INTRODUCTION

Cymbopogon martinii (Roxb.) Will. Watson (Poaceae), commonly known as palma rosa and Indian geranium, is a lemongrass native to South and Southeast Asia, especially India and Pakistan, and it is often cultivated for its oil (1). Phytochemical studies on volatile components of Cymbopogon martini revealed the presence of mono- and sesquiterpenes (2-4), together with some characteristic components as cymbodiacetal (5) and dihemiacetal bismonoterpenoids (6). The inflorescence of the plant is a good source of geraniol-rich essential oil, which is used to impart a rose-like aroma to a wide range of perfumes, soaps, cosmetics, toiletries and tobacco products (7). The plant has folkloric repute as abortifacient, analgesic, and aphrodisiac (8) and native healers use the plant for its astringent, carminative, emmenagogue, vulnerary, antispasmodic, stimulant and sudorific properties (9). It has been used to manage ache, snakebite, impotence, sore, cancer of stomach, liver and spleen, guinea worm, and pains (8), amenorrhea, fever, bleeding, wound, rheumatism (9), arthritis, alopecia, dermatosis, lumbago, biliousness, enterosisis and spasms (7). Moreover, it has also been known to be beneficial in diabetes, urinary tract infections and the plant is claimed to possess anti-inflammatory and diuretic properties (10).

Scientific studies on Cymbopogon martini demonstrated anthelmintic (11-12) antiseptic, antifungal (13-14) and insect-repellent (15-16) activities. It inhibits MAO activity in a competitive manner (17) and has wound healing properties (18). Cymbopogon martini has been reported to exert α-glucosidase inhibitory activity and helps in the management of postprandial glucose level (19). Volatile oil obtained from Cymbopogon martinii showed neuroprotective effect against cerebral ischemia and reperfusion-induced oxidative stress in rats, and showed therapeutic potential in cerebro-vascular diseases including stroke (20). Essential oil is reputed to improve stiff joints and lumbago, skin diseases, baldness and manage bilious complaints (21).

Despite its use in cardiovascular and gastrointestinal ailments, no study exists on rationalizing its use in these ailments. In this context, as part of our continuous studies on exploring medicinal flora of Pakistan for various activities (22-24), the present study was undertaken to validate traditional use of Cymbopogon martinii in the management of multiple ailments in traditional systems of medicines.

Key words: Cymbopogon martini, spasmylytic effect, bronchodilator effect, vasorelaxant effect, calcium, oxidative stress

MATERIALS AND METHODS

Collection, extraction of plant material and fractionation of extract

The leaves of Cymbopogon martinii were collected from Sadaqabad, Pakistan, in March 2012. The plant was identified by
the taxonomist, Professor Dr. Altaf Ahmad Dasti, in the Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan and a voucher specimen (P.Fl.108-3) was deposited in the herbarium of this Institute.

The plant material was shade dried and was rendered free of possible adulterant through manual picking. The dried herbal material was subsequently grinded into coarse powder. This powder was subsequently subjected to extraction by cold maceration and about 1 kg of the powdered material was soaked in 70% aqueous methanol in an amber glass container at 25°C for 7 days with occasional shaking. The soaked material was passed through a muslin cloth to get rid of the vegetative debris and fluid portion obtained was filtered through Whatman-I filter paper and the filtrate was evaporated to a thick, semi-solid mass of dark brown color at 37°C under reduced pressure on rotovapour (Buchi R-200 Switzerland) coupled with recirculation chiller (B-740) and vacuum pump (Buchi vac V-500). The approximate yield of the crude methanolic extract (Cm.Cr) was 6%. The fractionations of crude methanolic extract of Cymbopogon martinii was achieved by dissolving about 5 g of Cm.Cr in 20 ml of distilled water, followed by vigorous shaking with 20 ml of an immiscible organic solvent (dieloromethane) in a separating funnel. Individual fractions were collected in separate flasks and were evaporated by means of rotary evaporator under reduced pressure obtaining the dichloromethane (Cm.DCM) and the aqueous fractions (Cm.Aq).

Chemicals

Acetylcholine chloride, carbachol, potassium chloride, verapamil hydrochloride, phenylephrine, magnesium chloride, ethylene tetra-acetic acid (EDTA) were purchased from Sigma Chemicals Co. St Louis, MO, USA. Calcium chloride, glucose, magnesium sulphate, potassium dihydrogenophosphate, sodium bicarbonate, sodium dihydrogenophosphate and methanol were obtained from Merck, Darmstadt, Germany. Ammonium hydroxide, sodium chloride, and sodium hydroxide were purchased from BDH Laboratory supplies, Poole, England. The chemicals used in these experiments were of highest purity and the reagents of analytical grade.

Animals and housing conditions

All the experiments performed complied with the rulings of the Institute of Laboratory Animal Resources, Commission on Life Sciences (25), approved by the Ethical Committee of Bahauddin Zakariya University, Multan.

Animals (female and male) used in this study were local strain rabbits (1.0–1.8 kg). These were housed under controlled environmental condition (23–25°C) at the animal house of Faculty of Pharmacy, Bahauddin Zakariya University, Multan. The animals were provided with standard food and tap water ad libitum. The animals were deprived of food 24 h 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.

Isolated rabbit jejunum preparations

The crude methanolic extract of C. martinii (Cm.Cr) was tested for the possible presence of either spasmylocic or spasmogenic activity by using isolated rabbit jejunum preparations and responses were recorded through isotonic transducer by Power Lab Data Acquisition System (AD Instruments, Sydney, Australia) attached to a computer installed with Lab Chart Software (Version 7). For isolation of desired tissue, rabbit was dissected to remove jejunum and placed in Tyrode’s physiological salt solution maintained at 37°C and aerated with carbogen (95% O₂ and 5% CO₂). The tissues were cut into segments about 2 cm in length, rendered free of adhering mesenteries and subsequently suspended in isolated tissue baths containing Tyrode’s solution at 37°C and continuously aerated with carbogen. The composition of the Tyrode’s solution (mM) was: KCl (2.68), NaCl (136.9), MgCl₂ (1.05), NaHCO₃ (11.90), NaH₂PO₄ (0.42), CaCl₂ (1.8) and glucose (5.55). Under normal physiological environment, isolated rabbit jejunal preparations exhibit spontaneous rhythmic contractions, allowing testing of the antispasmodic (relaxant) effect without application of an agonist (26-33). The possible mechanism of the relaxant activity of the test materials was investigated through the relaxation of the observed sustained spasmic contractions following exposure to K⁺ (80 mM) (27). The test materials were applied in a cumulative manner to the sustained contractions to achieve concentration-dependent relaxant effects (26-27). The observed relaxant effect of the test materials on K⁺ (80 mM)-induced contraction was expressed as percent of the control contractile response.

Calcium channel blocking effect of the test substances were confirmed as reported in literature (31-33). Subsequent to an incubation period of 30 min, cumulative Ca²⁺ concentrations were applied to the tissue bath to obtain control calcium concentration-response curves (CRCs). The tissues were then washed and allowed to equilibrate with the Cm.Cr for 1 h and then the concentration response curves of Ca²⁺ were recorded and compared to the control curves. The CRCs of Ca²⁺ were recorded in the presence of different concentrations of the plant extracts in tissue bath.

Isolated rabbit tracheal preparations

Rabbit trachea was dissected out as described previously (31-33) and kept in Krebs solution having the following composition (mM): NaCl (118.2), NaHCO₃ (25.0), CaCl₂ (2.5), KCl (4.7), KH₂PO₄ (1.3), MgSO₄ (1.2) and glucose (11.7). The isolated rabbit tracheal preparations were mounted in 20 ml organ bath containing Krebs solution being maintained at 37°C and aerated with carbogen (95% O₂ + 5% CO₂). A preload tension of 1 g was applied and tissue preparations were allowed to be equilibrated for 1 hour prior to addition of any test material. The sustained contractions produced by carbachol (1 µM) and K⁺ (80 mM) were subsequently used for testing of different concentrations of the test material in a cumulative fashions. The isometric responses were recorded through a Power Lab Data Acquisition System (AD Instruments, Sydney, Australia) attached to a computer installed with Lab Chart Software (Version 7). The standard drug, verapamil, with Ca²⁺ channel blocking effect, was tested on carbachol- and K⁺ (80 mM)-induced spastic contractions for confirmation of possible mechanism of action.

Isolated rabbit aorta preparation

The effect of Cm.Cr on systemic vascular resistance was assessed on isolated rabbit aorta preparations. The descending thoracic aorta of rabbit was cut vertically in 2–3 mm width segments and was mounted in a tissue organ bath (Radnoti) containing Krebs solution aerated with carbogen at 37°C. A preload tension of 2 g was applied to each preparation and allowed to equilibrate for a period of 1 hour. The contractile effect of the test substance were studied on addition to tissue organ baths in a cumulative manner, whereas relaxant effect was studied following application to phenylephrine (1 µM)- and K⁺ (80 mM)-induced contractions. The changes in isometric tension of aortic rings were recorded by a force-displacement transducer.
(Model FORT100, WPI, USA) coupled to a Power Lab data acquisition system (AD Instruments, Sydney, Australia) and computer running Lab Chart software (version 7).

**Statistical analysis**

In isolated tissue experiments, data were expressed as the mean ± standard error of the mean (S.E.M.) and the median effective concentrations (EC$_{50}$) values with 95% confidence intervals (CI) were calculated by using the computer software Graphpad Prism Program (version 5.0), San Diego CA, USA. Concentration response curves were analyzed by non-linear regression of sigmoid response curve (variable slope). The statistic applied was the student’s t-test and P<0.05 was considered as significant.

**RESULTS**

**Effect on isolated rabbit jejunum preparations**

The Cm.Cr on application to spontaneous contractions of isolated rabbit jejunum preparations caused relaxation of spontaneous contractions at tissue bath concentration range of 0.01–5.0 mg/ml with an EC$_{50}$ value of 1.33 mg/ml (95% CI: 0.1707–1.8422 mg/ml; n=5) (Fig. 1a and b). Verapamil also inhibited the spontaneous contractions in isolated rabbit jejunum (Fig. 1b) with EC$_{50}$ value of 0.214 µM (95% CI: 0.1765–0.9601 µM; n=5). When tested against K$^+$ (80 mM)-induced contractions, Cm.Cr relaxed the K$^+$ (80 mM)-induced contractions at a tissue bath concentrations range of 0.01–5.0 mg/ml with an EC$_{50}$ value of 1.88 mg/ml (95% CI: 1.2801–2.2289 mg/ml; n=5) (Figs 1c and 2a). Verapamil also exhibited a similar pattern of relaxant effect against K$^+$ (80 mM)-induced contractions with an EC$_{50}$ value of 0.0357 µM (95% CI: 0.1765–0.9601 µM; n=5) (Fig. 1b). The pretreatment of isolated rabbit jejunum preparations with Cm.Cr (0.3–1.0 mg/ml) caused rightward shift of concentration response curves of Ca$^{2+}$ similar to that produced by verapamil (Fig. 3).

Activity directed fractionation of Cm.Cr revealed that the relaxant activity was more potent in the non-polar dichloromethane fraction (Cm.Dcm) on K$^+$ (80 mM)-induced contractions with EC$_{50}$ values of 0.863 mg/ml (95% CI: 0.2577–1.2983 mg/ml; n=5) in comparison with Cm.Aq which showed minor relaxation of the K$^+$ (80 mM)-induced contractions with EC$_{50}$ values of 9.313 mg/ml (95% CI: 6.3270–10.7805 mg/ml; n=5) (Fig. 2c).

**Effect on isolated rabbit tracheal preparations**

The Cm.Cr at respective tissue bath concentrations of 0.01–10.0 mg/ml and (0.01–5.0 mg/ml) exerted a relaxant effect on carbachol (CCh; 1 µM)- and K$^+$ (80 mM)-induced contractions (Fig. 4) in isolated rabbit tracheal preparations with respective EC$_{50}$ values of 2.84 mg/ml (95% CI: 1.223–2.595 mg/ml; n=5) and 0.805 mg/ml (95% CI: 1.414–1.990 mg/ml; n=5) (Fig. 5a). Verapamil also caused the relaxation of CCh (1 µM)- and K$^+$ (80 mM)-induced contractions with respective EC$_{50}$ values of 0.220 µM (95%: 0.04187–0.1862 µM; n=5) and 0.0721 µM (95% CI: 0.7150–1.062 µM; n=5) (Fig. 5b).

The organic fraction; dichloromethane (Cm.Dcm) exhibited complete relaxation of high K$^+$ (80 mM)-induced contractions of tracheal tissue at the dose of 1 mg/ml with EC$_{50}$ values of 0.3577 mg/ml (95% CI: 0.23464–0.6169 mg/ml; n=5), while aqueous fraction (Cm.Aq) did not exert complete relaxant effect even at the tissue bath concentration of 5 mg/ml with EC$_{50}$ values of 1.874 mg/ml (95% CI: 0.7014–3.100, n=5) (Fig. 5c). Similarly when these fractions were tested against carbachol (1 µM) induced contraction, Cm.Dcm exhibited more potent bronchodilation on tracheal tissue with EC$_{50}$ values of 0.2225 mg/ml (95% CI: 0.1659–0.2983 mg/ml) in comparison with Cm.Aq fraction which showed minor relaxation at higher concentration contractions with EC$_{50}$ values of 7.5052 mg/ml (95% CI: 5.3270–8.7805 mg/ml; n=5) (Fig. 5d).

**Effect on isolated rabbit aorta preparations**

The Cm.Cr exerted contractile effect after application to the isolated rabbit aortic preparations at concentration ranging from

![Fig. 1. Tracings presenting (a) spontaneous contraction of isolated rabbit jejunum and relaxant effect of the methanol extract of Cymbopogon martinii (Cm.Cr) on (b) spontaneous- and (c) high K$^+$ (80 mM)- induced tissue contraction. Extract was added in cumulative manner and values listed were the final tissue bath concentrations (n=5).](image)
0.01 to 10.0 mg/ml (Fig. 6a). However, the Cm.Cr at tissue bath concentration of 0.01–3.0 mg/ml exerted initially a contractile effect and then a relaxant effect (>5 mg/ml) on K⁺ (80 mM)-induced contractions in isolated rabbit aortic preparations with an EC₅₀ value of 0.219 mg/ml (95% CI: 3.285–3.905 mg/ml; n=3–4), whereas it caused a partial relaxation of phenylephrine (PE; 1 µM)-induced contractions (Fig. 6b).

**DISCUSSION**

*Cymbopogon martinii* is a species of grass in the lemongrass genus and possess numerous traditional applications in the south Asians communities for the relief of abdominal discomforts including diarrhea, dysentery and abdominal pain. The essential oil (palmarosa) extracted from the leaves of *Cymbopogon martini*...
has shown clinical usefulness in anthelmintic and antibacterial activities with the predominating component geraniol (65–83%), a monoterpenoid and an alcohol (11, 26). Although other components like citral, citronellol and linalool are present and possibly could contribute significantly in biological activities. In the recent years many pharmacological activities pertaining to garaniol and citral has been pointed out ranging from Ca^{2+} channel blockage to interference with enteric nervous system of gastrointestinal tract (27, 28). This voltage gated channel blockage potential of essential oil of *Cymbopogon martinii* ({Cm.Cr}) drew out attention to validate the folkloric uses of this lemongrass genus in specific types of visceral tissues in which calcium is a key player. Therefore, the possible presence of spasmolytic constituent(s) was investigated on isolated rabbit jejunum.

**Fig. 4.** Tracings showing relaxant effect of methanolic extract of *Cymbopogon martinii* (Cm.Cr) on (a) carbachol (1 µM)- and (b) high K⁺ (80 mM)-induced contractions in isolated rabbit tracheal preparation.

**Fig. 5.** Concentration dependant inhibitory effect of (a) methanolic extract of *Cymbopogon martinii* (Cm.Cr) (b) verapamil and (c and d) aqueous and dichloromethane fractions of Cm.Cr on carbachol (1 µM) and high K⁺ (80 mM)- induced contractions in isolated rabbit tracheal preparations (Values are the mean ± S.E.M., n=5).
 preparations, a model that permits the study of spasmolytic activity without the use of an agonist (27-33). Addition of Cm.Cr to spontaneously contracting isolated rabbit jejunum preparation inhibited spontaneous contractions, thus demonstrated an antispasmodic potential. It has been reported that spasmolytic effect on the part of medicinal plants likely can be mediated through blockade of Ca\(^2+\) channels (29, 36). The contractile elements in smooth muscle preparations including isolated rabbit jejunum preparations are activated on increased cytoplasmic free Ca\(^2+\) concentration (34). The increased intracellular Ca\(^2+\) level is likely to be mediated either influx through voltage dependent Ca\(^2+\) channels (VDCs) or released of Ca\(^2+\) from sarcoplasmic stores (38). The spontaneous movement of the intestine is regulated by the periodic depolarization and repolarization and when tissue is at maximal depolarization, the action potential is mediated via rapid influx of Ca\(^2+\) through VDCs (39). Thus, the observed relaxant effect of the Cm.Cr on the hyperactive smooth muscle preparation can be possibly mediated either through blockade of VDCs or through inhibition of Ca\(^2+\) release from sarcoplasmic stores. This finding is in agreement with the previously conducted study in which other variety of lemongrass i.e. Cymbopogan citratus and chief constituent citral has shown spasmytic effect on isolated rabbit ileum (26).

It has been reported that K\(^+\) (80 mM) causes the opening of VDCs, modifying the extracellular Ca\(^2+\) and resulting in the contraction of smooth muscle (38); therefore the substances capable to relax K\(^+\) (80 mM)-induced contractions is presumed to act as Ca\(^2+\) channel blocker (CCB). In order to assess whether antispasmodic effect of Cm.Cr is also mediated via a similar mechanism, the Cm.Cr was tested on K\(^+\) (80 mM)-induced contractions and the addition of Cm.Cr to tissue bath in a cumulative fashion (33) resulted in the relaxation of K\(^+\) (80 mM) induced contractions in isolated rabbit jejunum preparations. These findings were confirmed considering that the administration of Cm.Cr to isolated rabbit jejunum preparation resulted in a decrease in tissue response to Ca\(^2+\), resulting in rightward shift of the concentration response curves of Ca\(^2+\), similar to verapamil, a standard Ca\(^2+\) channel blocker (40). The Ca\(^2+\) channel blockers are known to be effective in hyperactive diseases of the gut (34) and this may validate the folkloric use of Cymbopogan martinii. The fractions of Cm.Cr, when explored for Ca\(^2+\) channel blocking activity, resulted in appearance of more pronounced activity in dichloromethane fraction as compared to aqueous, indicating that Ca\(^2+\) channel blocking activity is present among the non-polar plant constituents.

The leaves of Cymbopogan martini in the form of herbal tea has been used for the management of respiratory diseases like asthma and bronchitis and therefore, require validation. High K\(^+\) was used to induce contraction by opening of VDCs in a similar mechanism of jejunum tissue whereas, carbachol which is a M1 muscarinic receptor agonist induce contraction of tracheal tissue by stimulating predominantly Gq protein of trimeric G proteins of class that use upregulation of phospholipase C and, therefore, inositol trisphosphate and intracellular calcium as a signalling pathway (42). The Cm.Cr exerted relaxant effect on carbachol (1 µM)- and K\(^+\) (80 mM)-induced contractions in isolated rabbit tracheal preparations in a manner similar to verapamil and it is possible to hypothesize that this activity can be mediated through Ca\(^2+\) channels. The contractile effect of Cm.Cr indicates the ability of this extract to stimulate possibly adrenergic receptors which downstream augment calcium response and thus contraction of vascular smooth muscles (39). Further, Cm.Cr showed a dual effect (vasoconstriction-vasorelaxation) of the K\(^+\) (80 mM)-induced contractions in isolated rabbit aorta, whereas phenylephrine-induced contractions were partially relaxed. At higher dosage (>5.0 mg/ml) of Cm.Cr vasorelaxation effect might be mediated through the blockage of L-type Ca\(^2+\) and voltage gated K\(^+\) channels. This finding is in agreement with the recent published work by de Menezes-Filho et al., which demonstrated the blockage of Ca\(^2+\) and voltage gated K\(^+\) channels by geraniol (major component of Cymbopogan martini leaves) in mammalian myocardium (28). The isolated rabbit aorta preparations have been used for characterization of Ca\(^2+\) channel blocking activities (33) and tissues exposed to K\(^+\) (80 mM) showed contractions of smooth muscles via opening of voltage dependent Ca\(^2+\) channels (VDCs). The increase in intracellular Ca\(^2+\) due enhanced influx of Ca\(^2+\) can cause further

![Graphs showing (a) contractile effect of Cymbopogon martini (Cm.Cr) on isolated rabbit aortic tissue (without any pretreatment) in a concentration dependent manner (0.01–10.0 mg/ml) (b) application of Cm.Cr on pretreatment with high K\(^+\) (80 mM) aortic tissue caused dual effect; initial vasoconstriction (0.01–3.0 mg/ml) and then vasorelaxation (>5mg/ml).](image-url)
Ka+ release from sarcoplasmic reticulum (43, 44). Similarly, phenylephrine (PE) causes contraction of vascular smooth muscles due to raised cytosplasmic Ca2+ through two possible means, i.e., Ca2+ influx via receptor operated channels (ROCs) and subsequent release of Ca2+ from intracellular stores (37). The partial relaxation of phenylephrine-induced contractions on the part of Cm.Cr can be explained by focusing the fact that Cm.Cr, like other Ca2+ channel blockers, can only block Ca2+-influx through only VDCs and sparing other possible mechanism involved (38). Recently Su and colleagues (45) provided strong evidences of vasorelaxation of aortic ring by an involvement of other mechanisms like release of vasodilator substances from intact endothelium, or nitric oxide (NO) synthase or stimulation of muscarinic receptors. Therefore, vasodilator activity mediated by Cm.Cr against phenylephrine and high K+ could be the result of multiple other mechanisms apart from calcium antagonism. Nevertheless, the observed relaxant effect of Cm.Cr on vasculature may provide a scientific basis for the folkloric use of Cymbopogon martini in the management of cardiovascular ailments.

In conclusion, the crude methanolic extract of Cymbopogon martini Roxb. (Cm.Cr) has demonstrated antispasmodic, bronchodilator and vasodilator properties apart from calcium antagonism. Nevertheless, the observed relaxant effect of Cm.Cr on vasculature may provide a scientific basis for the folkloric use of Cymbopogon martini in the management of cardiovascular ailments.

Conflict of interests: None declared.

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