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Original Article

Comparison of antispasmodic effect of hydroalcoholic extract of *Dracocephalum kotschyi* Boiss. in rat uterus and ileum

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Abstract

Dracocephalum kotschyi Boiss. is a traditional medicine with antispasmodic activities. The objective of this research was to study antispasmodic activities of hydroalcoholic extract of *D. kotschyi* on rat isolated uterus contractions for comparison with isolated ileum. Hydroalcoholic extract was obtained from aerial part of *D. kotschyi* using percolation method. A portion of rat ileum or uterus was suspended in Tyrode's solution at 37 °C and gassed with O_2. Effect of *D. kotschyi* extract was assessed on ileum or uterus contractions induced by KCl (80 mM), acetylcholine (ACh, 500 nM), electrical field stimulation (EFS) or oxytocin (0.0005 IU/mL). The extract of *D. kotschyi* concentration-dependently inhibited ileum responses to KCl (IC_{50} = 65 ± 18 μg/mL), ACh (IC_{50} = 102 ± 18 μg/mL) and EFS (IC_{50} = 117 ± 29 μg/mL). The extract of *D. kotschyi* also concentration-dependently inhibited uterus responses to KCl (IC_{50} = 453 ± 64μg/mL), ACh (IC_{50} = 58 ± 9 μg/mL), EFS (IC_{50} = 22 ± 3 μg/mL) as well as oxytocin (IC_{50} = 70 ± 11 μg/mL). From this experiment it was concluded that *D. kotschyi* extract possesses antispasmodic activities on both smooth muscle of ileum and uterus. In comparison, the extract was more effective inhibitor of ACh and EFS responses in rat uterus than on the ileum. On the other hand, the extract was a more potent inhibitor of KCl response on rat ileum. However, the extract was found to be a potent inhibitor of oxytocin-induced contraction of rat uterus. These results indicate that *D. kotschyi* extract may contain components that might be useful lead compounds for prevention of uterus spasm.

Keywords: *Dracocephalum kotschyi*; Extract; Antispasmodic; Ileum; Uterus

INTRODUCTION

Dracocephalum (dragonhead) is a genus of about 60 to 70 species of flowering plants in the family Lamiaceae (1,2,3). They are annual or perennial herbaceous plants or subshrubs, growing to 15 to 90 centimeters tall (4). Eight species of *Dracocephalum* including *D. kotschyi*, *D. aucheri*, *D. moldavica*, *D. multicaule*, *D. polychaetum*, *D. subcaitatum*, *D. surmandimum* and *D. thymifolrum* are found in Iran (1,5). In traditional medicine, these plant species are used as carminative and tonic as well as for treatment of ailment such as congestion, headache, stomachache and liver diseases (6,7).

D. kotschyi is an aromatic medicinal plant which grows in clammy climate of high mountainous parts of Iran (4). Pharmacological studies have confirmed some medicinal properties of *D. kotschyi* including antinociceptive, anti-inflammatory (8,9) antihyperlipidemic (10), immunomodulatory (11) and anticancer (12-14) effects. Extract of this species is used as antispasmodic remedy in Iranian traditional medicine (3). It has been reported that the essential oil of *D. kotschyi* had strong spasmolytic activities on isolated ileum (15). The main components found in the essential oil were α-pinene, neral, geraniol, α-citral, limonene, cyclononadiene, terpinene-4-ol, linalool, carveol, myrcene, germacrene–D, isopinocarveol and α-terpineol (15-17). *D. kotschyi* hydroalcoholic extract also possessed potent antispasmodic activities (18). The constituents of the hydroalcoholic extract has

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also been separated and identified. These include, calycopterin, xanthomicrol, isokaempferide, luteolin, apigenin, luteolin 7-O-beta-D-glucopyranoside, lutcolin 3'-O-beta-D-glucuronide, apigenin 4'-O-beta-D-gluco-pyranoside, acacetin 7-O-beta-D-gluco-pyranoside and rosmarinic acid (19,20). The *D. kotschyi* extract concentration-dependently reduces the contractile responses of isolated rat ileum to neuronal stimulation (IC_{50} = 96 ± 7.1 μg/mL), exogenous acetylcholine (IC_{50} = 101 ± 9.5 μg/mL) or high concentration of KCl (IC_{50} = 36 ± 5.1 μg/mL) (18).

As *D. Kotschyi* extract has a potent antispasmodic effect on smooth muscle of rat ileum, it may have a similar activities on other smooth muscles. So far there is no report on the effect of *D. Kotschyi* extract on uterine contraction. Therefore, the aim of current study was to examine the effect of *D. kotschyi* extract on rat uterus contraction for comparison with rat ileum using *in vitro* isolated tissue preparation.

METHODS AND MATERIALS

D. kotschyi aerial parts were collected from Fereydun-shahr (in Isfahan province, Iran) and identified at the Botany Department of the Faculty of Sciences, University of Isfahan. A voucher specimen (1519) was deposited at the herbarium of the School of Pharmacy and Pharmaceutical Sciences of Isfahan University of Medical Sciences.

The plant materials were dried in shadow and ground to powder using electrical miller (Moulinex, France). The extract was prepared by percolation (21). From 150 g dried plant materials, 53.7 g dried extract was obtained.

Drugs and solutions

Acetylcholine hydrochloride was obtained from Sigma Co. (Germany), 17-β-estradiol valorate and oxytocin were purchased from Aburihan Pharmaceutical Co. (Iran). Salbutamol was supplied by Neolab limited (UK). 17-β-estradiol was prepared in cooking oil as 100 μg/mL stock solution for subcutaneous injection. *D. kotschyi* extract was made up as 50 mg/mL stock solution in dimethyl sulphoxide (DMSO), and diluted with distilled water to obtain 5 mg/mL and 500 μg/mL solutions. Acetylcholine (ACh) was prepared as 100 mM stock solution and acidified by 1% acetic acid. Further serial dilution was made in distilled water. Oxytocin was made up in distilled water to give 1 IU/mL stock solution. KCl (2 M) stock solution was made up in distilled water. Tyrode's solution composed of NaCl, 136.9; KCl, 2.68; $CaCl_2$, 1.8; $MgCl_2$, 1.05; $NaHCO_3$, 11.9; NaH_2PO_4, 0.42 and glucose, 5.55, (in mM) was made up in distilled water. Unless stated, all chemicals were from Merck (Germany).

Isotonic force measurements of the ileum and myometrial strips

Non-pregnant adult female Wister rats (200-250g) were obtained from School of Pharmacy and Pharmaceutical Sciences animal house in Isfahan. All animal experiments were approved by the Ethics Committee of Isfahan University of Medical Science and performed in accordance with National Institute of Health Guide for the Care and Use of Laboratory Animals. (22). Uterine horns were obtained from rats pretreated 24 h earlier with 17-β-estradiol (100 μg/kg, s.c.). On the day of experiment, a rat was killed and abdominal cavity was immediately opened with surgical scissors. Both horns of the uterus and a piece of ileum were clipped off and immediately placed into oxygenated Tyrode's solution for transporting to the laboratory where the tissues were continuously aerated with oxygen. The tissues were freed from the mesenteric and fat attachments. Uterus horns were cut into longitudinal strips of approximately 1 cm length. The dissected ileum was cut into several 2-3 cm long sections. The strips were mounted vertically in an organ bath (Harvard, England) in Tyrode´s solution, maintained at 37 °C, gassed continuously with oxygen and equilibrated for 30 min. Changes in length of preparation were recorded isotonically under 1 g tension and printed on a Harvard Universal Oscillograph (England) pen recorder device. The tissues were washed several times every 15 min and allowed to relax to a stable baseline.

Effect of *D. kotschyi* hydroalcoholic extract was examined on rat ileum and uterus

contractions suspended in the organ bath. Contractions were induced in both tissues by direct addition of KCl or ACh and application of local electrical field stimulation (EFS). In addition, in the case of uterus, relaxant effect of extract was also examined on oxytocin-induced contraction and compared with the standard drug salbutamol. Initially a number of pilot experiments were carried out for determination of effective concentration ranges of the extract.

Effect of extract on spasm evoked by KCl

KCl was added into organ bath to give final bath concentration of 80 mM. After 20 min equilibration time, *D. kotschyi* extract was added in a cumulative manner to the bath at 10-min intervals until a full concentration-effect curve was constructed. In the control groups, equivalent volume of *D. kotschyi* extract vehicle was added.

Effect of extract on spasm evoked by ACh

ACh was added into organ bath to give final bath concentration of 500 nM. After 30 s contact, the tissues were washed with fresh Tyrode's solution. This protocol was repeated at 10-min intervals until a consistent response was established. Then first concentration of *D. kotschyi* extract was added into the organ bath and 10 min later ACh response was assessed. Then next concentration of *D. kotschyi* extract was added using two-fold increments in concentration until a full concentration effect curve was constructed. In the control groups, the tissues were treated with equivalent volume of extract vehicle.

Effect of extract on spasm evoked by EFS

EFS were delivered through parallel platinum wire electrodes (10 cm long, 0.5 cm apart) in trains of rectangular pulses for one second. Initially several repeated stimuli were applied at 10-min intervals, until consistent responses were established. Then *D. kotschyi* extract was added and in presence of the extract, the tissue response to EFS was assessed. Then the next concentration of the extract was added using two fold increments in concentration until a full concentration-effect curve was constructed. In the parallel time-matched control groups, equivalent volume of the vehicle was added.

Effect of extract on spasm evoked by oxytocin

In the case of uterus, oxytocin was added into the bath to give final bath concentration of 0.0005 IU/mL. Oxytocin was in contact with the tissue for 5 min before it was washed off with fresh Tyrode's solution. After reproducible contraction were established the extract or equal volume of the vehicle were added directly into the organ bath at 10-min intervals. The effect of *D. kotschyi* extract was also examined on oxytocin-induced contraction with 2 fold increment in concentration in order to construct a full concentration-effect curve.

Full concentration-response curves were obtained using 6 to 11 different concentrations of examining agents. After maximum inhibitory effect was achieved, the tissue were washed with fresh Tyrode's solution and tested for reversibility of the response.

Measurements and statistical analysis

Contractile response to KCl, ACh and EFS were measured as maximum amplitude from the initial baseline and expressed as the percentage of the response prior to addition of the extract or vehicle. Assessment of oxytocin response was achieved by multiplying the amplitude of the spikes by the frequency over 10-min intervals and expressed as percentage of initial control group for each tissue. All the values are quoted as mean ± standard error of the mean (SEM).

Statistical significance were assessed using one-way analysis of variance (ANOVA) for repeated measures and when appropriate was compared with the control groups using unpaired Student's t-test. Differences were considered statistically significant for $P < 0.05$. Whenever appropriate, the IC_{50} value (drug concentration causing 50% of maximum inhibitory response), was calculated. Sigma Plot computer program (version 11) was used for statistical analysis and plotting the graphs.

RESULTS

Rat isolated ileum and uterus suspended in the fresh Tyrode's solution gradually relaxed to

a stable baseline over 10-20 min. The ileum strip produced relatively small and irregular spontaneous contractile activity which gradually faded away. On the other hand, the uterus strip produced relatively large rhythmic contraction with various amplitudes. Addition of KCl (80 mM) into the organ bath induced a sustained tonic contraction in both ileum and uterus smooth muscles. Addition of ACh (500 nM) into the organ bath produced a single rapid phasic contraction within 30 s contact times in both tissues. Application of EFS caused a single contractile response in rat uterus while produced a biphasic contraction in rat ileum as reported before (23-25). Addition of oxytocin (0.0005 IU/mL) potentiated both the frequency and amplitudes of the rhythmic contractions of the uterus.

Effect of extract on spasm evoked by KCl

D. kotschyi extract concentration-dependently inhibited the tonic contraction induced by KCl in both tissues (Fig. 1). However, the relaxant effect of ileum was observed with lower concentrations of the extract. Inhibitory effect of the extract on the ileum was started with bath concentration of 16 µg/mL and total relaxation was achieved with extract at 256 µg/mL in the bath (Fig. 1). The IC_{50} value of hydroalcoholic extract of *D. kotschyi* on the contractile response of KCl on ileum was 65 ± 17.6 µg/mL.

The relaxant effect of *D. kotschyi* on the uterus was not started until the bath concentration reached to 128 µg/mL (Fig. 1). Full inhibition of KCl response was only achieved with *D. kotschyi* extract concentration above 1 mg/mL. The IC_{50} value of hydroalcoholic extract of *D. kotschyi* on KCl response on the uterus was 453 ± 63.8 µg/mL. Following washing the tissues with fresh Tyrode's solution, the inhibitory effect of the extract was reversed. There were no statistically significant changes in the time-matched control groups treated with equivalent volume of vehicle (DMSO).

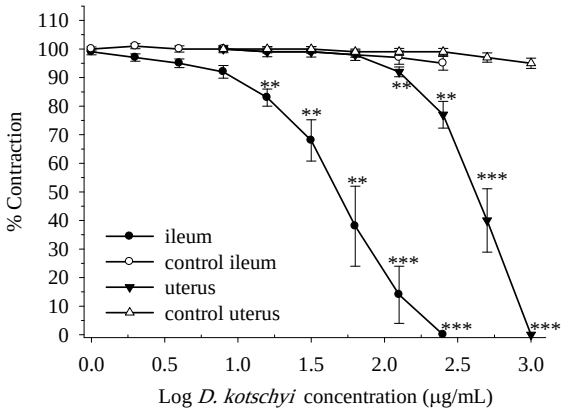

Fig. 1. Cumulative effect of *Dracocephalum kotschyi* extract on tension development to potassium chloride (KCl, 80 mM) on ileum and uterus of rats. Ordinate scale: tissues contractions expressed as percent of initial KCl response. Abscissa scale: $\log_{10}$ concentration of *D. kotschyi*. Lines drawn through the points, using two fold increments in concentration. The points are mean and the vertical bars show the SEM (n = 6). Stars shows statistical differences between each drug concentration with its corresponding vehicle-treated control. Keys: ***P* < 0.01, ****P* < 0.001 (Student's t-test). Maximum concentration of the vehicle (DMSO) in the bath was 7.5%.

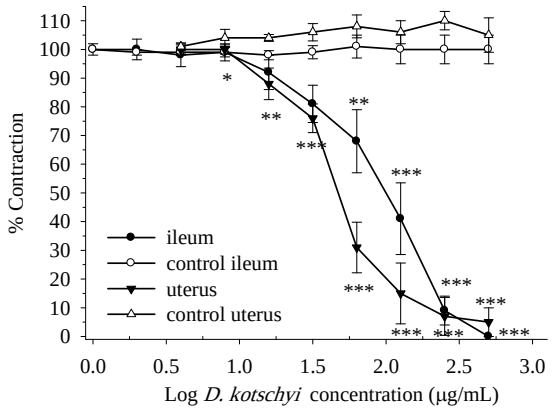

Fig. 2. Effect of *Dracocephalum kotschyi* extract on tension development to acetylcholine (ACh, 500nM) in ileum and uterus of rats. Ordinate scale: tissues contractions expressed as the percentage of initial ACh response. Abscissa scale: $\log_{10}$ concentration of *D. kotschyi*. Lines drawn through the points, using two fold increments in concentration. The points are mean and the vertical bars show the SEM (n=6). The oscillation in the response of vehicle treated control tissues was not statistically significant (ANOVA). Stars shows statistical differences between each drug concentration with its corresponding vehicle treated control. Keys: **P* < 0.05, ***P* < 0.01, ****P* < 0.001 (Student's t-test). Maximum concentration of vehicle (DMSO) in the bath was 2%.

Effect of extract on spasm evoked by ACh

The hyroalcoholic extract of *D. kotschyi* (8-512 µg/mL), concentration-dependently inhibited the ileum and uterus contractions induced by ACh (500 nM, Fig. 2). The extract at 512 µg/mL bath concentration diminished the contractile response to ACh in the ileum while 5% of original contraction of uterus was remained. The IC_{50} values for ileum and uterus were 102 ± 18 µg/mL and 58 ± 9 µg/mL respectively. The inhibitory effect of the extract on ACh responses was reversed following washing the tissue with fresh Tyrode's solution. There were no statistically significant changes in the time-matched control groups treated with equivalent volume of vehicle (DMSO).

Effect of extract on spasm evoked by EFS

D. kotschyi extract (20–640 µg/mL) concentration-dependently inhibited the ileum and uterus contractile responses to neuronal stimulation (EFS). At its highest concentration tested (512 µg/mL), the extract totally abolished the response to both biphasic responses of EFS in the ileum (Figs. 3 and 4). On the other hand, the relaxant effect of extract on the uterus was seen with much lower concentration but the total inhibition was not achieved even with bath concentration as high as 1 mg/mL (Fig. 3). The inhibitory concentration causing 50% of maximum responses were 117 ± 29 µg/mL and 22 ± 3 µg/mL for the ileum and the uterus respectively. The secondary contractile phase to EFS which was only seen in the ileum was also inhibited by the extract (IC_{50} = 40 ± 10 µg/mL) (Fig. 4). Following washing the tissue with fresh Tyrode's solution, the contractile responses to neuronal stimulation was gradually restored in both tissues. There were no statistically differences in the responses of vehicle treated time match control tissues over the course of studies (ANOVA).

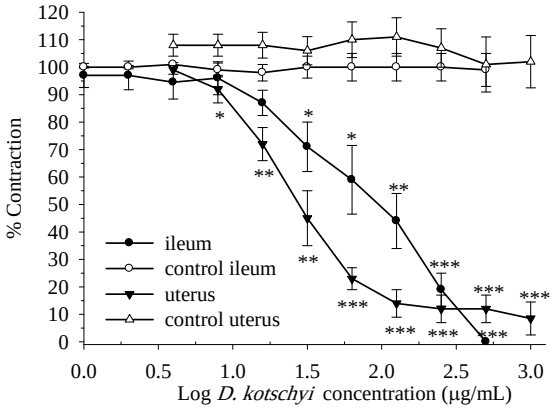

Fig. 3. Effect of *Dracocephalum kotschyi* extract on tension development to contractile response to electrical field stimulation (EFS, 6V, 50Hz, 1s duration),in the ileum and the uterus of rats. Ordinate scale: tissues contractions expressed as percentof initial EFS responses. Abscissa scale: $\log_{10}$ concentration of drugs *D. kotschyi*. Lines drawn through the points, using two fold increments in concentration. The points are mean and the vertical bars show the SEM (n=6). The fluctuations in the response of vehicle treated control tissues is not statistically significant (ANOVA). Stars shows statistical differences between each drug concentration with its corresponding vehicle treated control. Keys: $*P < 0.05$,$**P < 0.01$, $***P < 0.001$ (Student's t-test). Maximum concentration of vehicle (DMSO) in the bath was 2%.

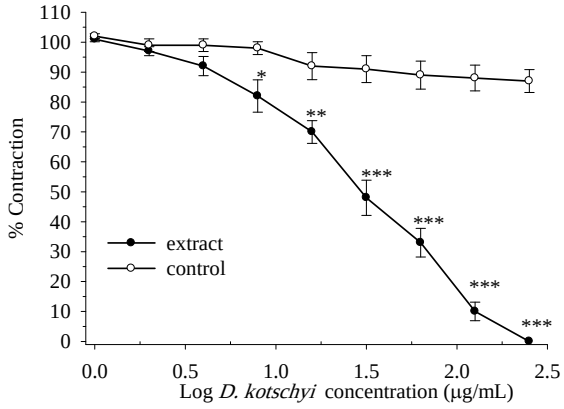

Fig. 4. Effect of *Dracocephalum kotschyi* extract on tension development to secondary contractile response to electrical field stimulation (EFS, 6V, 50Hz, 1s duration), in the ileum of rats. Ordinate scale: tissues contractions expressed as percentage ofof initial EFS responses. Abscissa scale: $\log_{10}$ concentration of drugs *D. kotschyi*. Lines drawn through the points, using two fold increments in concentration. The points are mean and the vertical bars show the SEM (n=6). The fluctuations in the response of vehicle treated control tissues is not statistically significant (ANOVA). Stars shows statistical differences between each drug concentration with its corresponding vehicle treated control. Keys: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ (Student's t-test). Maximum concentration of vehicle (DMSO) in the bath was 1%.

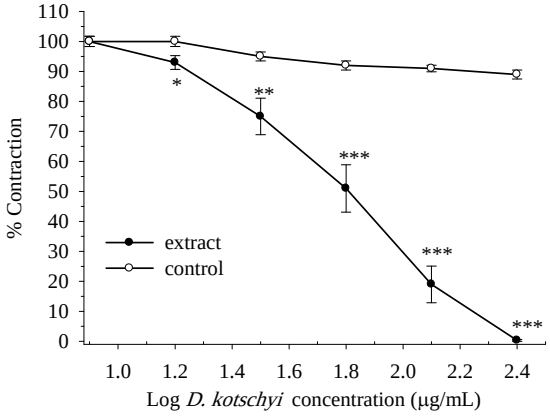

Fig. 5. Effect of *Dracocephalum kotschyi* extract on tension development to Oxytocin (0.0005IU/mL) in the uterus of rats. Ordinate scale: tissues contractions expressed as percentof initial oxytocin response. Abscissa scale: $\log_{10}$ concentration of *D. kotschyi*. Lines drawn through the points, using two fold increments in concentration. The points are mean and the vertical bars show the SEM (n=6). The small reduction in the response of vehicle treated control tissues was statistically significant ($P < 0.001$, ANOVA). Stars shows statistical differences between each drug concentration with its corresponding vehicle treated control. Keys: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ (*Student's t-test*). Maximum concentration of vehicle (DMSO) in the bath was 1%.

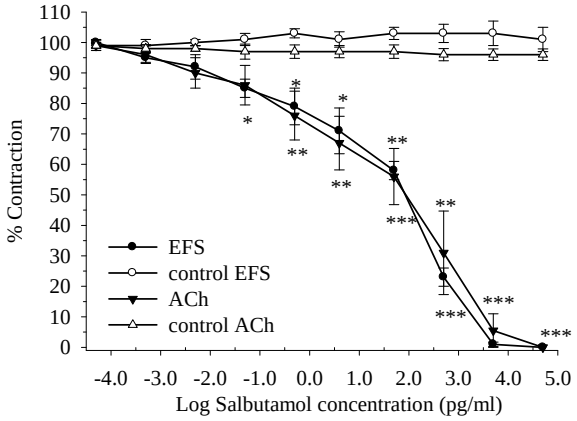

Fig. 6. Effect of salbutamol on tension development to ACh (500nM) and electrical field stimulation (EFS) in the rat uterus. Ordinate scale: tissues contractions expressed as % of initial contractile response. Abscissa scale: $\log_{10}$ concentration of salbutamol. Lines drawn through the points, using 10 fold increments in concentration. The points are mean and the vertical bars show the SEM (n=6). The small fluctuation in the response of vehicle treated control tissues was not statistically significant (ANOVA). Stars shows statistical differences between each drug concentration with its corresponding vehicle treated control. Keys: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ (Student's t-test).

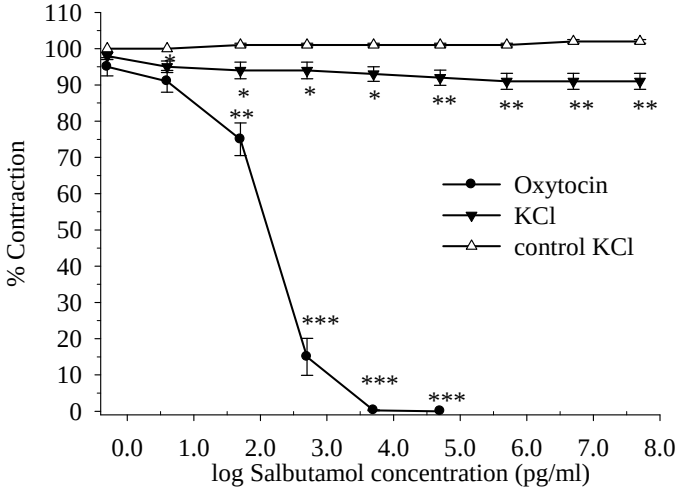

Fig. 7. Effect of salbutamol on tension development to oxytocin (0.0005IU/mL) and KCl (80mM) in the uterus of rats. Ordinate scale: tissues contractions expressed as % of initial contractile response. Abscissa scale: $\log_{10}$ concentration of salbutamol. Lines drawn through the points, using 10 fold increments in concentration. The points are mean and the vertical bars show the SEM (n = 6). There was no statistically significant change in the response of vehicle treated controls (ANOVA). Stars shows statistical differences between each drug concentration with its corresponding vehicle treated control. Keys: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ (Student's t-test).

Effect of extract on spasm evoked by Oxytocin

Hydroalcoholic extract of *D. kotschyi*, in a concentration-dependent manner, reduced both the amplitude and frequency of rhythmic contraction induced by oxytocin in rat isolated uterus. Full concentration-effect curve are presentd in Fig. 5. The IC_{50} value of hydroalcoholic extract of *D. kotschyi* on the contractile response of oxytocin on rat uterus was 70 ± 11 µg/mL. The inhibitory effect of *D. kotschyi* on the contractile response of oxytocin was reversed following removing the extract from the bath. There was a small reduction in the response of the tissues treated with equivalent volume of the vehicle (DMSO) over the course of the experiment (Fig. 5).

Effect of salbutamol on uterus contractions

Salbutamol as a β_2-adrenoceptor agonist was used as a standard inhibitor of uterine contraction. Salbutamol, in a concentration dependent fashion, inhibited uterus contraction induced by ACh, oxytocin and EFS (Figs. 6 and 7). Nevertheless, salbutamol only partially inhibited the KCl-induced contraction in rat uterus. Even at concentrations as high as 50 µg/mL, salbutamol only inhibited uterus contraction induced by KCl by 9 ± 2.2% (Fig. 7).

DISCUSSION

In traditional medicine various species of *Dracocephalum* are used for gastrointestinal disorders (26,27). Among these species, only *D. kotschyi* has been used as antispasmodic and analgesic as herbal medicine in Iran. Recent pharmacological investigation has shown that *D. kotschyi* is an inhibitor of rat ileum contraction both *in vivo* and *in vitro* (15,18,28). In this research antispasmodic effect of *D. kotschyi* extract on rat uterus contraction was investigated for comparison with that of the ileum. The first spasmogen used in this study was high concentration of KCl (80 mM). Addition of high concentration of KCl into extracellular fluid results is cell depolarization and activation of voltage-dependent L-type calcium channels (29). Increase in intracellular Ca^{2+} induces smooth muscle contraction (30,31). *D. kotschyi* extract inhibited contraction induced by KCl in both type of tissues, although, the extract was more effective on ileum than on uterus. In the case of KCl, comparison at IC_{50} level showed that *D. kotschyi* extract was 6.9 times more potent on ileum (Fig. 1). Salbutamol, which was used as a standard drug, only had minor inhibitory effect on KCl contraction in rat uterus (Fig. 7). Acetylcholine (ACh) which is a natural neurotransmitter in both ileum and uterus tissues was used as second spasmogen. ACh acts mainly on M_3 muscarinic receptors on smooth muscle cells and thereby increases phospholipase C activity and creation of IP_3 which induce intracellular Ca^{2+} release (30,31). *D. kotschyi* extract reversibly inhibited contractions induced in both types of tissues. Unlike the KCl response, salbutamol completely diminished the contractile response to ACh (Fig. 6). EFS was used as the more natural method of tissue contraction. Application of EFS not only causes release of ACh via stimulating parasympatic neurons embedded within smooth muscle but also may cause release of other natural neurotransmitters by stimulating non-adrenergic non-cholinergic neurons (32). The biphasic contraction of the ileum is due to the presence of complex network of enteric nerves system and release of various neurotransmitters (33). *D. kotschyi* extract inhibited monophasic EFS contraction of uterus as well as biphasic EFS contraction of ileum, indicating that *D. kotschyi* extract can inhibit contraction induced by natural neurotransmitters released during nerve stimulation. Although *D. kotschyi* extract totally removed the EFS responses in ileum, nevertheless about 10% of initial EFS response remained in the rat uterus probably due to the release of other substances during electrical filed stimulation.

Oxytocin is an endogenous hormone which causes contraction of pretreated uterus with estrogen (34). Oxytocin induces contraction by activating oxytocin receptors situated on uterine smooth muscle. Oxytocin receptors also are coupled with enzyme phopholipase C and release of Ca^{2+} from intracellular storages. *D. kotschyi* extract and salbutamol inhibited uterine contraction induced by oxytocin uterus.

Comparison of these results with the previous report of *D. kotschyi* extract on rat ileum (18) has shown the effect of the extract is reproducible. Comparison of potency of *D. kotschyi* extract on rat uterus indicates that the extract is more effective in inhibiting EFS, ACh and oxytocin responses than the KCl response. Comparison of inhibitory effect of *D. kotschyi* extract on isolated ileum and uterus smooth muscle contractions at IC_{50} level shows that the extract was more effective inhibitor of uterus contraction induced by oxytocin, ACh or EFS. Comparison of inhibitory effect of salbutamol with *D. kotschyi* extract indicates that the extract may have an advantage because it was more effective than salbutamol on inhibiting KCl-induced contraction. Therefore, examination of *D. kotschyi* extract on inhibition of uterine activity *in vivo* is recommended.

Salbutamol by activating β_2-adrenoceptor increases adenylyl cyclase activity and production of intracellular cAMP which inhibits contractile proteins in smooth muscles (35). Inhibition of KCl and ACh responses indicate that somehow release of both Ca^{2+} stores and Ca^{2+} entry is affected by *D. kotschyi* extract. This might be due to the presence of several active ingredients in the extract. Other possibility is that, the extract is acting on the final pathways of contractions and in this way smooth contraction is inhibited. At this point, definite conclusion about mechanism of action of the *D. kotschyi* extract is not possible and further research on elucidation of possible mechanism of action(s) of the *D. kotschyi* extract is recommended.

CONCLUSION

This study has shown that *D. kotschyi* extract is a potent relaxant of both uterus and ileum contractions and, if proven to be safe, it might be a suitable herbal remedy for control of preterm uterus contraction.

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