-dilation against carbachol induced broncho-constriction. Same kind of results are observed when TD. Cr, its crude extract was tested on isolated piece of trachea against contractions induced by carbachol. The application of carbachol. This effect was prevailing against contractions induced by carbachol. The inhibitory response was also shown by standard calcium channel blocker Verapamil against high K⁺ mediated contraction. When the Td DCM is applied to the isolated piece of tracheal tissue of rabbit. Similarly, Td aqueous shows the same result when applied to the tracheal contractions which are induced by carbachol.

Typha domingensis has a valuable reputation to treat cardiovascular diseases (Mathewson, 1985) Due to this reason rabbit aorta was used to check its effects and actions on blood vessel tone, cardiac rate and force of contraction. Td. Cr shows concentration mediated vaso-relaxant effect when given to phenylepherine treated aorta. Similarly, the Td DCM causes relaxation of already contracted aortic tissue with phenylepherine. Td aqueous show complete relaxation of PE induced vasoconstriction. Td DCM produces more predominant effect over Td. Cr and Td aqueous.

So, from these results it is proved that spasmolytic, bronchodilator and vasorelaxant effect of Typha domingensis is due to the Calcium channel blocking ability, but the other mechanisms involving ion channels and receptors should also be considered in further studies.

CONCLUSION

The current study shows anti-diarrheal, anti-asthmatic and antihypertensive potential of Typha domingensis. It is concluded from above mentioned results and discussion that Typha domingensis have the ability to block voltage mediated calcium channels, M-3 muscarinic receptors and α-receptors due to which plant possessed spasmylocic, bronchodilator and vasodilator potential. These medicinal herbs have important phytochemical constituents that may be isolated, purified and could be a potent new drug candidate for the management of other ailments. Further isolation/purification and characterization of these plants may lead to novel drug discovery.

REFERENCES

Aslam, F., 2013. Relaxation of smooth muscles is caused by blocking the Calcium channels as a result of inhibition of calcium influx. When these Voltage mediated channels are blocked, it is useful for the treatment of smooth muscle disorders for example hypertension, asthmatic cough and diarrhea (Aslam et al., 2016; Janbaz et al., 2015; Sanders et al., 2012; Somlyo and Somlyo, 2000).


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