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Full Length Article

Spasmolytic, Bronchodilator and Vasodilator Activities of Aqueous-methanolic Extract of *Ocimum basilicum*

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Abstract

The aqueous-methanolic extract of *Ocimum basilicum,* Linn was subjected to *in vitro* investigations for its possible antispasmodic, bronchodilator and vasodilator activities. The studies included testing on jejunum of rabbit, ileum of guinea-pig, trachea of rabbit, trachea of guinea-pig and aorta of rabbit. It produced relaxation of spontaneous as well as K^+(80 mM)-induced withering in jejunum of rabbit. The extract on application to guinea-pig ileum showed a concentration-dependent (3.0-10.0 mg/mL) contractile response which was blocked on addition of atropine (0.1 µM). It produced nonspecific relaxation of both the carbachol (1 µM) and high K^+ (80 mM)-induced contractions in isolated trachea of rabbit and guinea-pig in a manner comparable to verapamil. It also produced relaxation of phenylephrine-induced withering in rabbit aorta in a comparable fashion to verapamil. The observed spasmolytic, bronchodilator, and vasodilator effects on the part of The extract were likely to be mediated through Ca^{+2} channel blocking activities, whereas observed contractile response in isolated guinea-pig ileum preparations was likely to be mediated through cholinergic agonistic activity. These findings may validate the folkloric use of *O. basilicum* in constipation, vascular insufficiency and respiratory distress.

Keywords: Antispasmodic; Bronchodilator; Vasodilator; *Ocimum basilicum;* Sweet basil

Introduction

Sweet basil (*Ocimum basilicum*, L.) family Lamiaceae is an indigenous wildly growing but also cultivated herb in areas of central as well as south east Asia (Prajapatti *et al*., 2003). It is an erect, branched and aromatic herb of about 60-90 cm in height. The stems and branches are green or purple in colour. The leaves are simple, opposite, ovate-lanceolate, acuminate, toothed or entire, glandular and glabrous on both surfaces. The flowers are in whorled racemes, the terminal racemes are longer than the lateral and bracts are stalked. The corollas are 2-lipped about 8-13 mm long, glabrous or pubescent and white, pink or purplish in colour. The 2 mm long black fruits are nut less, ellipsoid and pitted (Prajapatti *et al*., 2003; Agharkar, 2004).

Extensive phytochemical investigations revealed the presences of essential oils (Grayer *et al*., 1996), i.e., phenylpropenes (Gang *et al*., 2001) including esters of cinnamic acid either methyl cinnamate (Siddiqui *et al*., 2007a), phenylpropanoid (Deschamps *et al*., 2010) and hydroxylated phenylpropanoids (Gang *et al*., 2002) including eugenol (Louie *et al*., 2007), methyleugenol (Miele *et al*., 2001), isoeugenol (Koeduka *et al*., 2006), chavicol (Vassão *et al*., 2006), methyl chaviocol and trans-anethole (Prajapatti *et al*., 2003), terpene (Yang *et al*., 2007) i.e., trans ß-ocimene (Johnson *et al*., 1999), geraniol (Iijima *et al*., 2004), linalool and linolyl acetate (Grayer *et al*., 1996), citral (Iijima *et al*., 2006), triterpenoids (Siddiqui *et al*., 2007b) like ursolic acid (Silva *et al*., 2008), steroidal glycoside (Siddiqui *et al*., 2007b), anthocyanin (Phippen and Simon, 2000) and carotenoid (Daly, 2010) among the plant constituents.

Several herbal preparations have been employed for therapeutic purposes (Ahmad *et al.*, 2012; Kim *et al.*, 2012; Ou *et al*., 2013). *O. basilicum* has traditionally been used for the management of a number of ailments pertaining to gastrointestinal tract i.e., colic, and dysentery (Duke, 1985); respiratory tract including asthma, bronchitis and cough (Germosén-Robineau, 1997) and cardiovascular diseases (Peirce, 1999).

Scientific investigations on plant material demonstrated anti-ulcer (Akhtar and Munir, 1989), anti-inflammatory activity (Singh, 1999), anti-platelet aggregation (Amrani e*t al*., 2009) and anti-parasitic (Santoro *et al*., 2007; Babar *et al*., 2012) activities.

The purpose of this learning was to investigate its potential pharmacological effect in gastrointestinal, respiratory and vascular systems to validate its folkloric use.

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Materials and Methods

Plant Materials

The aerial parts of *Ocimum basilicum* were collected from Multan, Pakistan in April, 2010. The plant was authenticated by Prof. Altaf Hussain Dasti, at Biology Department, BZU, Multan and voucher specimens were deposited at the herbarium. The plant material was rendered free from adulterated material and vegetative debris by hand picking and grinded to a coarse powder with the help of a special herbal grinder.

Antispasmodic Activity

The anti-spasmodic activity was estimated using isolated jejunum and ileum (Gilani *et al*., 2005; 2006).

Isolated rabbit jejunum: The relaxant effect of test material was calculated as % variation in spontaneous contractions of the jejunum of rabbit.

Isolated guinea-pig ileum: The contractile reaction to the test substances was surveyed as the percent of the maximal reaction prepared by acetylcholine (0.3 µM) as the control drug prior to the addition of test substances (Gilani and Aftab, 1992).

Determination of calcium channel blocking activity: The mechanism of the antispasmodic action was determined by the possible effects of the crude plant extracts on high K^+ (80 mM)-induced contractions as described by Farre *et al*. (1991).

Bronchodilator Activity

Isolated rabbit and guinea-pig tracheal preparations: The bronchodilator effect of the test material was studied on pre-contracted isolated tracheal preparation with carbachol.

Isoprenaline inhibitory CRCs: The relaxant graphs for isoprenaline were built against carbachol-induced contractions in trachea of guinea-pig in the lack and existence of the extract.

Vasodilator activities

Isolated rabbit aorta: The vasorelaxant/vasoconstrictive effects of the test materials were studied by addition in tissue organ baths containing pre-stabilized tissue in a cumulative manner.

Results

Effect of *O. basilicum* on Isolated Jejunum of Rabbit

The crude methanolic extract of *O. basilicum* produced a concentration-dependent (0.01-3 mg/mL) inhibition of the spontaneous and K^+ (80 mM)-induced withering (Fig. 1 and 3a). Verapamil also relaxed both spontaneous and K^+(80 mM)-induced contractions (Fig. 2 and 3b). The extract showed the shift of calcium concentration-response curves (CRC) towards right at 0.1-1.0 mg/mL (Fig. 4a). These results of plant extract were comparable with verapamil (the standard calcium channel blocker) (Fig. 4b).

Effect of *O. basilicum* Extract on Isolated Guinea-Pig Ileum Preparations

The extract on application to the isolated guinea-pig ileum preparations, exhibited a concentration-dependent contractile response at the concentration range of 3-10 mg/mL. (Fig. 5a and 6). Pretreatment of the tissue preparations with atropine (0.1 µM) completely blocked the contractile response to Ach and extract (Fig. 5b and 6), indicating that the extract may exert its contractile effect through activation of cholinergic muscarinic receptors similar to Ach.

Effect of *O. basilicum* Extract on Isolated Rabbit Tracheal Preparations

The extract on application to isolated rabbit tracheal preparations caused a concentration-dependent relaxant effect at a concentration-range of 0.1-3 mg/mL in carbachol (1 µM) and K^+(80 mM)-induced contraction (Fig. 7 and 9a). Verapamil also caused relaxation of carbachol (1 µM) and K^+(80 mM)-induced contractions (Fig. 8 and 9b).

Effect of *O. basilicum* on Isolated Guinea Pig Tracheal Preparations

The extract on application to isolated guinea-pig tracheal preparations, exhibited a concentration dependent relaxation (Fig. 10). Moreover, pretreatment of the isolated guinea-pig tracheal preparations with the extract (0.01–3.0 mg/mL) did not enhance the isoprenaline-induced relaxant response (Fig. 11).

Effect of Crude Extract of *O. basilicum* on Isolated Rabbit Aorta Preparations

The extract when applied on 1 µM phenylephrine and K^+ (80 mM)-induced contractility, showed concentration dependent relaxantion activity (Fig. 12).

Discussion

Resistance to the present compounds against management of different diseases has lead to search for the new candidates (Qadir and Malik, 2010; 2011). *O. basilicum*, exerted relaxant effect on isolated jejunum of rabbit. The possible relaxant activities of the test materials on gastrointestinal tract can be investigated in isolated rabbit jejunum preparations without use of any agonist (Gilani *et al*., 1994) and inhibition of the magnitude of the contractions. The observed relaxation of the gastrointestinal tract by the plant extract is likely to be mediated through calcium channel blockade (Gilani *et al.*, 2005; 2006). The raised cytoplasmic concentration of Ca^{+2} in smooth muscles

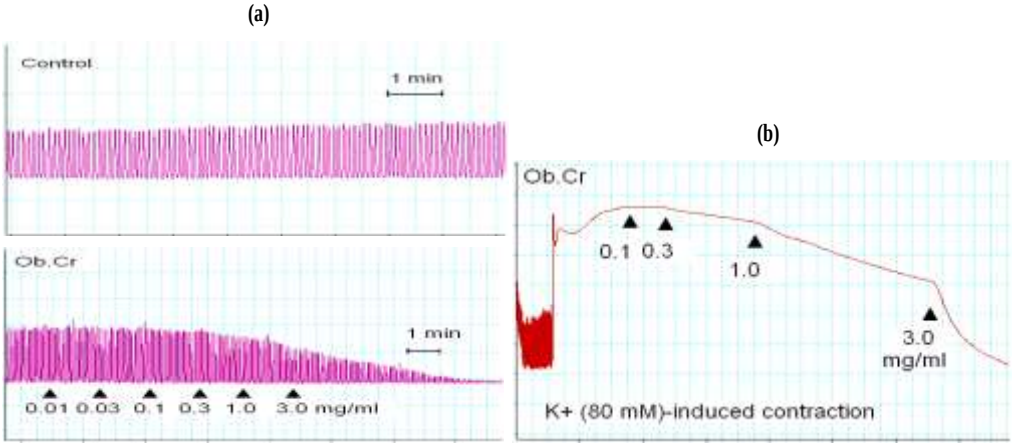

Fig. 1: Tracing showing inhibitory effects of crude extract of *Ocimum basilicum* (Ob.Cr) **(a)** on spontaneous and **(b)** K^+ (80 mM)-induced contractions of isolated rabbit jejunum preparation

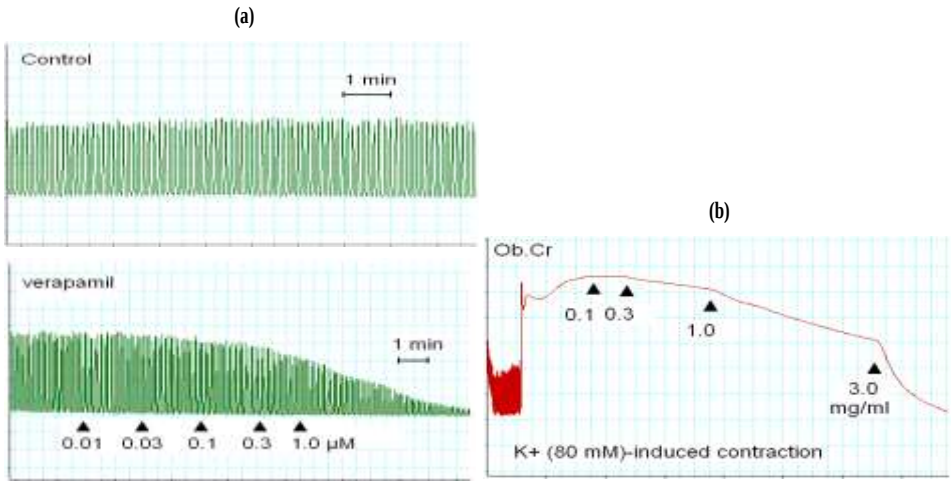

Fig. 2: Tracing showing inhibitory effects of verapamil on **(a)** spontaneous and **(b)** K^+ (80 mM)-induced contractions of isolated rabbit jejunum preparation

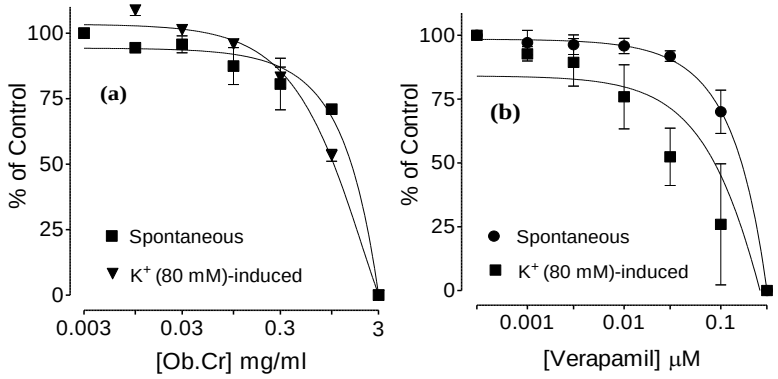

Fig. 3: Concentration-dependent inhibitory effects of crude extract of (a) *Ocimum basilicum* (Ob.Cr) and (b) verapamil on spontaneous and K^+(80 mM)-induced contractions of isolated rabbit jejunum preparation. Values are shown as mean ± S.E.M., n=5

like rabbit jejunum is associated with activation of the contractile mechanism (Karaki and Weis, 1984; Karaki *et al*., 1997) and this increase in cytoplasmic Ca^{2+} level is achieved through influx of Ca^{2+} via the voltage dependent Ca^{+2} channels (VDCs) or release of Ca^{+2} from sarcoplasmic stores (Godfraind *et al*., 1986). The spontaneous contractions in intestine are expression of the phenomenon of periodic depolarization/repolarization and the action

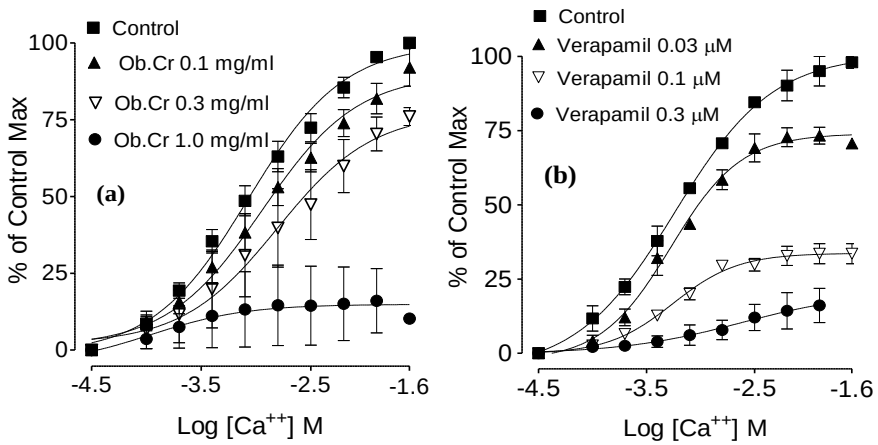

Fig. 4: Concentration-response curve of Ca^{+2} in the absence and presence of **(a)** crude extract of *Ocimum basilicum* (Ob.Cr) and **(b)** verapamil in isolated rabbit jejunum. Values are expressed as mean ± S.E.M., n=5

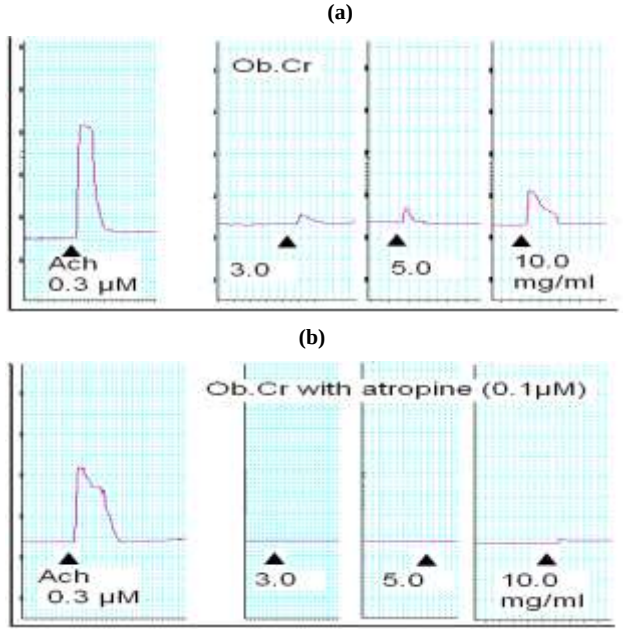

Fig. 5: Tracing showing contractile effect of *Ocimum basilicum* (Ob.Cr) in the **(a)** absence and **(b)** presence of atropine in guinea pig ileum preparations

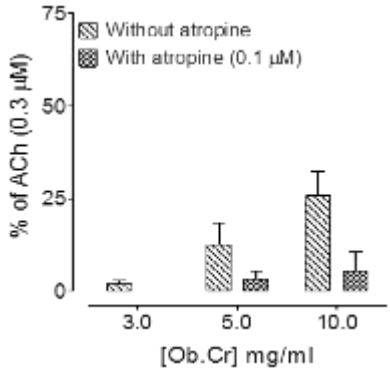

Fig. 6: Bar chart showing the concentration-dependent contractile effect of *Ocimum basilicum* (Ob.Cr) in the absence and presence of atropine in guinea pig ileum preparations. The values are shown as mean ± S.E.M., n= 5

potential is propagated via rapid influx of Ca^{+2} through VDCs at maximal depolarization of the tissues (Brading, 1981). Thus the observed relaxant effect of the extract on the isolated rabbit jejunum is likely to be mediated either through blockade of influx of Ca^{+2} via VDCs or by inhibition of Ca^{+2} release from sarcoplasmic stores.

The VDCs are reported to be opened in isolated smooth muscle preparations on exposure to high tissue bath concentrations of K^+(>30 mM), resulting in a rapid influx of extracellular Ca^{+2}, leading to contraction of the smooth muscles (Boltan, 1979; Godfraind *et al*., 1986). Hence, the substances capable to inhibit K^+-induced contractions are likely to be Ca^{+2} channel blockers. The possible mechanism of the observed relaxant effect of the extract was explored through application on high K^+-induced spastic contractions in isolated rabbit jejunum preparations and cumulative addition of the extract to the tissue baths resulted in concentration dependent relaxation of K^+-induced contractions in isolated rabbit jejunum preparations. These observations were in complete confirmation to the subsequent findings, where treatment of the isolated rabbit jejunum preparations with the extract resulted in decreased contractile responses to the added $CaCl_2$, hence produced rightward shift in the concentration response curves of $CaCl_2$, similar to that of verapamil (Fleckenstein, 1977).

Therefore, the relaxant activity of the test material on K^+-induced contractility, followed by displacement of concentration response curve for Ca^{2+} confirmed the Ca^{2+} channel blockage of the test material, which may provide the logical concepts for its utilization in problems involving hyperactive status of the gastrointestinal tract because the agents causing calcium channel blockage recognized to be

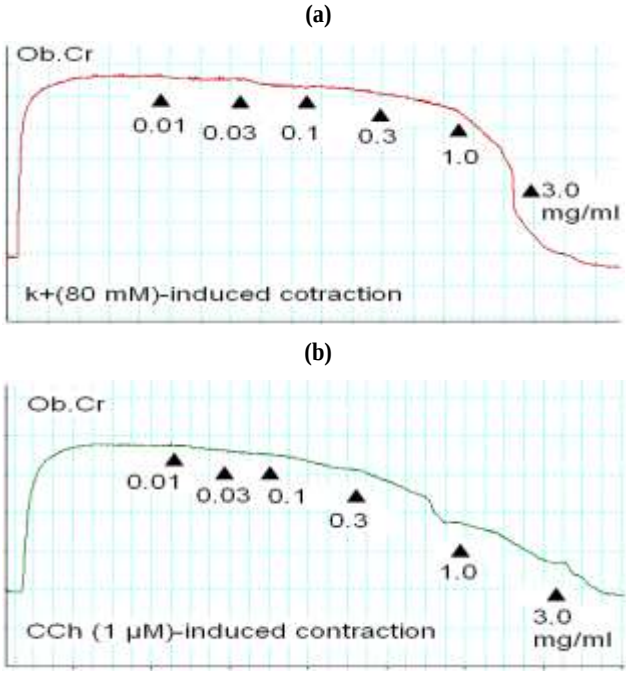

Fig. 7: Tracing showing relaxant effect of crude extract of *Ocimum basilicum* (Ob.Cr) and (a) on high K^+ (80 mM) and (b) CCh (1 µM)-induced contraction in isolated rabbit trachea

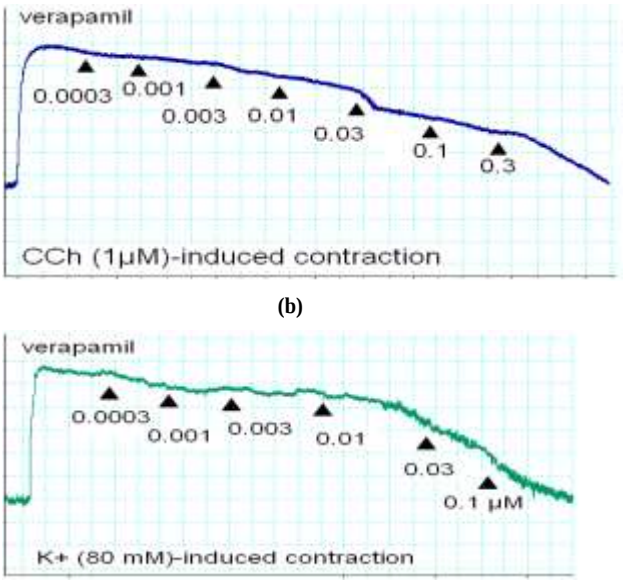

Fig. 8: Tracing showing inhibitory effect of crude extract of verapamil on (a) carbachol (1 µM) and (b) high K^+ (80 mM)-induced contractions in isolated rabbit tracheal preparations

efficient in agitated gut diseases (Brunton, 1996).

The extract on testing on isolated guinea-pig ileum exhibited a contractile response at elevated tissue bath concentrations. The observed contractile response was speculated to be mediated through cholinergic muscarinic receptor stimulation, which was confirmed subsequently by treating the guinea-pig ileum to atropine (0.1 µM) resulting

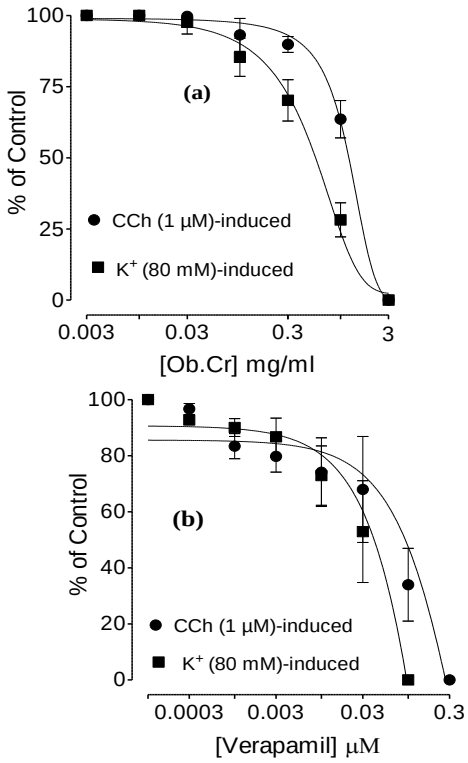

Fig. 9: Concentration-dependent relaxant effect of crude methanolic extract of (a) *Ocimum basilicum* (Ob.Cr) and (b) verapamil on high K^+(80 mM) and carbachol (1 µM)- induced contraction in isolated rabbit tracheal preparations. Values are shown as mean ± S.E.M., n=5

in entirely blockage of the contractile responses to both acetylcholine and the extract, demonstrating that the extract has contractility activity via stimulation of cholinergic muscarinic receptors similar to that of acetylcholine.

O. basilicum has folkloric repute of providing relief in asthma and bronchitis. The extract was tested for its possible relaxant activity on carbachol (1 µM) as well as K^+(80 mM)-induced contractility in trachea of rabbit and guinea-pig and founded to produce relaxation in both of the situations. The bronchodilator effect is likely to be mediated through Ca^{+2} channel blocking activity as calcium channel blockers are known to exert bronchodilator activities in hyper-reactivity of the respiratory tract (Ghayur, 2006). The extract also caused relaxation of the phenylephrine as well as K^+ (80 mM)-induced contractility in aorta of rabbit in a manner similar to verapamil and hence suggested its vasodilator effect possibly mediated through calcium channel blockade.

In conclusion, the observed spasmolytic, bronchodilator, and vasodilator effects on the part of the extract were likely to be mediated through Ca^{+2} channel blocking activities, whereas observed contractile response in isolated guinea-pig ileum preparations was likely to be mediated through cholinergic agonistic activity. These findings may validate the folkloric use of *O. basilicum* in constipation, vascular insufficiency and respiratory distress.

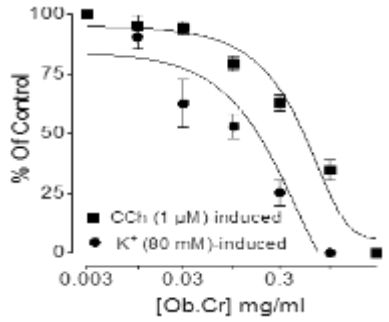

Fig. 10: Concentration dependent relaxant effect of the crude extract of *O. basilicum* (Ob.Cr) on carbachol (1 µM) and K^+(80 mM)-induced contractions in isolated guinea-pig tracheal preparations. Values are shown as mean ± S.E.M., n=5

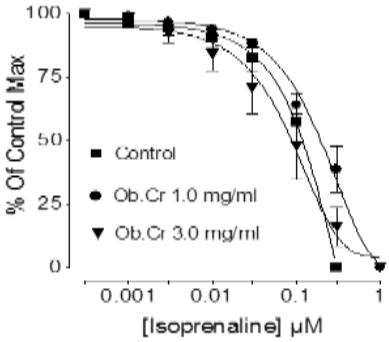

Fig. 11: Relaxant concentration–response curves of isoprenaline against carbachol (1 µM)-induced contractions in the presence of different concentrations of crude extract of *O. basilicum* (Ob.Cr) in isolated guinea-pig trachea. Symbols are mean ± SE, n=5

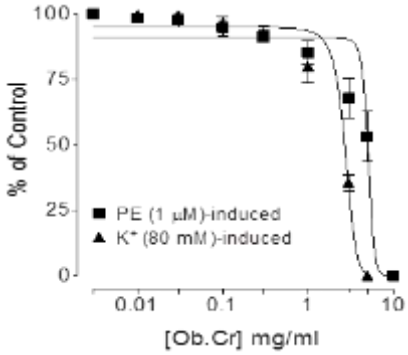

Fig. 12: Concentration dependent inhibitory effect of the crude extract of *Ocimum basilicum* (Ob.Cr) on phenylephrine (1 µM) and K^+(80 mM)-induced contractions of isolated rabbit aortic preparations. The values are shown as mean ± SE, n=5

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