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SPASMOLYTIC, BRONCHODILATORY AND ANTIOXIDANT ACTIVITIES OF *ERYTHRINA SUPEROSA* ROXB.

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Abstract: The present study was undertaken to explore the possible antioxidant, spasmolytic, bronchodilator and antioxidant activities of *Erythrina suberosa* Roxb. flowers. The crude aqueous methanolic extract of *Erythrina suberosa* Roxb. flowers (Es.Cr), on application to isolated rabbit jejunum preparations, caused concentration dependent relaxation of the spontaneous contractions as well as inhibition of K^+ (80 mM)-induced contractions, suggested that Es.Cr exhibited spasmolytic activity may possibly be mediated through Ca^{2+} channel blocking effect. This was confirmed further as Es.Cr treatment of the isolated rabbit jejunum preparations resulted in a rightward shift in the Ca^{2+} concentration-response curves in a manner similar to verapamil, a standard calcium channel blocker. Similarly, Es.Cr on application to isolated rabbit tracheal preparations; caused the concentration dependent relaxation of the carbachol (1 µM) and K^+ (80 mM)-induced contractions indicating its bronchodilator activity in a manner similar to verapamil. The crude methanolic extract of *Erythrina suberosa* exhibited antioxidant activity as manifested by strong scavenging activity on DPPH free radicals, whereas weaker scavenging activity was shown on NO free radicals in comparison with standard antioxidant quercetin, which is equally potent against both free radicals. It is concluded from this study that the crude aqueous methanolic extract of the flowers of *Erythrina suberosa* Roxb. possesses the antioxidant, spasmolytic and bronchodilator activities likely to be mediated through Ca^{2+} channel blocking mechanism.

Keywords: antioxidant, spasmolytic, bronchodilator, *Erythrina suberosa* Roxb., flowers

Erythrina suberosa Roxb. (Syn.: *Erythrina sticta*, Family: Fabaceae) is commonly known as Gul-e-nishtar (Urdu) and Coral tree (English). It is an ornamental tree, found in plains and hilly areas of India, Pakistan, Nepal and Bhutan (1, 2). It is medium sized deciduous tree with prickles on branches. The green, glabrous and broad (7.5–20 cm) leaflets; are matted with grey cottony pubescence on undersurfaces. The bright red flowers are axillary and terminal on the branches. The calyx is companulated to become 2-labiate. The upper stamens are free from the lower down. The pods are 12.5–15 cm long, terate and tapering at the ends. The black seeds are 4–5 in number (1, 3, 4).

In native systems of medicine, the roots are used as emmenagogue; whereas, the leaves are anthelmintic, cathartic, galactagogue and discutient. The fresh juice is used externally for dressing of ulcers and killing maggots in sores. The bark is diuretic, emmenagogue, expectorant, anthelmintic, spasmolytic, bronchodilator and anti-bilious. The bark decoction is used in the management of dysentery, worm infestation and as eye lotion in opthalmia. Flowers are used to reduce biliousness and ear troubles (2, 5).

The present study was undertaken to explore the possible antioxidant, spasmolytic, bronchodilator and antioxidant activities of *Erythrina suberosa* Roxb. flowers.

MATERIALS AND METHODS

Collection of plant materials

The flowers of *Erythrina suberosa* Roxb. were collected from residential area of Bahauddin Zakariya University, Multan, Pakistan in April 2010 and were identified by a kind cooperation of an expert taxonomist (Ms. Saima Shehzadi), Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan and a voucher specimen was deposited in herbarium (voucher no. P.FI 152).

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Preparation of crude extract

Subsequent to collection, the plant material was rendered free from any adulterated foreign material through manual picking and was subjected to shade drying on paper sheets in thin layers. These were also protected from exposure to intense bright sunlight to minimize the loss to constituents. The dried material was ground to coarse powder by an electric grinder specified for this task.

About 1 kg of dry powder was soaked in 3 liters of 70% aqueous methanol in amber colored glass bottle at 37°C for 7 days with occasional shaking. Then, material was first passed through muslin cloth and then filtered through Whatman no. 1 filter paper. The filtrate was concentrated in a rotary evaporator under reduced pressure at 40°C to dark brown material of thick syrup consistency with approximate yield of 13%. The extract was stored at 4°C to be used later on. It was dissolved in distilled water on the day of experiments to prepare the stock solution, which was diluted further for the purpose of investigations.

Drugs and chemicals

All the chemicals used in this research work were of the highest purity and of reagent analytical grade. Acetylcholine chloride, phenylephrine, atropine sulfate, carbachol, verapamil hydrochloride, potassium chloride and EDTA were purchased from Sigma Chemicals Co., St. Louis, MO, USA. Other chemicals: sodium chloride, sodium bicarbonate, potassium dihydrogen phosphate, sodium dihydrogen phosphate, glucose, calcium chloride, magnesium sulfate, magnesium chloride, hydrochloric acid, benzene, ethanol, acetic anhydride and diethyl ether were obtained from Merck, Darmastadt, Germany. Ammonium hydroxide, 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), quercetin, DMSO, sodium nitroprusside (SNP) solution and Griess reagent were from BDH Laboratory supplies, Poole, England. Stock solutions of chemicals were made in distilled water and were stored in refrigerator. The dilutions were made fresh on the day of experiment.

Experimental animals and their housing

Local breed albino rabbits (1–1.5 kg) of either sex were purchased from market and were housed in wooden cages being maintained at 23–25°C at the animal house of the Faculty of Pharmacy, Bahauddin Zakariya University, Multan. The animals were provided with fresh green fodder and water *ad libitum* but were deprived of food for hours prior to experiments. Rabbits were used for *in vitro* studies and were sacrificed following a blow on the back of the head. Experiments performed complied with the rulings of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council (1996) and adopted by Bahauddin Zakarya University, Multan.

Phytochemical analysis

Aqueous methanolic extract of *Erythrina suberosa* Roxb. flowers (Es.Cr) was subjected to phytochemical screening for the detection of saponins, tannins, phenols, coumarins, alkaloids and anthraquinones as possible constituents of the plant. The saponin presence was detected by the froth formation on vigorous shaking of the aqueous extract solution. Development of blue green or dark green coloration on mixing of aqueous $FeCl_3$ with extract solution indicated the presence of phenols and tannins. The coumarins as plant constituents were detected on emission of fluorescence in UV light from pieces of filter paper which were exposed to the vapors emerging from boiling aqueous solution of plant extract, subsequent to treatment with NaOH. The alkaloid presence was noted by the appearance of yellowish brown coloration on mixing of Dragendorff's reagent with HCl treated aqueous plant extract solution. The appearance of pink, violet or red coloration on exposure to NH_4OH of the mixture of benzene with aqueous solution of plant extract already acidified with 1% HCl was taken as the presence of anthraquinones among the plant constituents.

***In vitro* experiments**

Isolated rabbit jejunum preparations

The spasmolytic activity of the test material was assessed on spontaneously contracting isolated rabbit jejunum preparations (6, 7).

Subsequent to rabbit sacrifice, the rabbit abdomen was incised to extract the jejunum and 2 cm long pieces were cut and cleaned free of mesenteries and fecal masses. These were mounted in 15 mL tissue organ baths containing Tyrode's solution being maintained at 37°C and aerated with carbogen (mixture of 95% O_2 + 5% CO_2). The tissue responses were recorded isotonically *via* transducers and Data Acquisition Power Lab (AD Instruments, Sydney, Australia) attached to a computer installed with Lab Chart Software Version 7.

A preload of 1 g was applied to isolated rabbit jejunum preparation and was maintained as such throughout the experiment. The tissue was allowed to be equilibrated for 30 min, with double change of Tyrode's solution. The crude methanolic extracts of the plant was applied to spontaneously contracting

isolated rabbit jejunum preparations to explore its possible spasmolytic activity.

Determination of Ca^{2+} channel blocking activity

The observed relaxant activity of test material on spontaneous contractions of isolated rabbit jejunum preparations was speculated to be due to blockade of Ca^{2+} channels. In an attempt to explore the possible mode of action, the isolated tissue preparations was depolarized by exposing to high K^+ (80 mM), resulting in appearance of a sustained contraction (8). Subsequently, the test materials were added in installments to tissue bath to obtain a dose dependent relaxant effect. The relaxant effect of test material on K^+ (80 mM)-induced tissue contractions was expressed as percent of the K^+-mediated contractile response.

The Ca^{2+} channel blocking activity of the test material was confirmed further as isolated rabbit jejunum was allowed to stabilize in normal Tyrode's solution, which was then replaced by Ca^{2+} free Tyrode's solution to which EDTA (0.037 g/L) was added. The tissue was allowed to rest for 30 min in order to remove Ca^{2+} from the tissues. The tissue was further exposed to EDTA containing solution which was Ca^{2+} free and K^+ rich of following composition (g/L): NaCl (5.72), KCl (3.72), $MgCl_2$(0.1), $NaHCO_3$ (1.00), NaH_2PO_4 (0.05), glucose (1.00) and EDTA (0.37). After an incubation period of 40 min, the control concentration response curves (CRCs) of Ca^{2+} were made by gradual increase of Ca^{2+} concentrations in tissue bath. The control CRCs of Ca^{2+} were usually found to be superimposable after two cycles. Subsequently, the tissues were pretreated with different concentrations of test material for 60 min and CRCs of Ca^{2+} were determined in the presence of different concentrations of the test material.

Isolated rabbit tracheal preparations

The bronchodilator effect of the test material was assessed by using isolated rabbit tracheal preparations. The trachea was dissected, cleaned free of the fatty tissues and kept in Krebs solution. The tracheal tube was cut into rings about 2–3 mm width and having about two cartilages each. Each ring was cut open into longitudinal direction on the ventral side opposite to the smooth muscle, forming a tracheal strip having smooth muscle in its centre sandwiched between two cartilaginous portions on the edges. This strip was then mounted in 15 mL tissue organ bath containing Krebs solution maintained at 37°C and aerated with carbogen gas. A preload tension of 2 g was applied to each tracheal tissue and was maintained throughout the experiment. The isolated rabbit tracheal preparation was allowed to be equilibrated for 1 h. Afterwards, the isolated tissue preparations was exposed to sub-maximal doses of carbachol (1.0 µM) and K^+ (80 mM) for stabilization with a dose interval of 45 min until a constant response was obtained. The test material was applied on the obtained sustained contractions for possible relaxant effect. The doses of the test materials were added in cumulative fashion and the produced isometric responses were recorded by using Isometric Transducer attached to the Power Lab.

Antioxidant activity

DPPH free radical scavenging activity

The stable 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) was used for the determination of antioxidant activity (9). Different concentrations of test compounds in respective solvents were added at an equal volume (10 µL) to 90 µL of methanolic DPPH (100 µM) in a total volume of 100 µL in 96-well plates. The contents were mixed and incubated at 37°C for 30 min. The absorbance was measured at 517 nm. Quercetin was used as standard antioxidant. The experiments were carried out in triplicates. The decrease in absorbance indicated the increased free radical scavenging activity as determined by the following formula:

$$\text{Percent scavenging activity} = [100 - (\text{Abs. of test compound} / \text{Abs. of control})] \times 100$$

NO free radical scavenging activity

The NO free radical scavenging activity was determined following slight modifications of the method reported by Marcocci et al. (10). Different concentrations of each test sample in methanol or DMSO (50 µL) were added per well in 96 well plate. Then, 150 µL of 5 mM fresh aqueous sodium nitroprusside (SNP) solution was added per well. The contents were mixed and incubated at 37°C for 2 h. After incubation, 50 µL of Griess reagent (1% sulfanilamide in 5% H_3PO_4 and aqueous 0.02% naphthylethylene diamine dihydrochloride mixed in equal volumes) was added. Absorbance of the chromophore formed after diazotization and coupling was read after 20 min at 540 nm using Synergy HT BioTek (USA) 96 well plate reader. Quercetin was run as a control during the assay. For colored samples, change in absorbance was corrected. Results are expressed as the mean ± SEM of three independent experiments and percentage inhibition was calculated by the following formula:

$$\text{Inhibition (\%)} = [100 - (\text{Abs. of test compound} / \text{Abs. of control})] \times 100$$

Statistical analysis

All the data were expressed as the mean ± standard error of the mean (SEM) and the median effec-

Table 1. Antioxidant activity of crude methanolic extract of *Erythrina suberosa*.

Sample	DPPH scavenging activity	NO scavenging activity
Extract (0.5 mg/mL)	89.15%	21.50%
Quercetin	96.46%	90.21%

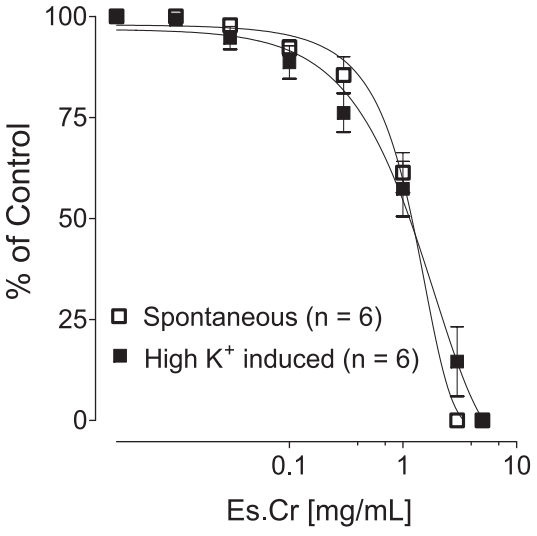

Figure 1. Concentration-dependent inhibitory effects of crude extract of the flowers of *Erythrina suberosa* (Es.Cr) on spontaneous and K^+ (80 mM)-induced contractions in isolated rabbit jejunum preparations. Values are shown as the means ± SEM, n = 6

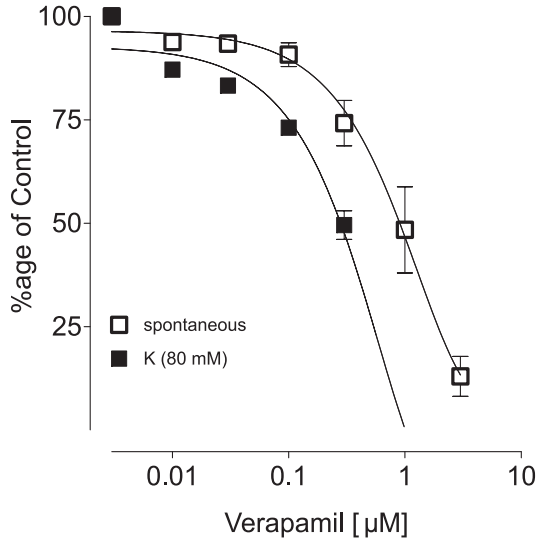

Figure 2. Concentration-dependent inhibitory effects of verapamil on spontaneous and K^+ (80 mM)-induced contractions in isolated rabbit jejunum preparations. Values are shown as the means ± SEM, n = 5

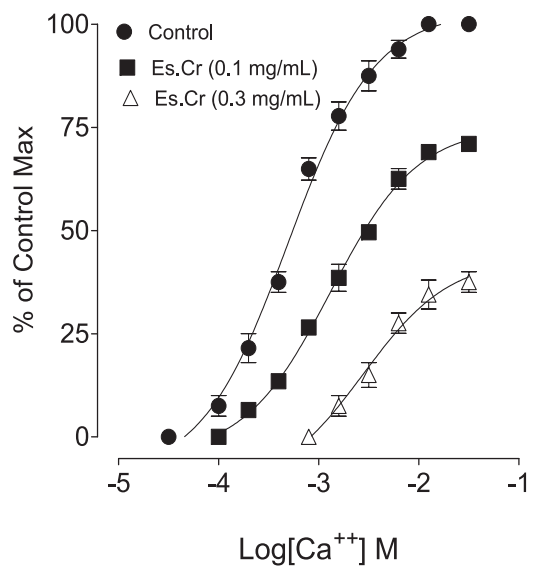

Figure 3. Concentration-response curve of Ca^{2+} in the absence and presence of increasing concentrations of crude extract of flowers of *Erythrina suberosa* (Es.Cr) in isolated rabbit jejunum preparations. Values are expressed as the means ± SEM, n = 5

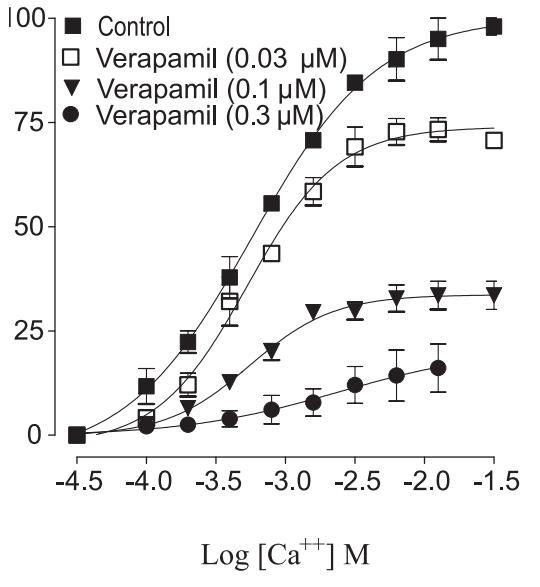

Figure 4. Concentration-response curve of Ca^{2+} in the absence and presence of increasing concentrations of verapamil in isolated rabbit jejunum preparations. Values are expressed as the means ± SEM, n = 5

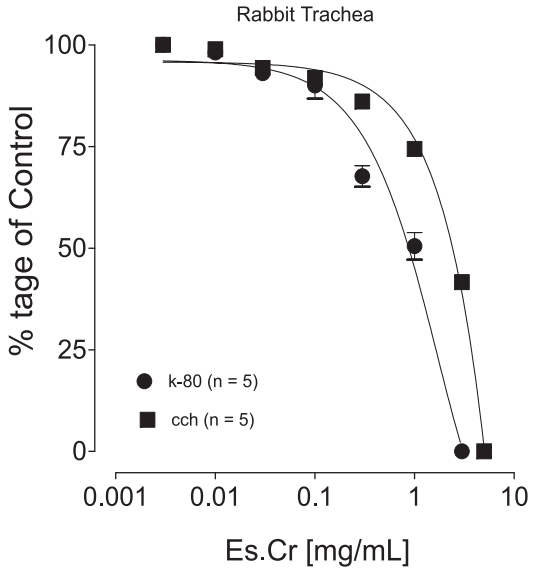

Figure 5. Concentration-dependent inhibitory effect of crude extract of flowers of *Erythrina suberosa* (Es.Cr) on high K^+ (80 mM) and carbachol (cch – 1 µM)-induced contraction in isolated rabbit tracheal preparations. Values are shown as the means ± SEM, n = 5

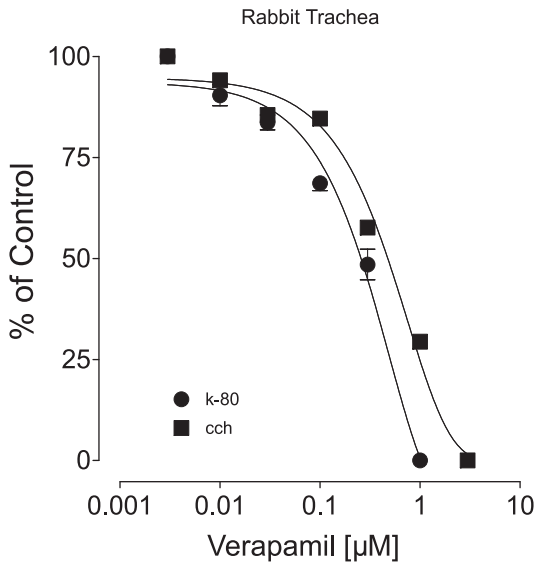

Figure 6. Concentration dependent inhibitory effect of verapamil on carbachol (cch – 1 µM) and K^+ (80 mM)-induced contractions of isolated rabbit tracheal preparations. Values are shown as the means ± SEM, n = 5

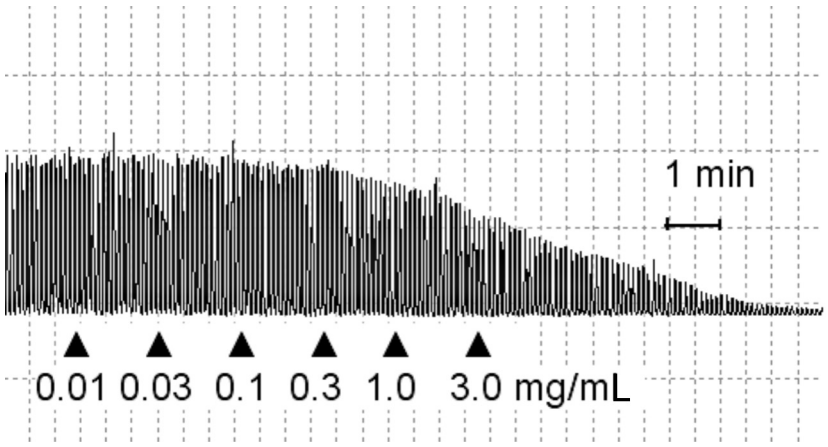

Figure 7. Determination of Ca^{2+} channel blocking activity

tive concentrations (EC_{50} values) are given with 95% confidence intervals (CI). The statistical parameter applied was the χ^2-test with $p < 0.05$ considered as significantly different.

RESULTS

Effect on isolated rabbit jejunum preparations

The Es.Cr caused inhibition of both spontaneous and K^+ (80 mM)-induced contraction in isolated rabbit jejunum preparations in a concentration-dependent (0.01–3.0 mg/mL) manner with EC_{50} value of 1.217 mg/mL (95% CI; 1.063–1.392; n = 5) and 1.184 mg/mL (95% CI; 0.8644–1.621; n = 5), respectively (Fig. 1). The relaxation of K^+ (80 mM)-induced contractions suggested that Es.Cr may possess calcium channel blocking activity. Verapamil, a standard calcium channel blocking agent, also relaxed both spontaneous and K^+ (80 mM)-induced contractions in a concentration dependent fashion

with EC_{50} values of 0.9680 µM (95% CI; 0.7484–1.252; n = 5) and 0.3064 µM (95% CI; 0.2278–0.4122; n = 5), respectively (Fig. 2). The Es.Cr caused the rightward shift of Ca^{2+} concentration-response curves at the concentration range of 0.03–0.1 mg/mL in a manner similar to verapamil (Fig. 3 and 4), thus, confirming the presence of Ca^{2+} channel blocking activity on the part of the crude methanolic extract of *Erythrina suberosa* Roxb.

Effect on isolated rabbit tracheal preparations

The Es.Cr caused relaxation of both K^+ (80 mM) and carbachol (1.0 µM)-induced contractions in a concentration-dependent manner with EC_{50} values of 0.9543 mg/mL (95% CI; 0.6929–1.314; n = 5) and of 2.685 mg/mL (95% CI; 2.355–3.062; n = 5), respectively (Fig. 5). Verapamil also caused relaxation of K^+ (80 mM) and carbachol (1 µM)-induced contraction with EC_{50} values of 0.26917 mg/mL (95% CI; 0.1738–0.3941; n = 5) and 0.4618 mg/mL (95% CI; 0.3157–0.6756; n = 5), respectively (Fig. 6), indicating that crude methanolic extract of *Erythrina suberosa* Roxb. exerted broncho-dilating properties likely to be mediated through Ca^{2+} channel blocking activity.

Antioxidant activity

The Es.Cr exhibited 89.15% scavenging of DPPH activity at a concentration of 0.5 mg/mL, whereas standard antioxidant quercetin demonstrated 96.46% activity at comparable concentrations. On the other hand, it showed 21.50% scavenging of NO free radicals as compared to quercetin, which exhibited 90.21% activity. Thus, Es.Cr demonstrated strong scavenging activity on DPPH free radicals and weak scavenging activity on NO free radicals (Table 1).

DISCUSSION

The crude methanolic extract of *Erythrina suberosa* (Roxb*)* flowers (Es.Cr) was tested for the presence of different groups of chemical constituents and it was found to contain tannins, anthraquinone, sterols, terpenes, flavonoids, saponins and phenolic compounds.

Erythrina suberosa (Roxb.) bark has traditionally been used as laxative, cathartic, anti-dysentery, spasmolytic and stomachic but presently, the crude methanolic extract of *Erythrina suberosa* flowers are subjected to evaluation for possible spasmolytic, bronchodilatory and antioxidant activities. The spasmolytic effect was evaluated on isolated rabbit jejunum preparations because it is a hyperactive spontaneous contracting preparation, which allows study of spasmolytic effects without use of any agonist (11). Application of crude methanolic flower extract on spontaneously contracting isolated rabbit jejunum preparation did not show any resulted in inhibition of the spontaneous contractions, thus showing an antispasmodic or spasmolytic effect. It has been observed that spasmolytic effect on the part of plant extracts is usually mediated through Ca^{2+} channel blocking mechanism (11–13). The contractile elements in smooth muscle preparations including rabbit jejunum are activated by increased cytoplasmic concentration of free Ca^{2+} (14, 15). The increase in intracellular Ca^{2+} levels is mediated through voltage dependent Ca^{2+} channels (VDCs) or release of Ca^{2+} from sarcoplasmic stores (16). The spontaneous movement of the intestine is regulated by the periodic depolarization and repolarization. In the state of maximal depolarization, the action potential is mediated through rapid influx of Ca^{2+} through VDCs (17).

Thus, the relaxant effect of the crude methanolic extract of *Erythrina suberosa* flowers seen on the hyperactive smooth muscle preparation is possibly mediated either through blockade of VDCs or through inhibition of Ca^{2+} released from sarcoplasmic stores.

It has been reported that a high dose of K^+ (> 30 mM) causes the opening of VDCs, causing an influx of extracellular Ca^{2+}, which results in contraction of smooth muscle (16, 18) and the substances which inhibits K^+-induced contractions are considered as Ca^{+2} channel blocker (CCB). To demonstrate whether observed antispasmodic or spasmolytic effects of Es.Cr are also mediated *via* similar mechanisms, the extract was tested on high K^+-induced contractions. High tissue bath concentration of K^+ (80 mM) was applied to cause depolarization in tissue and addition of Es.Cr into the tissue bath in a cumulative fashion (19) caused concentration dependent inhibition of K^+-induced contractions in isolated rabbit jejunum preparations. These findings were confirmed further, following treatment of isolated rabbit jejunum preparations with the extract and resulting in a decrease in tissue response to $CaCl_2$ and rightward shift of the Ca^{2+} concentration response curves in a manner similar to a standard CCB (verapamil) (20, 21).

The observed effect of crude extract of Es.Cr to inhibit K^+-induced contractions, followed by displacing effect of high concentrations of Ca^{2+}, suggests the presence of Ca^{2+} channel blocking activity of this plant, therefore, the speculation of possible involvement of Ca^{2+} influx antagonistic mechanism is confirmed. The

plant extract has similar pattern of inhibitory effect with comparable potency against spontaneous and K^+-induced contractions, while verapamil, a standard Ca^{2+} antagonist, was relatively selective in its inhibitory effect on K^+-induced contraction, a typical characteristic of Ca^{2+} antagonist. Verapamil causes dose dependent inhibition of calcium entry and its effect is reversed by Ca^{2+} (21) and similar reversal was observed in our experimental settings, which might explain the use of plant in hyperactive disease status of the gut. Traditionally, bark of *Erythrina suberosa* plant has been used in the treatment of abdominal disorder. The present study provides a scientific base of its use in such conditions because the Ca^{2+} channel blockers are known to be effective in hyperactive gut diseases (22).

Diverse plants are used in traditional medicine for respiratory tract disease, bronchitis, and cough. The bronchodilator potential of crude methanolic extract of *Erythrina suberosa* flowers was investigated on sustained contractions induced on addition of carbachol (1 µM) and high K^+ (80 mM) to the tissue baths containing isolated rabbit tracheal preparations. The cumulative addition of Es.Cr produced a concentration dependent relaxation of both CCh (1 µM) and K^+ (80 mM)-induced contractions, suggestive of bronchodilator effect mediated possibly through CCB. Many medicinal plants have shown bronchodilator activity on tracheal chain due to CCB. Interestingly, Ca^{+2} channel blockers are known to be useful as tracheal relaxant in disorders characterized by hyperresponsiveness of the respiratory tract (23, 24).

The crude methanolic extract of *Erythrina suberosa* flowers exhibited antioxidant activity as manifested by demonstration of strong scavenging activity on DPPH free radicals and weak scavenging effect on NO free radicals as compared to quercetin as standard antioxidant being equally effective against both free radicals.

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