April 2012

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J Nat Med (2012) 66:292–301
DOI 10.1007/s11418-011-0605-z

ORIGINAL PAPER

Pharmacological explanation for the medicinal use of *Juniperus excelsa* in hyperactive gastrointestinal and respiratory disorders

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Received: 8 July 2011 / Accepted: 2 September 2011 / Published online: 3 December 2011

Abstract Crude extract of *Juniperus excelsa* (JeExt), which tested positive for the presence of anthraquinone, flavonoids, saponins, sterols, terpenes and tannin, exhibited a protective effect against castor oil-induced diarrhoea in mice at 100–1000 mg/kg. In rabbit jejunum preparations, JeExt (0.01–1.0 mg/mL) caused relaxation of spontaneous and K^+ (80 mM)-induced contractions at similar concentrations to papaverine, whereas verapamil was relatively more potent against K^+. JeExt (0.03–0.3 mg/mL) shifted Ca^{2+} concentration–response curves to the right, like papaverine or verapamil. JeExt (0.003–0.01 mg/mL) caused a leftward shift of isoprenaline-induced inhibitory concentration–response curves, similar to papaverine. JeExt (1.0–30 mg/kg) caused suppression of carbachol (CCh, 100 μg/kg)-induced increase in inspiratory pressure of anaesthetized rats. In guinea-pig trachea, JeExt (0.001–3.0 mg/mL) relaxed CCh (1 μM)- and high K^+-induced contractions and shifted isoprenaline-induced inhibitory curves to the left. This study suggests that *Juniperus excelsa* possibly exhibits a combination of Ca^{2+} antagonist and phosphodiesterase inhibitory effects, which provides a pharmacological basis for its traditional use in disorders of gut and airways hyperactivity, such as diarrhoea, colic and asthma.

Keywords *Juniperus excelsa* · Ca^{2+} channel blocker · PDE inhibitor · Gut and airways disorders

Introduction

Juniperus excelsa Bieb. (Cupressaceae/Coniferae), commonly known as "pencil cedar/Juniper" and locally as "Dhup Guggal" is found throughout the eastern Mediterranean from northeastern Greece and southern Bulgaria across Turkey to Syria and the Caucasus mountains at an altitude of 2000–4000 m. It also occurs in Alborz and other mountains of Iran, east to northwestern Pakistan and Oman [1, 2]. *Juniperus excelsa* is used in folk medicine to treat diarrhoea, abdominal spasm, asthma [3], fever, gonorrhoea, headache and leucorrhoea [4, 5] as well as being considered useful as an antihypertensive, diuretic, appetizer, carminative, stimulant, anticonvulsant and flavouring agent [6]. Phytochemical studies on the plant revealed the presence of (+)-cedrol, (+)-sabinene, (+)-limonene, menthene, terpinene-4-ol, α-cedrene, β-cedrene, *p*-cymene, β-phellandrene, α-copaene, muurolene, β-guaiene, guaiazulene [7], α-thujene, α-fenchene, camphene, α-phellandrene, γ-3-carene, α-terpinene, *trans*-ocimene, γ-terpinene, terpinolene, endo-fenchol, *cis*-pinene hydrate, α-campholenal, *trans*-pinocarveol, camphor, borneol, γ-terpineol, naphthalene, α-terpineaol, myrtenol, verbenone, *trans*-carveol, endo-fenchyl acetate, piperitone, bornyl acetate, carvacrol, β-cubebene, thujopsene α-cadinene, α-humulene, β-acoradiene, β-cadinene, γ-muurolene [8], toluene, tricyclene,

M. Khan was on leave from University of Malakand for the PhD study.

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thujene, pinene, camphene, triene cycloheptane 1,3,5-trismethylene, β-myrcene, *o*-allyl toluene, *m*-cymene, *d*,*l*-limonene, α-pinene oxide, α-terpinolene, 3-thujanone and α-campholene aldehyde [9].

Despite the fact that extensive phytochemical research has been carried out on *Juniperus excelsa,* reports related to pharmacological investigation are limited, only citing its antibacterial [10] and antifungal [11] activities. In the present research, we provide evidence that *Juniperus excelsa* exhibits antidiarrheal, antispasmodic and bronchodilatory activities, occurring via a combination of Ca^{2+} channel blockade and phosphodiesterase (PDE) inhibitory pathways, which explains the medicinal use of *Juniperus excelsa* in hyperactive gut and airways disorders such as diarrhea, colic and asthma.

Materials and methods

Plant material and extraction

The aerial parts (stem + leaves) of *Juniperus excelsa* were collected from northern areas of Pakistan (Chitral) in September 2007. The plant was identified with the help of a taxonomist, Dr. Ilyas Iqbal, Department of Botany, University of Malakand, KPK, Pakistan. A voucher specimen (UOM/BGH/150) has been submitted to the herbarium of the same university. Plant material was cleaned, shade-dried and coarsely ground. The powdered material (580.27 g) was soaked in aqueous methanol (70%) at room temperature (25 ± 2.0°C) for 3 days with occasional shaking. It was filtered through muslin cloth and then through Whatman qualitative grade 1 filter paper [12]. The procedure of maceration and filtration was repeated twice more. All the filtrates were combined and evaporated to dryness in a rotary evaporator under reduced pressure (−760 mmHg) at 35–40°C to obtain crude extract of *Juniperus excelsa* (JeExt), yielding approx. 24%. JeExt was dissolved in normal saline/distilled water for use in in-vivo and in-vitro experiments.

Chemicals

Acetylcholine chloride (ACh), carbachol (CCh), isoprenaline, loperamide, papaverine and verapamil were purchased from Sigma Chemical Co., St Louis, MO, USA. Aminophylline, pentothal sodium (thiopental) and castor oil were obtained from GlaxoSmithKline, Abbott Laboratories and KCL Pharma, Karachi, Pakistan, respectively. Chemicals used for making physiological salt solutions were: potassium chloride (Sigma), calcium chloride, glucose, magnesium chloride, magnesium sulfate, potassium dihydrogen phosphate, sodium bicarbonate, sodium dihydrogen phosphate (Merck, Darmstadt, Germany) and sodium chloride (BDH Laboratory Supplies, Poole, UK). The chemicals used in phytochemical analysis include: acetic anhydride, aluminum chloride, ammonium hydroxide, ferric chloride (Sigma), benzene, chloroform, hydrochloric acid and petroleum ether (BDH). All chemicals used were of analytical grade and dissolved in distilled H_2O/saline.

Phytochemical screening

Preliminary investigation of the plant extracts for the presence of various phytochemical classes, such as saponins, coumarins, sterols, terpenes, flavonoids, anthraquinones and tannins was done according to reported methods [13]. The presence of saponins was detected based on the appearance of froth upon vigorous shaking of diluted samples. The observation of yellow fluorescence under ultraviolet light on examination of filter paper previously exposed to the vapours from boiling plant material indicated coumarins. For the detection of sterols and terpenes, plant material was treated with petroleum ether and subsequently extracted with chloroform. The appearance of green to pink (for sterols) and pink to purple colours (for terpenes) was then noted after treatment of the chloroform layer with acetic anhydride and concentrated HCl in succession. Plant material was noted as positive for flavonoids when it gave a yellow colour with aluminum chloride reagent, and for tannins when green or black colour was produced with aqueous ferric chloride. Lastly, for detecting anthraquinones, the extract was dissolved in 1% HCl, then in benzene, and observed if the extract showed a pink, violet or red colour with ammonium hydroxide.

Experimental animals

Rabbits (1–1.2 kg), guinea pigs (500–550 g), Sprague–Dawley rats (200–250 g) and BALB/c mice (20–25 g) of local breed and either sex were used for this study and were housed at the Animal House of the Aga Khan University, maintained at 23–25°C and given a standard diet and tap water. Rabbits starved for 24 h were killed by a blow to the back of the head, and guinea pigs by cervical dislocation. The experiments complied with the rules of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council [14] and were approved by the Ethical Committee of the Aga Khan University.

Castor oil-induced diarrhea

Mice were fasted for 24 h before the experiment. Animals were housed in individual cages and divided in five groups, each containing 10 mice. The first group received saline (10 mL/kg, orally) and served as a negative control. The

doses of the test extract were selected on a trial basis and three increasing doses of extract, 100, 300 and 1000 mg/kg, were given orally to three different groups. One group of mice was treated with loperamide (10 mg/kg, orally) as a positive control. One hour after treatment, each animal received 10 mL/kg of castor oil orally through a feeding needle. Afterward, the cages were inspected for the presence of diarrhoea droppings; their absence was noted as a positive result, indicating protection from diarrhoea at that time [15].

Rabbit jejunum

The rabbit abdomen was opened and the jejunum was dissected out, kept in normal Tyrode's solution and cleaned of mesenteries [16]. Each segment of about 2 cm length was suspended in a 10 mL tissue bath containing Tyrode's solution (pH 7.4), maintained at 37°C and aerated with a mixture of 95% O_2 and 5% CO_2 (carbogen). The composition of Tyrode's solution was (in mM): NaCl: 136.9, KCl: 2.7, $MgCl_2{\cdot}6H_2O$: 0.5, $NaHCO_3$: 11.9, $NaH_2PO_4{\cdot}2H_2O$: 0.32, $CaCl_2$: 1.8, and glucose: 5.05. One end of the segment was attached to a metal tissue hook and the other was attached by a cotton thread to an isotonic Bioscience transducer, connected to a Student oscillograph (Harvard Apparatus, Holliston, MA, USA). An initial load of 1 g was applied to each tissue and was allowed to equilibrate for 30 min before the addition of any drug. Following the equilibration period, each preparation was then stabilized with a sub-maximal concentration of ACh (0.3 μM) at 3 min intervals until constant responses were recorded. The inhibitory effects of test substances were measured as the percent change in spontaneous contractions of the jejunum. For the determination of the Ca^{2+} channel blockade effect, high K^+ (80 mM) was used to depolarize the preparations, as described by Farre et al. [17]. High K^+ (>30 mM) is known to cause smooth muscle contractions through the opening of voltage-dependent Ca^{2+} channels, thus allowing influx of extracellular Ca^{2+} causing a contractile effect; a substance causing inhibition of high K^+-induced contraction is considered to be a blocker of Ca^{2+} influx through L-type Ca^{2+} channels [18]. Once the induced contraction achieved a plateau (usually within 7–10 min), the test material was then added in a cumulative fashion to obtain concentration-dependent inhibitory responses. To confirm the Ca^{2+} antagonist action of the test substance, the tissue was allowed to stabilize in normal Tyrode's solution, which was then replaced with Ca^{2+}-free Tyrode's 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 Tyrode's solution, having the composition (in mM): NaCl: 91.03, KCl: 50, $MgCl_2{\cdot}6H_2O$: 0.50, $NaHCO_3$: 11.9, $NaH_2PO_4{\cdot}2H_2O$: 0.32, glucose: 5.05, and EDTA-$Na_2{\cdot}2H_2O$: 0.1. Following an incubation period of 30 min, control concentration–response curves of Ca^{2+} were obtained. When the control Ca^{2+} concentration–response curves were found to be superimposable (usually after two cycles), the tissue was pretreated with the test drug for 1 h. The concentration–response curves of Ca^{2+} were reconstructed in the presence of different concentrations of the test material to observe the Ca^{2+} antagonist effect. The PDE inhibitory effect was studied indirectly by constructing isoprenaline-induced inhibitory concentration–response curves against CCh-induced contractions in the absence and presence of the test substance, as PDE-inhibitors are known to potentiate the effect of isoprenaline [19, 20].

Bronchodilatory activity

Rats were anaesthetized with sodium thiopental (Pentothal, 80–100 mg/kg, intraperitoneally), then incubated with a tracheal tube and ventilated 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 [15]. A polyethylene catheter was inserted into the jugular vein for drug administration. Changes in airways resistance (mmHg) were measured by a pressure transducer (MLT-1199) connected to the side arm of tracheal cannula and recorded by PowerLab 4/25 with running chart software via a Quad bridge amplifier (ADInstruments, 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 administration of CCh. The responses were expressed as the percent reduction of the CCh-induced bronchospasm.

Guinea pig trachea

Trachea was dissected from guinea pigs and kept in normal Kreb's solution. The tracheal tube was cut into rings, 2–3 mm wide, each containing about two cartilages. Each ring was opened by a longitudinal cut on the ventral side opposite the smooth muscle, forming a strip with smooth muscle in the centre and cartilaginous portions on the edges [21]. The preparation was mounted in a 20 mL tissue bath containing Kreb's solution (pH 7.4) at 37°C and aerated with carbogen. The composition of Kreb's solution was (in mM): NaCl: 118.2, $NaHCO_3$: 25.0, $CaCl_2$: 2.5, KCl: 4.7, KH_2PO_4: 1.2, $MgSO_4{\cdot}7H_2O$: 1.2, and glucose: 11.7. A tension of 1 g was applied to tracheal strips continuously throughout the experiment. The tissues were allowed to equilibrate for 1 h before the addition of any drug. CCh (1 μM) was used for the stabilization. When sustained contractions were obtained in

the preparations using the spasmogens, such as CCh and/or K^+, the relaxant effect of the test substance was assessed by adding it in a cumulative fashion. As in the jejunum, isoprenaline concentration–response curves were constructed in trachea as described previously [22]. Isometric responses were recorded on a Grass model 7 Polygraph (Grass Instrument Company, Quincy, MA, USA).

Acute toxicity test

Mice were divided into groups of five mice each. The test was performed using increasing doses of the plant extract, given orally in 10 mL/kg volume to different test groups. Another group of mice was administered saline (10 mL/kg, orally) as negative control. The mice were allowed food ad libitum and kept under regular observation for lethality recorded after 24 h.

Statistical analysis

The data expressed are mean ± standard error of mean (SEM, n = number of experiment) and median effective concentrations (EC_{50}) with 95% confidence intervals. The statistical parameters applied were the chi-squared test for antidiarrhoeal assay, one-way analysis of variance followed by Dunnett's test for bronchodilatory activity, and Student's t test to compare the curves of the in-vitro experiment results. $P < 0.05$ was considered statistically significant. Concentration–response curves were analyzed by non-linear regression using GraphPad software (GraphPAD, San Diego, CA, USA).

Results

Phytochemical analysis

JeExt was found to contain anthraquinone, flavonoids, saponins, sterols, terpenes and tannin.

Effect on castor oil-induced diarrhea

JeExt exhibited a dose-dependent (100–1000 mg/kg) protective effect against castor oil-induced diarrhoea in mice. The negative control group (saline-treated) did not show any protection against castor oil-induced diarrhea. Pretreatment of animals with the plant extract showed 50% protection from diarrhoea at 100, 70% at 300 and 80% protection at 1000 mg/kg ($P < 0.05$ vs. saline group at each dose). Loperamide (10 mg/kg) showed 100% protection from diarrhoea ($P < 0.01$ vs. saline group) in the positive control group (Table 1).

Effect on jejunum

The plant extract, papaverine and verapamil all caused concentration-dependent inhibition of spontaneous contractions with varying potencies (Fig. 1). Figure 2 shows the comparative inhibitory effect against spontaneous and K^+ (80 mM)-induced contractions. JeExt was found to be equally effective against spontaneous and K^+ (80 mM)-induced contractions ($P > 0.05$) with EC_{50} values of 0.047 (0.04–0.08, $n = 7$) and 0.051 mg/mL (0.04–0.07, $n = 6$), respectively, as shown in Fig. 2a. Papaverine also showed a similar pattern of non-specific inhibitory response

Table 1 Effect of the crude extract of *Juniperus excelsa* (*JeExt*) on castor oil (*C. oil*, 10 mL/kg)-induced diarrhoea in mice

Treatment (mg/kg, orally)	No. of mice with diarrhea (n = 10)	Protection (%)
Saline (10) + C. oil	10	0
JeExt (100) + C. oil	5*	50
JeExt (300) + C. oil	3*	70
JeExt (1000) + C. oil	2*	80
Loperamide (10) + C. oil	0**	100

* $P < 0.05$, ** $P < 0.01$ compared to saline group, chi-squared test

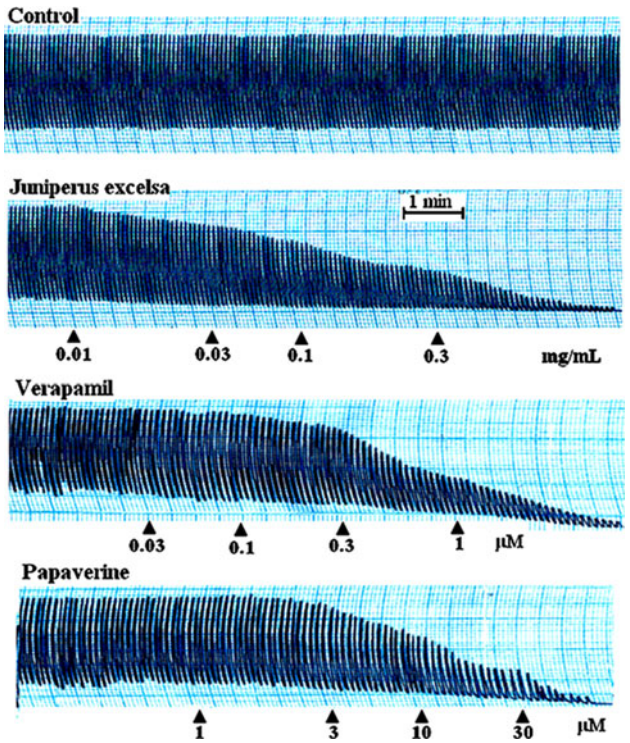

Fig. 1 Tracings showing comparative spasmolytic effect of the crude extract of *Juniperus excelsa*, papaverine and verapamil on spontaneously contracting isolated rabbit jejunum preparations. Test materials were added into the tissue bath in a cumulative fashion and the concentrations shown are the final bath concentrations

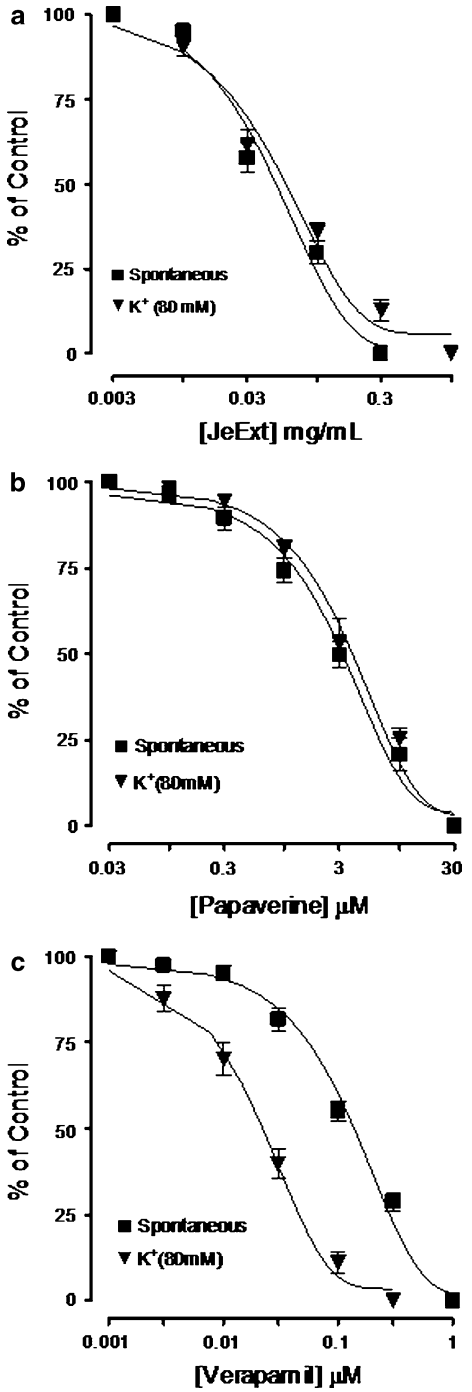

Fig. 2 Concentration-dependent inhibitory effect on spontaneous and K^+ (80 mM)-induced contractions of **a** crude extract of *Juniperus excelsa* (JeExt), **b** papaverine and **c** verapamil in isolated rabbit jejunum preparations. Values shown are mean ± SEM (n = 3–7)

(Fig. 2b) with EC_{50} values of 3.05 (2.7–4.3, n = 4) and 3.35 µM (3.1–4.6, n = 4), respectively, whereas verapamil was found to be more potent against K^+ (80 mM)-induced contractions ($P < 0.05$) with an EC_{50} value of 0.027 µM (0.02–0.04, n = 3) as compared to spontaneous contractions [0.14 µM (0.11–0.21, n = 3)], as shown in Fig. 2c.

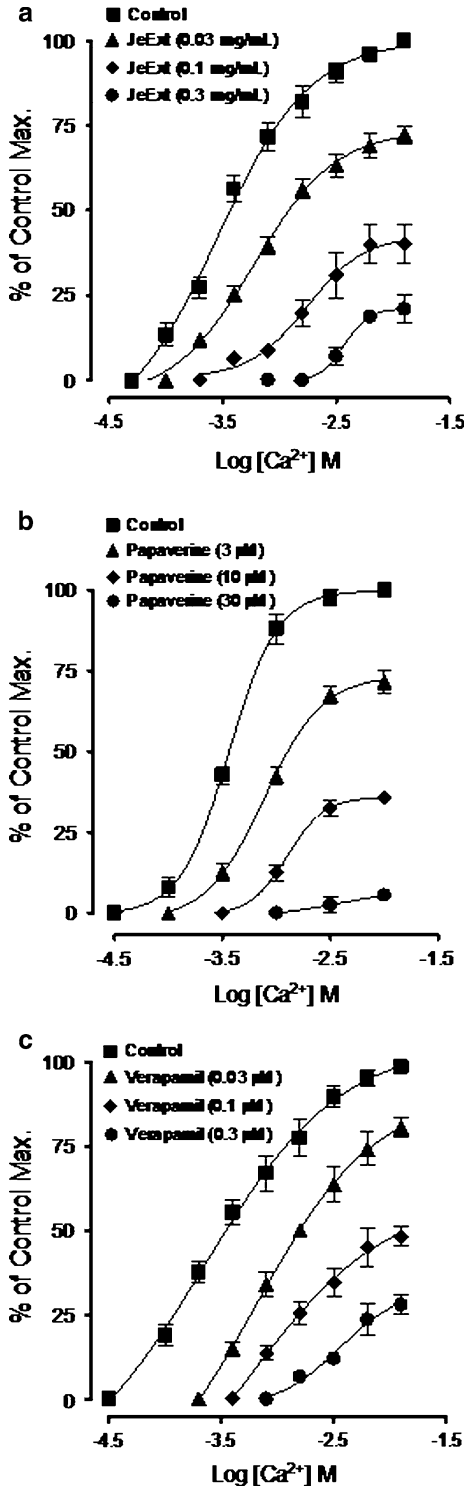

Fig. 3 Concentration–response curves of Ca^{2+} in the absence and presence of the increasing concentrations of **a** crude extract of *Juniperus excelsa* (JeExt), **b** papaverine and **c** verapamil in isolated rabbit jejunum preparations. Values shown are mean ± SEM (n = 3–6)

Pretreatment of tissue with JeExt (0.03–0.3 mg/mL) caused a rightward shift in the Ca^{2+} concentration–response curves accompanied by suppression of the maximum contractile

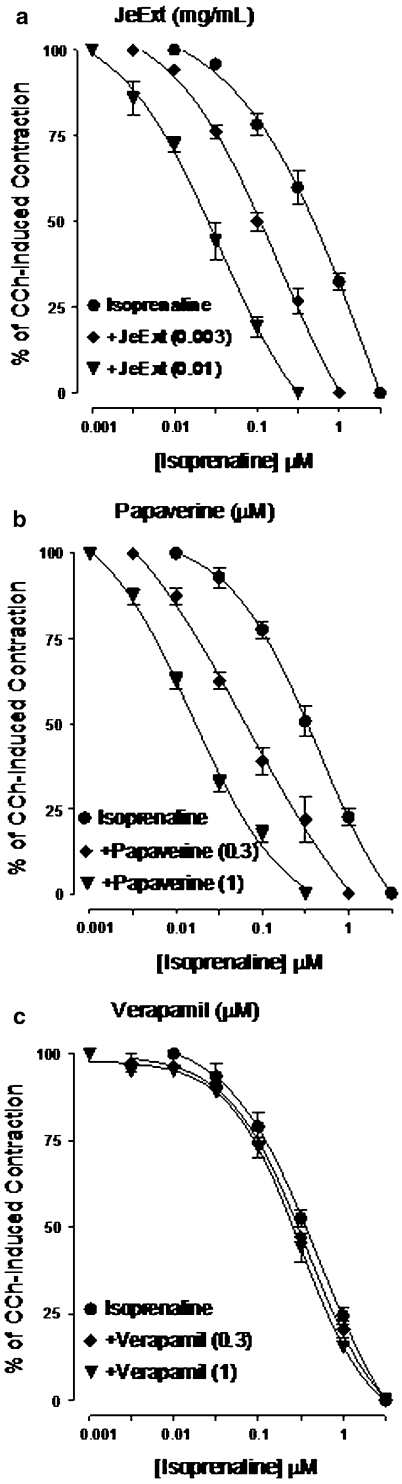

Fig. 4 Inhibitory concentration–response curves of isoprenaline against carbachol (CCh)-induced contractions in the absence and presence of different concentrations of **a** crude extract of *Juniperus excelsa* (JeExt), **b** papaverine and **c** verapamil in isolated rabbit jejunum preparations. Values shown are mean ± SEM ($n = 3$–4). The curves obtained by pretreatment of tissues with JeExt and papaverine are significantly different from the respective isoprenaline control curves ($P < 0.05$), while that obtained in the presence of verapamil is not significantly different ($P > 0.05$), Student's t test

effect (Fig. 3a), similar to that caused by papaverine (3–30 μM; Fig. 3b) and verapamil (0.03–0.3 μM), as shown in Fig. 3c. When tested for possible interaction with isoprenaline, pretreatment of the tissues with JeExt (0.003–0.01 mg/mL) shifted the isoprenaline-induced inhibitory concentration–response curves to the left, showing a potentiating effect (Fig. 4a). Papaverine caused a similar concentration-dependent (0.3–1.0 μM) leftward shift in the concentration–response curves of isoprenaline (Fig. 4b), while verapamil (0.3–1.0 μM) did not alter the inhibitory response to isoprenaline (Fig. 4c).

Effect on carbachol-induced bronchoconstriction

JeExt at doses of 1, 3, 10 and 30 mg/kg caused 3.4 ± 1.2, 10.2 ± 1.1, 25.7 ± 5.9 and 45.14 ± 6.5% ($n = 5$–7), respectively, suppression of CCh (100 μg/kg)-induced increase in inspiratory pressure of anaesthetized rats (Fig. 5a). Aminophylline was used as a positive control, and inhibited the CCh (100 μg/kg)-mediated bronchoconstriction at 1, 3, 10 and 30 mg/kg by 8.0 ± 4.4, 19.3 ± 7.4, 28.0 ± 9.2 and 53.2 ± 11.8% ($n = 8$), respectively (Fig. 5b).

Effect on trachea

JeExt, papaverine and verapamil were all found devoid of any stimulant action when screened on the tracheal resting baseline. When tested against CCh (1 μM)- and K^+ (80 mM)-induced contractions, JeExt caused concentration-dependent inhibition with EC_{50} values of 0.04 (0.03–0.14, $n = 4$) and 0.21 mg/mL (0.18–0.30, $n = 4$), respectively, as shown in Fig. 6a. Similarly, papaverine had an inhibitory effect against CCh (1 μM)- and K^+ (80 mM)-induced contractions (Fig. 6b) with EC_{50} values of 0.82 (0.61–0.93, $n = 5$) and 2.4 μM (1.7–3.4, $n = 5$), respectively. Verapamil was found to be more potent in its inhibitory effect against K^+ (80 mM)-induced contractions with an EC_{50} value of 0.03 μM (0.02–0.04, $n = 4$) compared to CCh-induced contractions [0.87 μM (0.53–1.44, $n = 3$)], as shown in Fig. 6c. Pretreatment of tracheal preparations with JeExt shifted the isoprenaline-induced inhibitory concentration–response curves to the left (Fig. 7a) in a concentration-dependent manner (0.03–0.3 mg/mL), similar to that caused by papaverine (1.0–10 μM), showing a potentiating effect (Fig. 7b), while verapamil (0.03–0.3 μM) did not alter the inhibitory response to isoprenaline (Fig. 7c).

Acute toxicity

The three different groups of mice were given JeExt in the graded doses of 1, 5 and 10 g/kg, respectively, and the

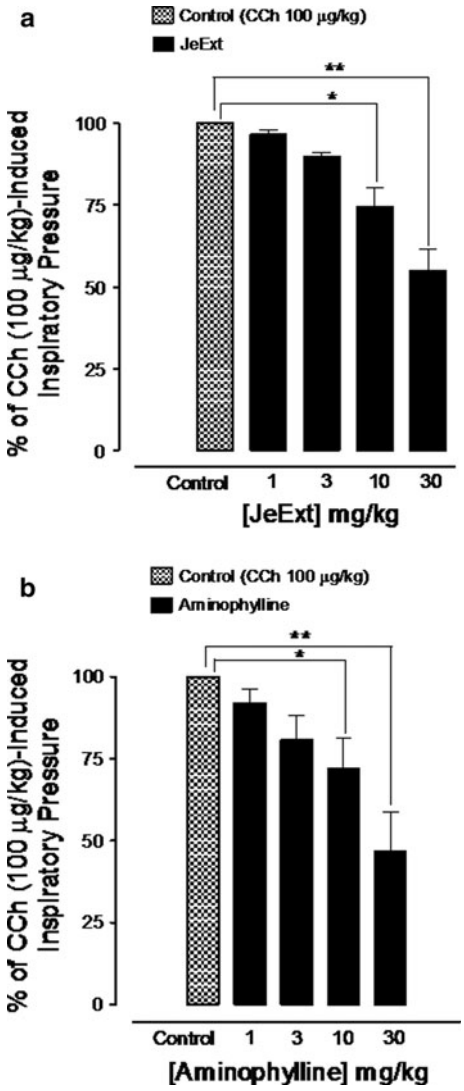

Fig. 5 Dose-dependent effect of **a** crude extract of *Juniperus excelsa* (JeExt) and **b** aminophylline on carbachol (CCh)-mediated bronchoconstriction in anaesthetized rats. Values shown are mean ± SEM ($n = 5$–8), $^{*}P < 0.05$, $^{**}P < 0.01$ versus control (carbachol), one-way analysis of variance, followed by Dunnett's test

animals were observed for mortality after 24 h of drug administration. The extract did not cause any mortality up to the dose of 10 g/kg.

Discussion

In view of the traditional use of *Juniperus excelsa* in hyperactive gut disorders, such as diarrhoea, its extract was evaluated for possible antidiarrhoeal action in mice and the underlying pharmacological mechanism was elucidated using isolated intestinal tissue. In the castor oil-induced diarrhoea model, *Juniperus excelsa* extract showed a protective effect in a dose-dependent fashion, similar to that

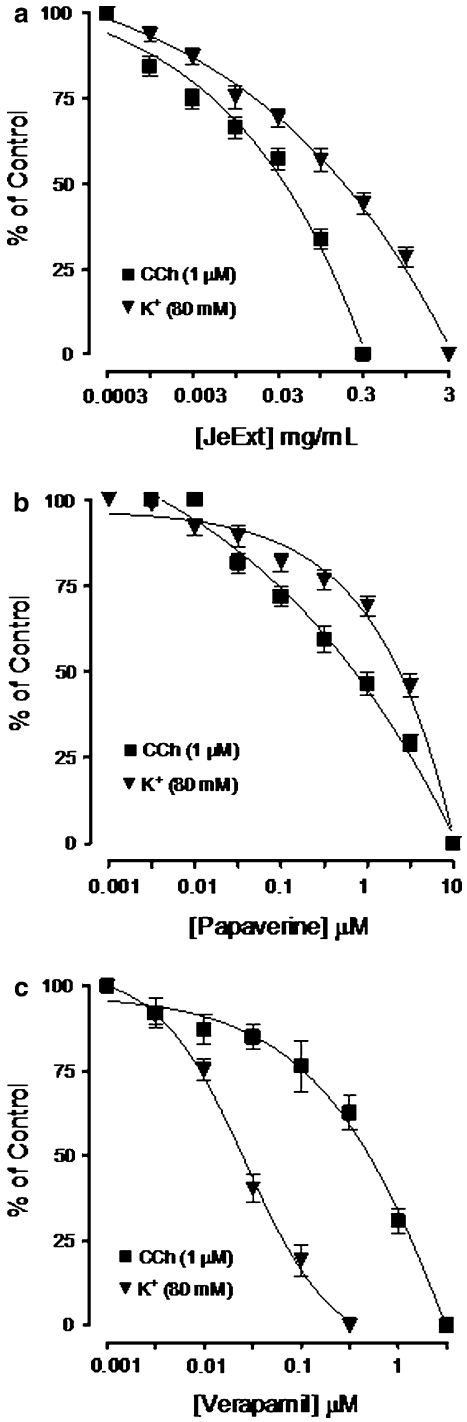

Fig. 6 Concentration–response curves showing effect of **a** crude extract of *Juniperus excelsa* (JeExt), **b** papaverine, and **c** verapamil on carbachol (CCh)- and K^{+}-induced contractions in isolated guinea-pig tracheal preparations. Values shown are mean ± SEM ($n = 3$–5)

caused by loperamide, a standard antidiarrhoeal drug [23]. Castor oil induces diarrhoea as a result of the action of ricinoleic acid formed during the hydrolysis of oil, which produces changes in the transport of electrolytes and water, leading to the generation of massive contractions of the

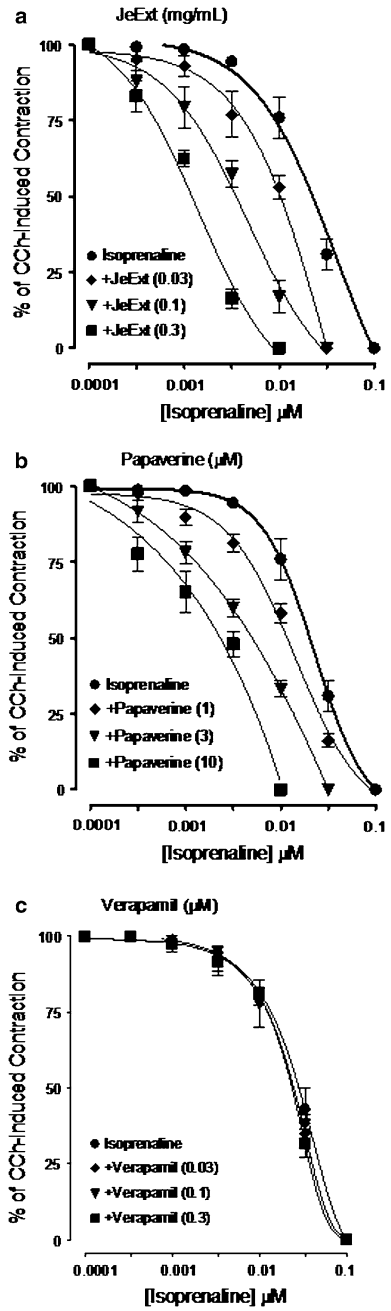

Fig. 7 Inhibitory concentration–response curves of isoprenaline against carbachol (CCh)-induced contractions in the absence and presence of different concentrations of **a** crude extract of *Juniperus excelsa* (JeExt), **b** papaverine and **c** verapamil in isolated guinea-pig tracheal preparations. Values shown are mean ± SEM ($n = 4$–5). The curves obtained by pretreatment of tissues with JeExt and papaverine are significantly different from the respective isoprenaline control curves ($P < 0.05$), while that obtained in the presence of verapamil is not significantly different ($P > 0.05$), Student's t test

intestine [24]. Thus a potential antidiarrhoeal agent may exhibit its antidiarrhoeal effect by inhibiting bowel contractions. The antidiarrhoeal activity of JeExt following oral administration appears to be due to the presence of gastrointestinal relaxant component(s) in *Juniperus excelsa.*

To study the possible mechanism of the gut inhibitory effect, we used isolated rabbit jejunum preparation, which is known to exhibit spontaneous rhythmic contractions, thus allowing the testing of relaxant (antispasmodic) activity directly without use of an agonist [25]. In jejunum, JeExt inhibited both spontaneous and high K^+-induced contractions with similar potency. Papaverine, PDE and Ca^{2+} influx inhibitor [26] caused similar patterns of inhibitory effect with comparable potency against spontaneous and K^+-induced contractions, while verapamil, a standard Ca^{2+} antagonist [27], was relatively selective in its inhibitory effect on the K^+-induced contractions, a typical characteristic of a Ca^{2+} channel blocker [16, 20]. The presence of a Ca^{2+} antagonist effect was further confirmed when pretreatment of the tissue with plant extract shifted the Ca^{2+} concentration–response curves to the right, similar to that caused by verapamil or papaverine. However, a similar inhibitory pattern of the plant extract against spontaneous and K^+-induced contractions, like that of papaverine and unlike that of verapamil, suggests that it may have additional mechanism(s) involved in the spasmolytic effect, such as PDE inhibition. The PDE inhibitory effect was confirmed when the extract potentiated the isoprenaline-induced relaxant effect, similar to that caused by papaverine, while verapamil was found to lack any such effect. However, the possibility of β_2-adrenergic receptor stimulation or adenyl cyclase activation mechanisms cannot be ignored. PDE inhibitors increase the intracellular level of cyclic adenosine monophosphate through inhibition of PDE, considered to be a relaxant in the smooth muscles and a stimulant in heart [28]. The observed antidiarrhoeal and antispasmodic effects of *Juniperus excelsa* justify its traditional use in diarrhoea and colic and are in accordance with expectation, as both Ca^{2+} antagonists and PDE inhibitors are known to possess antidiarrhoeal and antispasmodic activities [23, 25, 29].

Based on the folkloric reputation of *Juniperus excelsa* application in asthma, it was tested for a possible bronchodilatory effect in anaesthetized rats. JeExt dose-dependently suppressed CCh-evoked bronchospasm, similar to aminophylline (soluble salt of theophylline), a well-known bronchodilator [30]. *Juniperus excelsa* was further studied in isolated tracheal preparations to explore the pharmacodynamics of its brochodilatory effect. The plant extract, papaverine and verapamil all caused relaxation of trachea precontracted with CCh and high K^+. Unlike the plant extract and papaverine, verapamil was found to be comparatively more potent against high K^+- than against CCh-induced contractions, as expected from Ca^{2+} antagonists [31]. Pretreatment of tracheal tissues with the plant extract shifted the isoprenaline-induced inhibitory concentration–response curves to the left, similar to that observed in gut

preparations, indicating the presence of additional PDE inhibitory bronchodilatory substance(s) in *Juniperus excelsa*. Interestingly, the PDE inhibitory effect of the plant extract in jejunum was observed at lower concentrations to that in tracheal preparation. The possible explanation could be localization of different PDE subfamilies in the two physiological systems [32–34], though species difference cannot be ruled out. The usefulness of PDE inhibitors in asthma is well established [35, 36], though the major limitation is cardiac stimulation as a side-effect [37]. Interestingly, Ca^{2+} antagonists have also been shown to be useful in bronchoconstriction [38] and are known to exhibit a cardiosuppressant effect [39]. The co-existence of Ca^{2+} channel blocker constituents with PDE inhibitor(s) in *Juniperus excelsa* is perhaps meant by nature to offset the tachycardia associated with PDE inhibitors when used alone. This finding strengthens the concept that natural remedies known to possess synergistic and/or side-effect-neutralizing potential in addition to cost-effectiveness offer merit in evidence-based studies [40]. Thus the presence of the combined inhibitory effect on PDE and Ca^{2+} channels might account for the medicinal use of *Juniperus excelsa* in airways hyperactivity and asthma.

The presence of phyotchemicals, anthraquinone, flavonoids, saponins, sterols, terpenes and tannins may be partly responsible for the reported pharmacological effects of *Juniperus excelsa.* However, further in-depth studies are required to probe the nature of its chemical constituents and the molecular basis of its biological activities. In the acute toxicity study, JeExt was found to be safe up to the dose of 10 mg/kg, which indicate a safety profile of the plant at a wide dose range.

In conclusion, these results reveals that the crude extract of *Juniperus excelsa* possesses antidiarrhoeal, antispasmodic and bronchodilatory effects, mediated through a combination of spasmolytic mechanisms, such as inhibition of Ca^{2+} influx and PDE enzyme(s). Thus, this study provides a sound mechanistic background to the medicinal use of *Juniperus excelsa* in hyperactive gut and airways disorders, like diarrhoea, abdominal spasm and asthma. Moreover, its in-vivo antidiarrhoeal and bronchodilatory activities prove the effectiveness of the plant in such conditions, which is a step forward towards the evidence-based medicinal use of phytomedicine.

Acknowledgments This study was partially supported by Higher Education Commission of Pakistan. Munasib Khan was on leave from University of Malakand for the PhD study.

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