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The Airway Relaxant Effect of Horse Purslane, *Trianthema portulacastrum,* Linn. (Aizoaceae), is Mediated through Dual Blockade of Muscarinic Receptors and Ca^{2+} Influx

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SB and AHG designed and supervised the study. Authors SA and SRM carried out the experimental work, data analysis and literature search. The manuscript was drafted by author SB and finalized by author AHG. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The horse purslane, *Trianthema portulacastrum,* Linn. (Aizoaceae), is used in traditional systems of medicine for the treatment of asthma. The current investigation was aimed at exploring possible mechanisms underlying the potential bronchodilator effect of *Trianthema portulacastrum (T. portulacastrum)*.
Methodology: The whole plant extract of *T. portulacastrum* was studied on rats for its *in-vivo* bronchodilator activity and on isolated rabbit trachea, to find out the mechanistic basis of the therapeutic effect. The data were analysed using Student's *t*-test.

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Results: *T. portulacastrum* crude extract dose-dependently (3 - 30 mg/kg) inhibited carbachol-induced bronchoconstriction in anaesthetised rats, similarly to aminophylline. When tested on rabbit trachea, the plant extract inhibited carbachol (1 µM) and high K^+ (80 mM)-induced contractions in a fashion similar to dicyclomine, indicating the presence of airway-relaxant activity, possibly mediated through blockade of calcium channels and muscarinic receptors. The presence of a dual muscarinic and Ca^{2+} channel inhibitory mechanism was confirmed when the crude extract, caused a rightwards shift of carbachol and Ca^{2+} concentration-response curves, similar to dicyclomine.
Conclusions: This investigation indicates that the *T. portulacastrum* extract possesses bronchodilator activity that is possibly mediated through a combination of an anti-muscarinic effect and calcium channel blockade, providing a scientific basis for its medicinal use in asthma.

Keywords: Trianthema portulacastrum; asthma; bronchodilator; muscarinic receptor antagonist; Ca^{2+} antagonist.

1. INTRODUCTION

T. portulacastrum, Linn. (Aizoaceae), commonly known as horse purslane, is a fast-growing wild weed, native to Southeast Asian countries, Tropical America and Africa. The plant grows abundantly in crop fields, gardens, wastelands and on riversides of Pakistan, India, Bangladesh and Sri Lanka particularly during rainy seasons, where it is commonly consumed as a cooked vegetable [1, 2]. Being a good source of fibre, protein, riboflavin, nicotinic acid, ascorbic acid, magnesium, phosphorus and iron, *T. portulacastrum* possesses significant nutritional potential [3-5]. It is also a valuable herb in traditional systems of medicine practised in Southeast Asian countries. All parts of the plant are considered therapeutically effective for various diseases. *T. portulacastrum*, as a whole plant, is used as analgesic, purgative, stomachic and for the treatment of anaemia, bronchitis, asthma, piles, inflammation, itching, chronic ulcer, night blindness and diseases of liver, blood and skin [6]. The root is cathartic, irritant, abortifacient and is effective against cough, asthma and phlegmatic fevers. Leaves, due to their diuretic property, are recommended in oedema and dropsy while an extract from these plant parts is used as an antidote to alcohol poisoning, as a vermifuge and for the treatment of rheumatism [7-9].

Pharmacological studies with various extracts and phytoconstituents of *T. portulacastrum* have confirmed the presence of a number of medicinal activities in this plant. *T. portulacastrum* has been reported to possess antilithic, antihyperglycemic, antibacterial and antifungal, hepatoprotective, anthelmintic, antihyperlipidemic and central analgesic activities [10-18]. The protective role of *T. portulacastrum* against diethylnitrosamine–induced experimental hepatocarcinogenesis has been established [19]. *T. portulacastrum* leaf extract has also been shown to possess wound healing property possibly mediated through antioxidant and anti-inflammatory effects [20].

Phytochemical analyses have identified the presence of steroids, flavonoid, alkaloids, carbohydrates, tannins and terpenes in *T. portulacastrum* [21]. Ecdysterone, the most widely occurring phytoecdysone, has been identified as the principal constituent of *T. portulacastrum* [22]. Other phytoconstituents isolated from *T. portulacastrum* include Trianthenol, 5, 2'-dihydroxy-7-methoxy-6, 8-dimethylflavone and leptorumol [13, 23]. The plant also contains potassium salts, punarnavine and trianthemine. Its root contains saponin glycosides, vanillic acid, P-hydroxybenzoic acid, ferrulic acid, trans-cinnamic acid, caffeic acid, protocatechuic acid, o-coumaric acid, while pyrogallic acid is reported to be present in leaves and stem of the plant [24, 25].

T. portulacastrum has been used in indigenous medicinal systems for respiratory problems including bronchitis and asthma [6]. However, there is no study available in the literature rationalising its medicinal use and defining the precise mode of action in such disorders. This study was therefore designed to provide the pharmacological basis for the traditional use of *T. portulacastrum* in asthma.

2. MATERIALS AND METHODS

2.1 Plant Material and Preparation of Crude Extract

For the purpose of this study, which was carried out from June to December, 2013, the whole plant of *T. portulacastrum* was collected from the

fields of Bahauddin Zakariya University, Multan (Pakistan) and was identified by an expert taxonomist of the Institute of Pure & Applied Biology, Bahauddin Zakariya University, Multan. A specimen of the plant has been deposited at the herbarium of the same institute (voucher no. P. Fl 235-3). The plant material was washed with tap water and subjected to shade drying. The dried plant material (200 g) was ground into coarse powder through an electrically driven device and the powder was soaked in a mixture of methanol and water at a ratio of 7:3 (v/v) for three days in amber coloured glass bottles with occasional shaking. The soaked material was passed through a double-layered muslin cloth to remove vegetative debris, and the obtained fluid was subsequently filtered through filter paper [26]. The residue was re-soaked for the next three days and the procedure repeated twice. The filtrates were evaporated on a rotary evaporator (R-210, BUCHI, Switzerland) under reduced pressure (-760 mmHg) at 37ºC to yield a dark brown paste, the *T. portulacastrum* crude extract (Tp.Cr) that constituted 11% of the starting material. Tp.Cr was solubilised in normal saline for *in vivo* studies and in 10% dimethyl sulfoxide (DMSO) for all *in vitro* experiments.

2.2 Drugs and Reagents

Carbachol (CCh), dicyclomine and verapamil hydrochloride were purchased from Sigma Chemical Co., St Louis, Mo, USA. Among chemicals used in the physiological salt solutions, potassium chloride and ethylenediamine tetra-acetic acid (EDTA) were purchased from Sigma Chemicals Co., calcium chloride, glucose, magnesium chloride, magnesium sulphate, potassium dihydrogen phosphate, hydrochloric acid, sodium dihydrogen phosphate, sodium bicarbonate and DMSO from Merck, Darmstadt, Germany, and sodium chloride, sodium hydroxide and ammonium hydroxide from BDH Laboratory Supplies, Poole, England. All chemicals used were of analytical grade. The vehicle used for solubilisation of drugs had no effect in the control *in vivo* and *in vitro* experiments. The stock solutions and their dilutions were made fresh at the day of the experiment.

2.3 Animals Used in the Experiments

Sprague-Dawley rats and rabbits of local breed and either sex were housed in the Animal House of the Aga Khan University, Karachi, maintained at 23-25ºC and 12 h light/dark cycle. Animals were provided tap water and standard diet. Rats were used for the *in vivo* bronchodilator study while tissues isolated from rabbits were used for the *in vitro* experiments. Animals had free access to water throughout the study, but food of the rabbits was withdrawn 24 h prior to the experiments. Rabbits were sacrificed by a blow on the back of the head followed by cervical dislocation. Experiments performed complied with the rulings of the Institute of Laboratory Animals Resources, Commission on Life Sciences, National Research Council [27].

2.4 Effect on CCh-induced Bronchospasm

Rats, anaesthetised with sodium thiopental (Pentothal, 80-100 mg/kg, i.p.), underwent endotracheal intubation followed by ventilation with a volume ventilator (Miniature ideal pump, Bioscience, UK) adjusted at a rate of 70-80 strokes/min to deliver 7-10 ml/kg of room air [28]. A polyethylene catheter was inserted into the jugular vein for drug administration. Changes in airway resistance (mm of Hg) were measured by a pressure transducer (MLT-1199) connected to the side arm of the tracheal cannula and recorded by PowerLab 4/25 with running chart software via a Quad bridge amplifier (AD Instruments, Bella Vista, NSW, Australia). Bronchoconstriction was induced with CCh (100 µg/kg), which was reversed within 7-10 min. The test drug was given to the animals 5-8 min prior to the administration of CCh. The responses were expressed as the percent reduction of the CCh-induced bronchospasm.

2.5 Experiments on Isolated Rabbit Trachea

The trachea was dissected out and kept in Krebs' solution. The trachea was cleaned of the surrounding fatty tissues and cut into rings of 2-3 mm in width (containing 2 cartilages), as described by Arshad et al. [29]. Each ring was then opened by a longitudinal cut on the side opposite to the smooth muscle layer in such a way that smooth muscles were in between the C-shaped cartilaginous part. The isolated preparations were then mounted in tissue baths containing Krebs' solution and aerated with carbogen at 37°C. The composition of Krebs' solution was (mM): NaCl 118.2, $NaHCO_3$ 25.0, $CaCl_2$ 2.5, KCl 4.7, KH_2PO_4 1.3, $MgSO_4$ 1.2 and glucose 11.7 (pH 7.4). A tension of 1 g was applied to the tracheal strips continuously and equilibrated for 1 h before the addition of any

chemical substance. CCh (1μM) was used to stabilise the preparations until constant responses to successive treatments were achieved (usually after 3-4 exposures). The bronchodilator activity of the plant extract and control drugs was studied on CCh (1μM) or high K^+-induced sustained contractions by adding them in a cumulative manner.

To determine the antimuscarinic activity of Tp.Cr and the control drugs, cumulative concentration-response curves (CRCs) of CCh were constructed using increasing concentrations of the agonist. When a 3-fold increase in concentration produced no further increment in response, the tissue was washed to re-establish the baseline tension. The CCh CRCs were repeated in the presence of increasing concentrations of crude extract, dicyclomine and verapamil [30], respectively Isometric responses were recorded by a force transducer (model FORT100) coupled to a Transbridge (model TBM4M, World Precision Instruments, Hertfordshire, UK) and a PowerLab data acquisition system (model ML845, AD Instruments, Sydney, Australia) and computer running Chart software (version 6).

For the determination of Ca^{2+} channel blocking (CCB) activity, high K^+ (80 mM) was used to depolarise the preparations as described previously [31]. K^+ (80 mM) was added to the tissue bath, which produced a sustained contraction. Once a plateau of the induced contraction was achieved, the test material was added in a cumulative fashion to obtain concentration-dependent inhibitory responses [32]. To confirm the Ca^{2+} antagonist activity of the test substances, the tissue was allowed to stabilise in normal Krebs' solution, which was then replaced with Ca^{2+}-free Krebs' solution containing EDTA (0.1 mM) for 30 min in order to remove Ca^{2+} from the tissues. This solution was further replaced with K^+-rich and Ca^{2+}-free Krebs' solution, having the following composition (mM): KCl 50, NaCl 91.04, $MgCl_2$ 1.05, $NaHCO_3$ 11.90, NaH_2PO_4 0.42, glucose 5.55 and EDTA 0.1. Following an incubation period of 30 min, control CRCs of Ca^{2+} were obtained. When the CRCs of Ca^{2+} were found super-imposable (usually after two cycles), the tissue was pretreated with the crude extract for 60 min to test for a possible CCB effect. The CRCs of Ca^{2+} were reconstructed in the presence of different concentrations of Tp.Cr and of the control drugs: dicyclomine and verapamil.

2.6 Statistical Analysis

All experiments have been repeated 3 to 4 times and the data are expressed as mean ± standard error of the mean (S.E.M.) and the median effective concentration (EC_{50}) with 95% confidence intervals (CI). Concentration-response curves of CCh and Ca^{2+} are analysed by non-linear regression using the GraphPad program (GraphPAD, SanDiego, CA, USA). The statistical analysis is performed by applying Student's *t*-test. Difference of $P < 0.05$ is considered statistically significant.

3. RESULTS

3.1 Bronchodilator Activity

Tp.Cr, at the doses of 3, 10 and 30 mg/kg, caused suppression of the CCh (100 μg/kg)-induced increase in respiratory pressure of the anaesthetised rats (Fig. 1a), similar to the positive control drug, aminophylline (Fig. 1b).

3.2 Effect on Rabbit Trachea

Tp.Cr caused inhibition of both CCh (1 μM) and high K^+ -induced contractions in isolated rabbit trachea, however, the CCh-induced contraction was inhibited with a lower EC_{50} value of 0.20 mg/ml (0.15-0.25, 95% CI) than the K^+-induced contraction, which was inhibited at 0.85 mg/ml (0.64-1.14), as shown in Fig. 2a. Similarly, dicyclomine, the positive control drug, inhibited CCh and high K^+ -induced contractions with EC_{50} values of 0.14 (0.10-0.18) and 1.80 (1.26-2.58) μM, respectively (Fig. 2b). On the other hand, verapamil inhibited K^+ -induced contraction with a lower EC_{50} value of0.02 (0.01-0.03) μM than the CCh-induced contraction, 0.06 (0.05-0.08) μM, as shown in Fig. 2c.

Tp.Cr produced rightwards parallel displacement of the CCh CRCs without suppression of the maximum contractile response at 0.03 mg/ml, followed by a non-parallel shift with suppression of the maximum effect at 0.1 mg/ml (Fig. 3a). Dicyclomine (0.01-0.03 μM) also exhibited a similar pattern of shifting CCh CRCs (Fig. 3b), whereas verapamil, at 0.03 and 0.1 μM, shifted CCh CRCs rightwards, suppressing the maximum response at both concentrations (Fig. 3c).

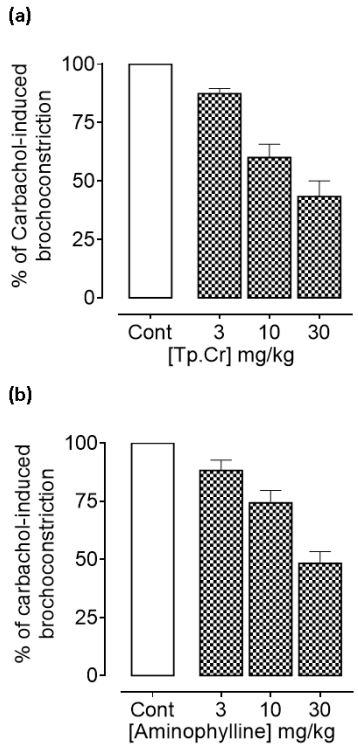

Fig. 1. Bar-diagram showing the bronchodilator effect of (a) the crude extract of *Trianthema portulacastrum* (Tp.Cr) and (b) aminophylline on carbachol-induced bronchoconstriction in normal rats under anaesthesia. Values shown are mean ± S.E.M, n=3

Tp.Cr also shifted Ca^{2+} cumulative CRCs, constructed in rabbit tracheal preparations, towards the right, at concentrations of 0.1, 0.3 and 1 mg/ml (Fig. 4a). The control drugs, dicyclomine and verapamil, similarly caused a rightwards displacement of the Ca^{2+} curves (Fig. 4b and 4c).

4. DISCUSSION

Based on the medicinal use of *T. portulacastrum* Linn. in respiratory disorders resulting from increased airway resistance, the crude extract of the plant was tested for a possible bronchodilator effect. Tp.Cr pretreatment caused protection against CCh-induced bronchoconstriction in anaesthetised rats in a dose-dependent manner, similar to the standard bronchodilator drug, aminophylline [33]. CCh is known to cause airway constriction through stimulation of muscarinic receptors, subtype M_3 [34]. Interestingly, Tp.Cr was found slightly more efficacious than aminophylline at all doses tested.

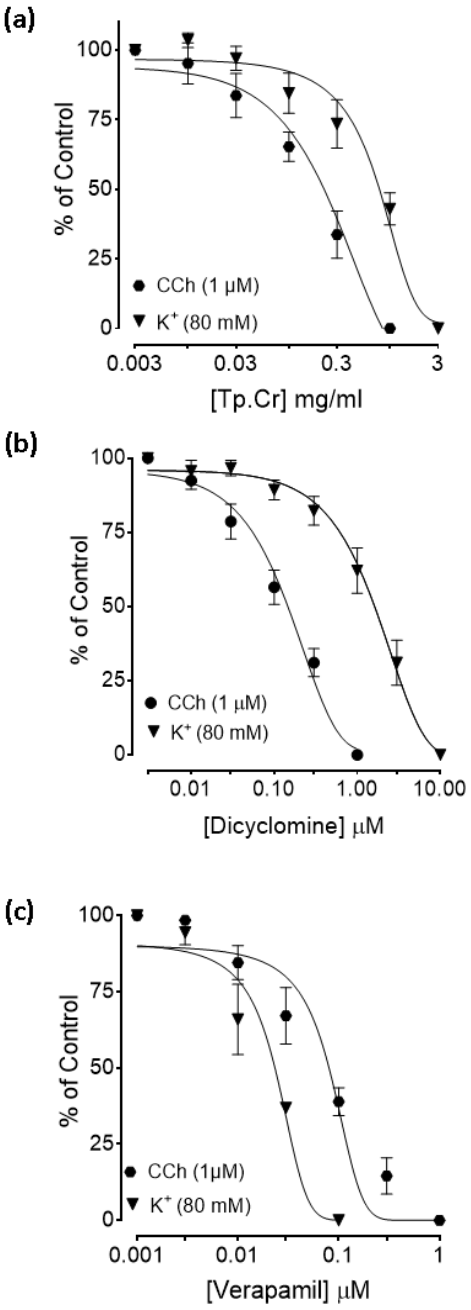

Fig. 2. Concentration-dependent inhibitory effects of (a) the crude extract of *Trianthema portulacastrum* (Tp.Cr), (b) verapamil and (c) dicyclomine on K^+ (80 mM) and carbachol (1 μM)-induced contractions in isolated rabbit trachea. Values shown are mean ± S.E.M., n=3-4

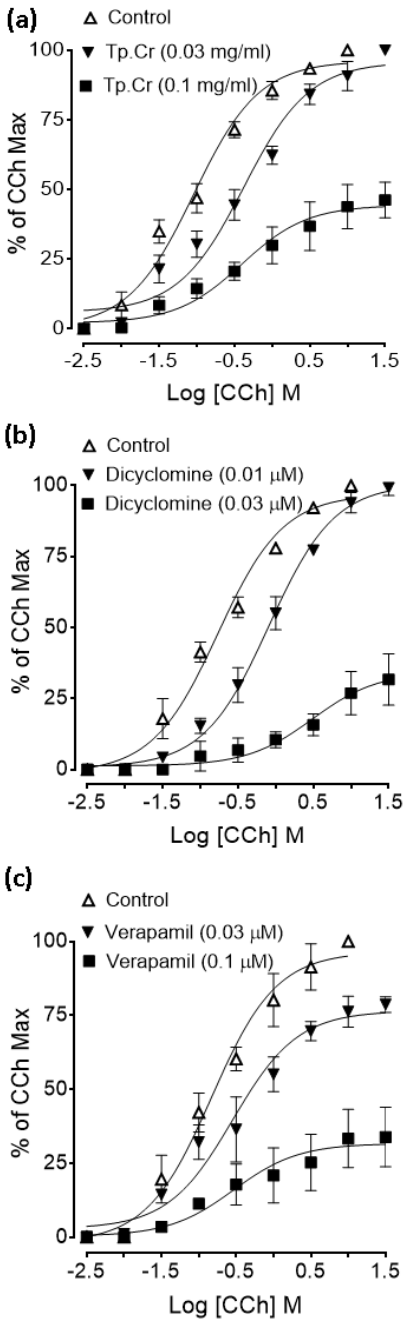

Fig. 3. Concentration-response curves of carbachol in the absence and presence of (a) crude extract of *Trianthema portulacastrum* (Tp.Cr), (b) dicyclomine and (c) verapamil in isolated rabbit tracheal preparations. Values are expressed as mean ± S.E.M., n = 3-4

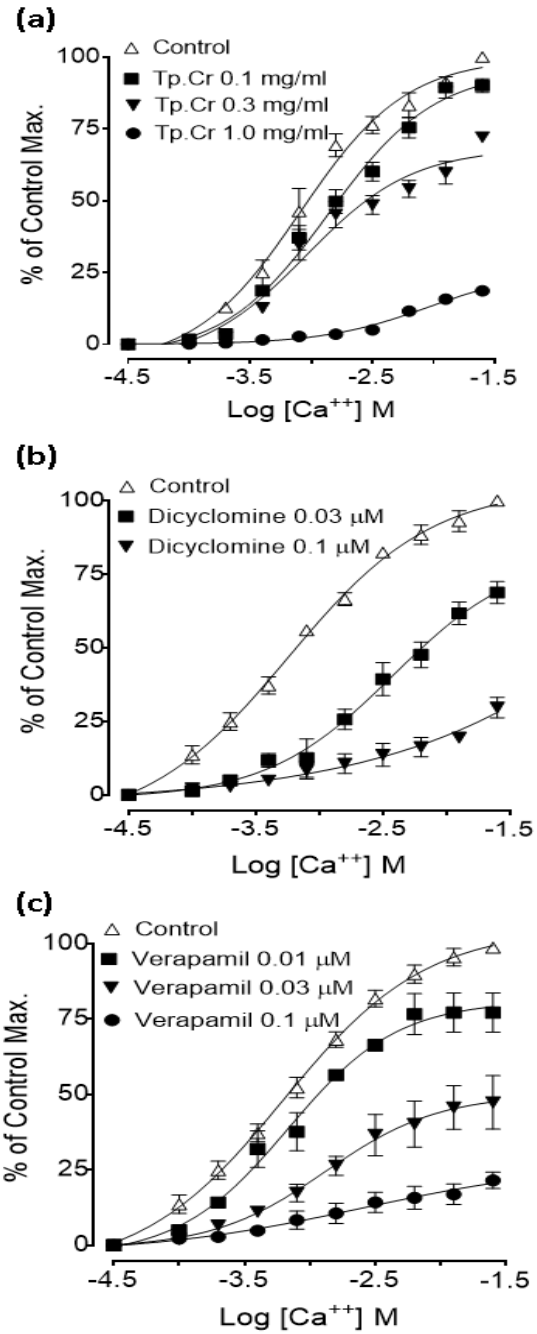

Fig. 4. Concentration-response curves of Ca^{2+} in the absence and presence of (a) crude extract of *Trianthema portulacastrum* (Tp.Cr), (b) dicyclomine and (c) verapamil in isolated rabbit trachea. Values are expressed as mean ± S.E.M., n = 3-4

It is customary to use isolated tissue preparations to explore the mechanistic basis of a given pharmacological effect [28,35]. Tp.Cr was studied on isolated rabbit trachea to investigate the possible mode of action of the bronchodilator effect. Experimental studies have shown that medicinal plants commonly exhibit their bronchodilator effect through synergistic combinations of activities, frequently involving Ca^{2+} channel blockade and muscarinic receptor antagonism [28,36,37]. Therefore, Tp.Cr was tested against CCh and high K^+- induced contractions in isolated rabbit trachea. Although Tp.Cr inhibited both the contractions, it was found more potent against CCh than high K^+, as reflected by a lower median effective concentration required against former than latter, similar to dicyclomine, a dual blocker of muscarinic receptors and Ca^{2+} influx [30,38]. Contrary to the effect of Tp.Cr and dicyclomine, verapamil, a pure Ca^{2+} channel blocker, was more potent against high K^+ than CCh- induced contractions. These results thus suggest the presence of anticholinergic and CCB activities in *T. portulacastrum*.

The anticholinergic component of the plant extract was further investigated by testing the effect of Tp.Cr on CCh CRCs, where it caused a parallel displacement of CCh-curves without suppression of the maximum effect at the lower concentration, followed by non-parallel shift with suppression of the maximum effect at next higher concentration, pointing towards the presence of non-competitive inhibition [39], known with Ca^{++} antagonists [40]. Dicyclomine also shifted the CCh-curves to the right in a fashion similar to that of the crude extract. The presence of CCB activity in *T. portulacastrum* was confirmed when Tp.Cr caused a rightwards shift in Ca^{2+} CRCs similar to both dicyclomine and verapamil. Ca^{2+} antagonists are known to be effective in hyperactive respiratory disorders, while, similarly, anticholinergic agents are now considered important bronchodilators for the treatment of asthma, and this unique combination of activities may offer more effective bronchodilation [41,42]. *T. portulacastrum* has previously been reported to possess anti-oxidant and anti-inflammatory activities [20]. In this study, the effect of the plant extract is investigated on an animal model of CCh-induced acute bronchoconstriction, therefore the reported activities are less likely to contribute towards broncho-dilatation in this case, however, these effects may play a beneficial role in the case of chronic asthma [43].

5. CONCLUSION

From the results of this study, it is concluded that *Trianthema portulacastrum* whole plant possesses bronchodilator activity, possibly mediated through a combination of an antimuscarinic effect and blockade of Ca^{2+} influx. These findings provide the scientific basis for the medicinal use of *Trianthema portulacastrum* in asthma and identify this plant as a potential source of lead compounds for drug development.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The study was approved by the Animal Ethics Committee, Bahauddin Zakariya University, Multan.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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