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ORIGINAL ARTICLE

VASODILATOR, BRONCHODILATOR AND SPASMOLYTIC ACTIVITIES OF *SPILANTHES ACMELLA*

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ABSTRACT

Spilanthes acmella Murr. (*Sa. Murr*) is belong to family *asteraceae* known as akarkara in common language and widely distributed perennial herb in South Asia (particularly in India and Pakistan); customarily used by local population for the management of multiple illnesses including gastrointestinal, respiratory and vascular disorders but commonly used for toothache and to stimulate flow of saliva, tongue paralysis and sore mouth and the present study was undertaken to validate these folkloric uses. The application of a methanol extract of the plant (*Sa. Murr*) to isolated rabbit jejunum preparation exhibited relaxation through decrease in magnitude and frequency of spontaneous contractions. The (*Sa. Murr*.) also exerted relaxant effect on high K^+ (80 mM) induced contractions in isolated rabbit jejunum preparations. The (*Sa. Murr*.) also caused concentration-dependent relaxation in spontaneous and K^+ (80 mM) induced contractions which are comparable to effect produced by verapamil. The (*Sa. Murr*.) caused shifting of the Ca^{2+}-curves toward right, suggesting the presence of a Ca^{2+} channel blocking activity. Subsequently, the *Sa. Murr*., caused relaxation of CCh (1 μM) and K^+ (80 mM) induced contractions in isolated rabbit tracheal preparations, suggesting that the observed relaxant effect can be mediated through anti-muscarinic and/or Ca^{2+} channel blocking activities. The *Sa. Murr*. tested against phenylephrine (PE; 1 μM) and K^+ (80 mM) induced contractions exhibited relaxation of isolated rabbit aortic preparations. The above-mentioned studies provided a scientific basis for the folkloric use of *Spilanthes acmella* Murr. in the management of multiple ailments in traditional systems of medicines.

Keywords*:* *Spilanthes acmella*, spasmolytic effect, bronchodilator effect, vasorelaxant effect, calcium.

INTRODUCTION

Medicinal plants contain a diverse group of highly valuable and readily available resource of bioactive metabolites like alkaloids, tannins, essential oils and flavonoids which are used in medicinal practices since long time. The World Health Organization has estimated that about 80 % of the population in developing countries is unable to afford modern medicine and rely on traditional plant-based medicines. Due to therapeutic efficiency and freely availability, medicinal plants are used as source of alternative medicine in many diseases and conditions. There is need of detailed examination of many medicinal plants for their pharmacological and therapeutic value. *Spilanthes acmella* Murr. is used to treat mouth ailments, stomatitis, anti-inflammatory, antiseptic, analgesic, antioxidant, cytotoxic, constituent of beauty care cosmetics, larvicidal and insecticidal properties Rheumatism, inflammation, stimulant and as sialagogue, tongue paralysis, stomatitis, toothache, headache, asthma, fever, sore throat and hemorrhoids and gum infections, potential vasodilator, antioxidant and immune-modulator, diuretic, antibacterial and anti-

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inflammatory activities, Toothache and to stimulate flow of saliva, tongue paralysis and sore mouth, locally against itching and psoriasis, Analgesic and local anaesthetic (Chakraborty *et al.,* 2011) Containing amino acids, alkaloids and N-isobutylamides (spilanthol, undeca-2E,7Z, 9E-trienoic acid isobutylamide and undeca-2E-en-8,10-diynoic acid isobutylomide) (Russel *et al.,* 1999). 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 (Apilak *et al*., 2009), the present study was undertaken to validate traditional use of *S. acmella* Murr. in the management of gastrointestinal, respiratory and cardiovascular. This was our aim of study to find out the different pharmacological and therapeutic effects of *S. acmella* Murr with the support of histo-pathological investigations.

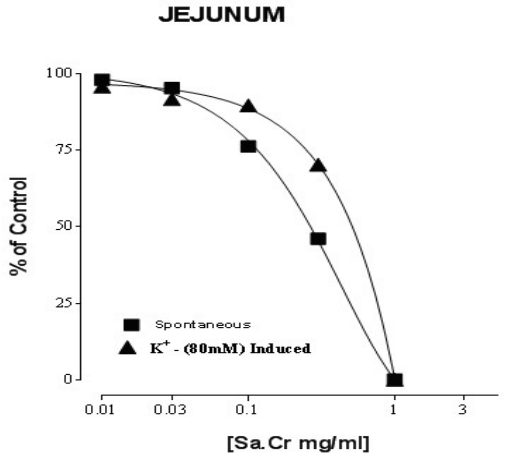

Fig. 1: Effect of crude extract of Spilanthes *acmella* (Sa.Cr) on spontaneous and K^+(80mM)-induced contractions on isolated rabbit jejunum preparations Values are expressed as the mean ± SEM**.**

MATERIALS AND METHODS

Collection, identification and extraction of plant material

Aerial part of *S. acmella* Murr. was purchased from herbal store of the local market and was identified by a taxonomist (Dr. Zafarullah) from the Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan with voucher specimen #. 1214/BZU/15. The plant material was rendered free from soil and adulterated materials, shade dried at 40°C and was rendered free of possible adulterant through manual picking. Then grinded into coarse and passes through sieve 40. The dried herbal material was subsequently grinded into coarse powder. The powdered material was soaked in an amber glass container having methanol for seven days at room temperature 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-1 filter paper then filtered and subjected to evaporation under reduced pressure on a rotary evaporator (Rota-vapor Buchii, Japan) at 45ºC with rotation of 3.0 rpm and pressure 0.07 MPA to thick paste coupled with recirculation chiller (B-740) and vacuum pump (Buchi vac V-500). The approximate yield of the crude methanolic extract (Sa.Cr) was 6%. Dried extract of plant was transferred in air tight container and preserved in biomedical freezer (Sanyo biomedical freezer, MDF-U333, Japan).

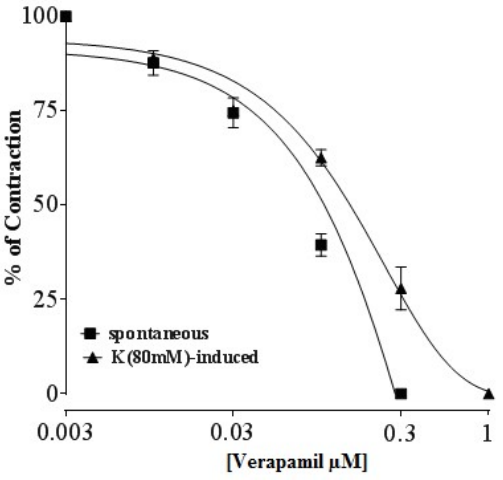

Fig. 2: Effect of Verapamil on spontaneous and K^+ (80mM)-induced contractions on isolated rabbit jejunum preparations Values are expressed as the mean ± SEM.

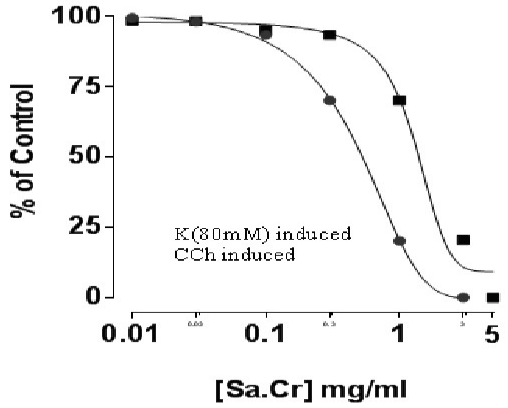

Fig. 3: Effect of crude extract of *Spilanthes acmella* (Sa.Cr) on CCh(1µM) and K^+(80mM)-induced contractions on isolated rabbit tracheal preparations Values are expressed as the mean ± SEM.

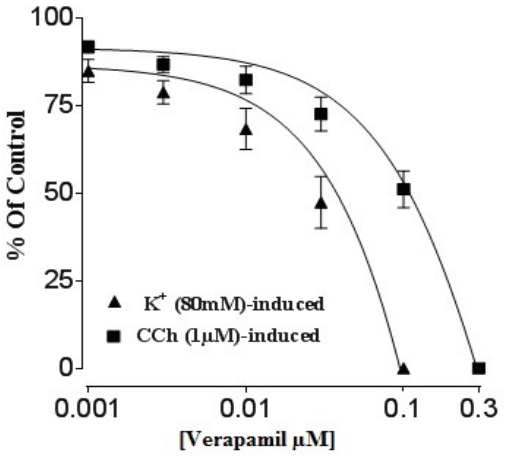

Fig. 4: Effect of Verapamil on CCh(1µM) and K^+(80mM)-induced contractions on isolated rabbit tracheal preparations Values are expressed as the mean ± SEM.

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 dihydrogenphosphate, sodium bicarbonate, sodium dihydrogenphosphate 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.

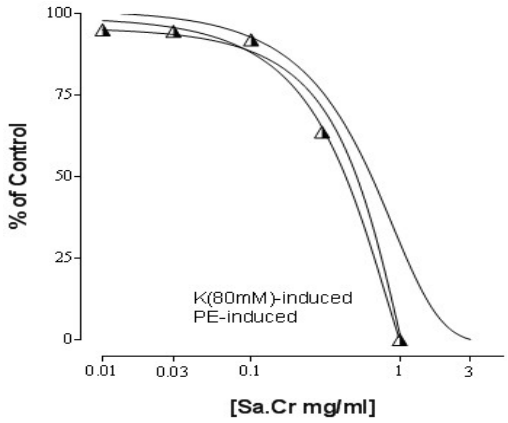

Fig. 5: Effect of crude extract of *Spilanthes acmella* (Sa.Cr) on PE(1µM) and K^+(80mM)-induced contractions on isolated rabbit aortic preparations Values are expressed as the mean ± SEM.

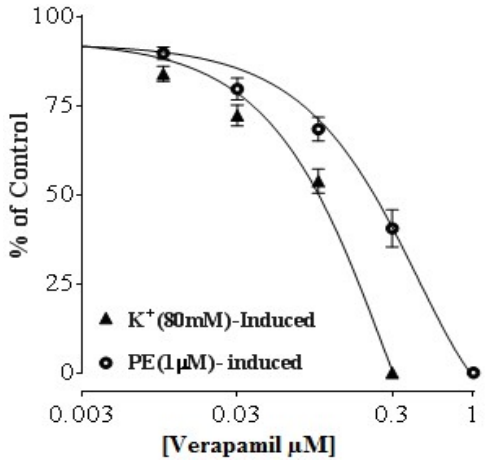

Fig. 6: Effect of Verapamil on PE(1µM) and K^+(80mM)-induced contractions on isolated rabbit aortic preparations Values are expressed as the mean ± SEM.

Animals and housing conditions

All the experiments performed complied with the rulings of Institute of Laboratory

Animal Resources, Commission on Life Sciences (NRC, 2011), 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 *S. acmella* Murr. was tested for the possible presence of either spasmolytic 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) (Janbaz *et al.,* 2015a). 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% O_2 and 5% CO_2). The tissues was 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 jejunum preparations exhibit spontaneous rhythmic contractions, allowing testing of the antispasmodic (relaxant) effect without application of an agonist. The possible mechanism of the relaxant activity of the test materials were investigated through the relaxation of the observed sustained spasmodic contractions following exposure to K^+ (80 mM). The test materials were applied in a cumulative manner to the sustained contractions to achieve concentration-dependent relaxant effects. 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 . Subsequent to an incubation period of 30 min, cumulative Ca^{2+} 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 *S. acmella* Murr. for 1 h and then the concentration response curves of Ca^{2+} were recorded and compared to the control curves (Farre *et al.,* 1991). The CRCs of Ca^{2+} 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), $NaHCO_3$ (25.0), $CaCl_2$ (2.5), KCl (4.7), KH_2PO_4 (1.3), $MgSO_4$ (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% O_2 + 5% CO_2). 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) (Janbaz *et al.,* 2015). The standard drug, verapamil, with Ca^{2+} 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 *S. acmella* Murr. 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 (Janbaz *et al.,* 2014). 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^+ (80mM)-induced contractions. The changes in isometric tension of aortic rings were recorded by a force-displacement transducer 860 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 (EC50 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 AND DISCUSSION

Effect on isolated rabbit jejunum preparation

The methanolic extract of *S. acmella* caused relaxation of the spontaneous contractions in isolated rabbit jejunum contractions at concentration range of 0.01-1.0mg/ml with EC50 value of 0.523mg/ml. The extract also caused relaxation of K^+ (80mM)-induced contractions at concentration range of 0.01-1.0mg/ml with an EC_{50} value of 3.825mg/ml. The verapamil (standard drug) caused relaxation of the spontaneous contractions of jejunum at concentration range of 0.01-0.3mg/ml with EC_{50} value of 0.712μM and K^+ (80mM)-induced contractions at concentration range of 0.01-1.0mg/ml with EC_{50} value of 0.204μM.

Effect on isolated rabbit tracheal preparations

The methanolic extract caused complete relaxation of carbachol (1uM) and K^+ (80mM)-induced contractions in isolated rabbit tracheal preparations concentration-dependent manner, with respective EC_{50} values of 0.617mg/ml and 0.594mg/ml. Similarly, verapamil (standard drug) caused relaxation of carbachol (1μM) and K^+ (80mM)-induced contractions in isolated rabbit tracheal preparations concentration dependent manner, with the respective EC_{50} values of 0.310μM and 0.064μM.

Effect on isolated rabbit Aorta preparations

The methanolic extract on cumulative addition to isolated tissue baths caused a concentration-dependent relaxation of phenylephrine (1uM) and K(80mM)-induced contractions in isolated rabbit aorta, with respective EC50 values of 0.894mg/ml and 3.373mg/ml. Similarly, verapamil caused relaxation of phenylephrine (1μM) and K(80mM)-induced contractions in isolated rabbit aorta with the respective EC_{50} values of 0.402μM and 0.37μM.

S. acmella is commonly known as toothache plant. The methanolic extract caused relaxation of spontaneous contractions in isolated rabbit jejunum preparations in a concentration-dependent manner. The extract was tested on K^+ (80mM)-induced contractions in isolated rabbit jejunum preparations to explore the possible mechanism of the observed relaxant activity on spontaneous contractions, because smooth muscles when exposed to K^+(80mM) are known to exhibit contractile response due to influx of extracellular Ca through opening of voltage dependent slow Ca channels. The

extract exerted a concentration-dependent relaxant effect on K^+ (80mM)-induced contractions, which may be due to blockade of Ca channels.

S. acmella has been traditionally used in the treatment of respiratory diseases. The methanolic extract was tested for its possible bronchodilator activity on carbachol and K^+ (80mM)-induced contractions in isolated rabbit tracheal preparations. The extract exerted a concentration-dependent relaxant effect on both of carbachol (1uM) and K(80mM)-induced contractions. The observed bronchodilator activity may possibly be mediated through blockade of Ca^{2+} channels. Interestingly, Ca^{2+}-channel blocking is known to be useful as tracheal relaxant in disorders characterized by hyper responsiveness of respiratory tract.

Moreover, the extract caused relaxation of both phenylephrine (1uM) and K^+ (80mM)-induced contractions in isolated rabbit aorta preparations in a concentration-dependent manner. Phenylephrine causes an increase in tone of vascular smooth muscles by an increase in Ca^{2+} influx via two means, that is, influx of Ca^{2+} via receptor operator channels and through release of intracellular Ca^{2+}. Hence, the observed relaxation of both phenylephrine (1uM) and K^+ (80mM)-induced contractions may be viewed as nonspecific vasodilator action likely to be mediated through the blockade of Ca^{2+} channels and Ca^{2+} channel blocking agents are being prescribed as vasodilators in management of cardiovascular disorders.

On the basis of above mentioned, this data contribute to validate the folkloric use of *S. acmella* for the management of gastrointestinal and respiratory and cardiovascular ailments.

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