Antispasmodic effect of hydroalcoholic and flavonoids extracts of *Dracocephalum kotschyi* on rabbit bladder

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ABSTRACT

Introduction: *Dracocephalum kotschyi* extract has antispasmodic activities on smooth muscle including ileum, uterus and trachea. The objective of this research was to investigate antispasmodic activity of hydroalcoholic and flavonoids extracts of *D. kotschyi* on rabbit bladder contractions.

Methods: Rabbits were euthanized by carbon dioxide asphyxiation and the whole bladder was dissected out and immersed in the Tyrode's solution. Longitudinal bladder strips were mounted vertically in an organ bath at 37°C and gassed continuously with O_2. Bladder strips were contracted with acetylcholine (ACh), KCl, or electrical field stimulation (EFS). Isotonic tension of the tissue was recorded before and after addition of hydroalcoholic or flavonoids rich extracts of *D. kotschyi*. Nifedipine and propantheline were used as standard drugs.

Results: Standard drug propantheline, prevented bladder phasic contraction induced by ACh (1μM) without affecting KCl response. On the other hand, cumulative addition of nifedipine attenuated the tonic contractions induced by KCl (20mM) on bladder smooth muscle. Hydroalcoholic and flavonoids extracts of *D. kotschyi* at concentration ranges of 10-320 μg/mL in a concentration dependent way inhibited bladder tonic contraction induced by KCl (n=6). Both extracts also in a concentration-dependent manner relaxed EFS and ACh-induced contractions (range, 20–1280 μg/mL) of bladder smooth muscle *in vitro*. Complete inhibition was achieved with the highest used concentrations of the extracts. The inhibitory effect of the extract was reversible following washing the tissues with fresh Tyrode's solution.

Conclusion: This study clearly demonstrated that *D. kotschyi* extracts were able to prevent contractions induced by ACh, KCl or EFS in isolated rabbit bladder. This means that people consuming this medicinal plant may face urinary retention which could be a problem for patients with prostate hypertrophy. On the other hand, this plant might be useful in patients with urinary incontinence. However, its usefulness must be assessed in the controlled clinical trials.

Introduction

Dracocephalum kotschyi Boiss is a native Iranian flowering plant in the family Lamiaceae (1-3). *D. kotschyi* (Zaringiah in Persian) is a perennial herbaceous plant with stems growing between 10 and 20 cm tall (1-3). Although Zaringiah is mainly used as food flavoring herb among Iranian population, nevertheless infusion or decoction of Zaringiah traditionally is used by local people for various ailments (4). Zaringiah is not well documented in the Iranian traditional medicine books and only in a few texts has referred to its anti-inflammatory and anti-rheumatism benefits (1,2).

Zaringiah is enriched in various constituents including essential oils, flavonoids and monoterpene glycosides (5-10). The essential oils and flavonoids components seem to possess the active pharmacologically substances

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(7-11). Geranial, limonene, α-pinene, citral, α-terpineol, caryophyllene, terpinyl, Gerania, cyclononadiene, isopinocarveol, terpinene-4-ol, linalool, carveol, myrcene, germacrene-D, etc, accounts for major identified constituents of essential oil (6-8). It has been reported that antinociceptive and anti-inflammatory effects of essential oil is due to action of limonene and α-terpineol (9). Ethanolic extract of *D. kotschyi* also possess potent anti-inflammatory properties (11). Many constituents of the extract also have been identified. Some of these constituents including calycopterin, xanthomicrol, isokaempferide, luteolin, apigenin, luteolin 7-O-beta-D-glucopyranoside, lutcolin 3'-Obeta-D-glucuronide, apigenin 4'-O-beta-D-glucopyranoside, acacetin 7-O-beta-D glucopyranoside, rosmarinic acid are its known components (10, 12).

In modern medicine *D. kotschyi* has widely been studied for treatment of diseases other than inflammatory conditions. For instant, *D. kotschyi* extract has been introduced as one of the components in the drug called Spinal-Z in Iran for treatment of cancer (13). The anti-tumor activity of *D. kotschyi* extract has been attributed to its constituent calycopterin and xanthomicrol although anti-proliferative properties for other constituents are also reported (14-16). Immunomodulatory activity is another reported action of *D. kotschyi* extract, which might be associated with its anticancer and anti-inflammatory properties of this plant (16,17).

Modern pharmacological research confirms that *D. kotschyi* has strong antispasmodic activity both *in vitro* and *in vivo* (8,18-22). For example, *D. kotschyi* essential oil and extract has potent antispasmodic activity on isolated ileum (8, 18, 20) and has been shown to reduce intestinal meal transit in vivo (19,22). In addition, *D. kotschyi* prevents uterus contraction induced by KCl, oxytocin, acetylcholine (ACh) and neuronal stimulation (21). Furthermore, flavonoids extract of *D. kotschyi* inhibits tracheal contraction induced by KCl and ACh and because of its antispasmodic and anti-inflammatory properties, it was suggested as a suitable herbal remedy for treatment of asthma (23). The antispasmodic of the extract has been suggested to be due to flavonoids constituents such as apigenin and luteolin (20,23). So far there is no report on pharmacological action of *D. kotschyi* on bladder contraction. Therefore, the objective of this research was to investigate spasmolytic effect of *D. kotschyi* extract on isolated bladder smooth muscle contraction *in vitro*.

Materials and Methods
Pharmacognosy studies
Aerial parts of Zaringiah were collected from cultivated farm on the base of Shahan Kooh in Feridonshar (Isfahan-Iran) during flowering season in year 2017. The plant was identified as *D. kotschyi* by the botanist Mr Asfa from Department of Natural Resources in Isfahan province. A herbarium voucher has been deposited in the School of Pharmacy and Pharmaceutical Sciences (No. 1519).

The plant materials were dried in shade and grained into fine powder using an electric grinder (Keep, Korea). Hydroalcoholic extract was prepared by maceration technique (24). 300 g of the plant powder was mixed with 70% ethanol in order to moisturize the plant material and left at room temperature. Two hours later, the plant materials were packed in a percolator and ethanol (70%) was added with ratio of 1 to 8 (2.4 L ethanol) making sure that the entire plant materials were soaked in the ethanol. The top of percolator was covered and left for 3 days at room temperature. On the third day, the percolator tap was opened and the extract was collected from bottom of percolator. The process of extraction was repeated thrice. All the collected extracts were added together and concentrated under reduced pressure at 60°C with rotary apparatus (Heidolph, Germany). The yield of hydro-alcoholic extract was calculated.

The flavonoids extract was prepared from concentrated hydro-alcoholic extract using solvent in solvent fractionations technique (25-27). One hundred grams of hydro-alcoholic fraction of *D. kotschyi* extract was dissolved in mixture of chloroform and water (1L:1L) and shaken for 20 minutes. This mixture was poured into a decanter and allowed the solvents to be separated. The lower chloroform phase was carefully decanted. The middle phase (semisolid phase) was also removed. Equivalent volume of chloroform was added into the remaining aqueous phase and the process repeated thrice. The chloroform fractions were added together and concentrated with rotary apparatus under 30°C. The remaining aqueous solvent was further extracted with ethyl acetate solution (1:1 ratio) as described above. Extraction was repeated 5 times and ethyl acetate phase was separated and the solvents were evaporated with rotary apparatus at 50°C. The percentage yield of the ethyl acetate extract was calculated (W/W).

Assessment of total phenolic contents
Folin-Ciocalteu's phenol reagent test was used for assessment of total phenolic contents of the extracts (28). Gallic acid was used as standard phenol compound. Gallic acid was dissolved in 10% ethanol as 5 mg/mL stock solution and further diluted with distilled water in order to prepare stock solutions of 500, 250, 150, 100 and 50 μg/mL. In a test tube 20 μL of test solution, 100 μL of Folin-Ciocalteu's phenol reagent and 300 μL of sodium carbonate (200 mg/mL) were mixed with 1.58 ml distilled water and stored at dark. After 2 hours incubation at room temperature, standard cuvette was filled with this solution and light absorption was read at 765 nm wavelength with a light spectrophotometer (Jenway 5105, UV/V, England). Calibration curve for gallic acid was drawn by plotting each concentration of gallic acid against its light absorption and straight line was fitted. The extracts samples were

prepared as with gallic acid and after reading their light absorption at 765 nm wavelength, concentration of total phenolic content was calculated from the standard graph.

Pharmacological studies
Drugs and solutions
Tyrode's solution was made in distilled water with the following composition (mM): NaCl (136.6), NaHCO$_3$ (11.9), glucose (5.55), KCl (2.68), CaCl$_2$ (1.8), MgCl$_2$ (1.05) and NaH$_2$PO$_4$ (0.42). Hydroalcoholic and flavonoids extracts were made up in dimethylsulphoxide (DMSO) as 80 mg/mL and 40 mg/mL stock solutions, respectively. Further dilution was made in 50% DMSO. Nifedipine was made up as 2 mg/mL stock solution in DMSO. Further dilution was made in DMSO or distilled water as appropriate. Propantheline was made up as 4.5 µg/mL stock solution in distilled water. Further dilution was prepared in distilled water. KCl and ACh were prepared as 2M and 100µM stock solutions in distilled water, respectively.

Antispasmodic investigation
Local rabbits bred in open field farm in Isfahan, was located in the School of Pharmacy and Pharmaceutical Sciences animal house at room temperature with full access to food and water. On each morning, a rabbit was placed in glass chamber (25×40×30 cm) and euthanized by carbon dioxide asphyxiation. Then, the abdominal cavity was opened and bladder was identified and carefully dissected out and immersed in oxygenated Tyrode's solution. Isolated rabbit bladder was cut in four separate longitudinal strips. One end of strip was tied into a hook and placed in an organ bath (Palmer, England) at 37°C and gassed continuously with oxygen. Other end of the tissue was tied into isotonic transducer (Harvard) and held under 1 g tension. Bladder contractions were recorded on Harvard Universal Oscillograph (England). Following calibration of Oscillograph, the tissue was washed several times and allowed to relax into a stable basal tension. Initially, the tissue response to a single concentration of ACh (1µM) was determined. Only the tissues with appropriate response to ACh were used in this research.

Smooth muscle contractions of rabbit bladder strips were induced either by addition of KCl (20mM), ACh (1µM) or electrical field stimulation (EFS, 6 V, 50 Hz and 1 second duration). KCl was added directly into the organ bath and contraction recording continued at least for 15 minutes before drugs (nifedipine, propantheline) or extracts (hydro-alcoholic, flavonoids) were added in a cumulative manner. Drugs were added at 5-minute intervals while extract was added at 12-minute intervals. Effects of drugs and extracts on ACh and EFS response were studied in a non-cumulative manner. Each drug or extract concentration was at least 5 minutes in contact with the tissue before its effect was evaluated. Full concentration response curves were constructed in each case. In the parallel time-matched control tissues effects of equivalent volume of extract vehicle were examined.

Statistics and data analysis
Bladder contractions were assessed as maximum amplitude of induced contraction from the baseline and expressed as percentage of initial response prior to addition of the test drug. Data are presented as mean ± standard error of mean (SEM) for each group of results. For statistical analysis, one-way analysis of variance (ANOVA) or unpaired Student's *t* test were used for comparison with corresponding time matched vehicle treated control group. *P* value less than 0.05 was considered statistically significant.

Results
Pharmacognosy studies
Hydro-alcoholic extract of *D. kotschyi* had a dark green color. The yield of the hydro-alcoholic extract was calculated 21% (W/W). Total phenolic contents of the extracts are summarized in Table 1. The most phenolic contents were found in the ethyl acetate extract and therefore recognized as flavonoids rich extract. The yield of flavonoids extract from the hydro-alcoholic extract was calculated as 13.9% (W/W). This means that flavonoids extract accounts for 2.9% of the dried plant materials.

Pharmacological studies
Isolated strips of rabbit bladder suspended in the organ bath gradually relaxed into a relatively stable tension and exhibited spontaneous contractile activities which gradually subsided down over the time. Addition of KCl (20mM bath concentration) produced a tonic sustained contraction which was maintained over the course of studies. Addition of ACh induced a single phasic contraction, reaching to its peak within 30 seconds contact time. Following washing the tissue with fresh Tyrode's solution it relaxed back to the baseline. Application of EFS produced a sharp contraction which quickly returned toward the baseline following cessation of stimulation.

Propantheline (60 pg/mL to 8 ng/mL) in a concentration dependent manner inhibited phasic contraction induced by ACh (IC$_{50}$ = 0.5 ± 0.16 ng/mL) without affecting

Table 1. Total phenolic contents of the *Dracocephalum kotschyi* extracts

% Phenolic contents (n=3)	
Hydro-alcoholic extract	1.27 ± 0.09
Ethyl acetate extract	2.4 ± 0.11
Chloroform extract	0.06 ± 0.006
Remained aqueous extract	0.69 ± 0.09

Folin-Ciocalteu's phenol reagent test was used for assessment of total phenolic contents and gallic acid was used as standard phenol compound. Light absorption was read at 765 nm wavelength.

KCl response (Figure 1). Higher concentrations of propantheline (40 ng/mL & 80 ng/mL) also had no effect on KCl induced contraction of rabbit bladder (Figure 2). Propantheline at concentrations which inhibited ACh response caused no significant inhibitory effect on EFS response. However, at higher concentrations (40 ng/mL and 80 ng/mL) partially but significantly reduced EFS contractile response of rabbit isolated bladder strip (Figure 2).

Unlike, propantheline, nifedipine (100 pg/mL to 1.6 µg/mL bath concentrations) inhibited KCl induced contractions in rabbit bladder strips with IC_{50} value of 37 ± 10.3 ng/mL (Figure 3). Nifedipine at similar concentration ranges also inhibited ACh induced responses. However, complete inhibition was not achieved and at the highest bath concentration used still 12% of initial contraction remained (Figure 3). Nifedipine also inhibited bladder response to EFS but complete inhibition was not achieved. The inhibitory concentration was seen at 25 ng/mL bath concentration and maximum inhibition was seen with 6.4 µg/mL bath concentration. Increasing the concentration to 25 µg/mL had no further inhibitory effect and still 8% of initial response remained (Figure 3). The IC_{50} values can be seen in Table 2.

Hydroalcoholic and flavonoids extracts of *D. kotschyi* in a concentration dependent manner inhibited KCl induced tonic contraction in rabbit bladder strips. Initial inhibitory effect was observed with 10 µg/mL extracts in the bath and complete inhibition was achieved with 320 µg/mL bath concentrations (Figure 4). The IC_{50} values are compared in Table 2. Following washing the tissue with fresh Tyrode's solution, contractile response of bladder strip to KCl gradually was restored. Addition of equal volume of extract vehicle (DMSO) only at the highest used
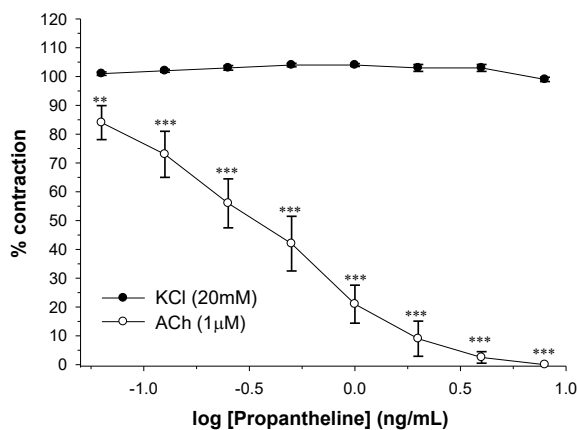

Figure 2. Effects of high concentrations of propantheline on KCl (n = 4) and electrical field stimulation (EFS, n = 6) responses of rabbit isolated bladder strips. Data are presented as mean ± SEM. Asterisk symbols show statistically significant inhibition in comparison with the initial control group (**P<0.01, ***P<0.001, Student's *t* test).

Figure 3. Effect nifedipine on KCl, acetylcholine (ACh) and electrical field stimulation (EFS) induced contractions on rabbit isolated bladder strips. Results are expressed as % of initial contraction prior to addition of nifedipine. Data are presented as mean ± SEM (n = 6). Nifedipine was 5 min in contact with the tissue before its effect was evaluated. Inhibition of contraction in all cases was statistically significant (ANOVA, P<0.001).

Figure 1. Effect of propantheline on acetylcholine (ACh) and KCl induced contractions in rabbit isolated bladder strips. Propantheline was added into bath with 2 fold increment in concentration. Data are presented as mean ± SEM (n=6). Stars shows statistically significant changes in the ACh response (**P<0.01, ***P<0.001, Student's *t* test). There are no statistically significant changes in the KCl response over the course of study (ANOVA).
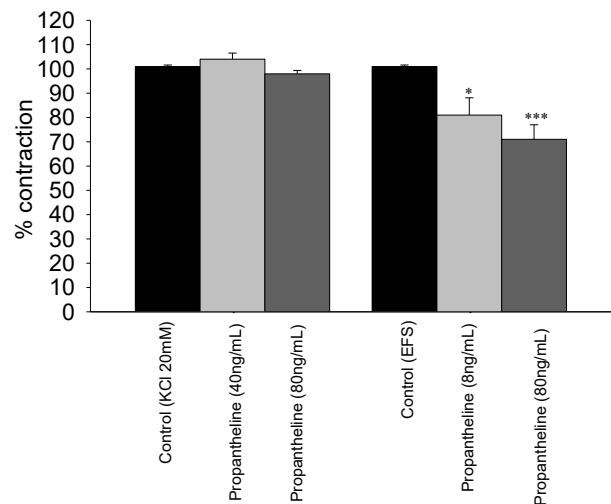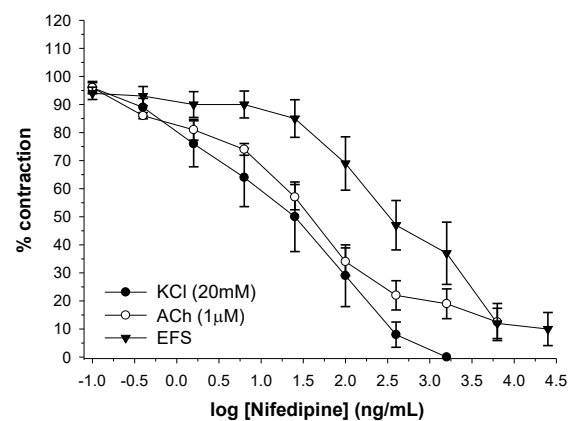
concentration slightly reduced KCl response (Figure 4).

Addition of *D. kotschyi* extracts into the organ bath at 5 minutes intervals gradually attenuated phasic contraction induced by ACh. Inhibitory effect of hydro-alcoholic extract was seen with 160 µg/mL bath concentration and ACh response was removed with 1.28 mg/mL extract in the bath (Figure 5). The inhibitory effect of flavonoids extract was started with 80 µg/mL extract in the bath and at bath concentration of 640 µg/mL almost abolished (Figure 5). The IC_{50} values are presented in Table 2. The inhibitory effects of the extracts on ACh responses were reversible following washing the tissues with fresh Tyrode's solution.

Table 2. Comparison of inhibitory concentrations causing 50% of maximum response (IC$_{50}$ value) of nifedipine and *Dracocephalum kotschyi* extracts

	IC$_{50}$ values		
	KCl (20mM)	**ACh (1μM)**	**EFS**
Nifedipine	37±10.3 ng/mL	30±6 ng/mL	0.4±0.18μg/mL
Hydro-alcoholic extract	53±6 μg/mL	243±38 μg/mL	170±15 μg/mL
Flavonoids extract	102±9 μg/mL	190±58 μg/mL	105±27 μg/mL

In the control group treated with vehicle (DMSO), ACh response was slightly potentiated (Figure 5).

Both hydro-alcoholic and flavonoids extracts of *D. kotschyi* in a concentration dependent way reduced rabbit bladder strips responses to EFS. First sign of inhibition was observed with 10 μg/mL extract in the bath. However, complete inhibition was not achieved and with bath concentration of 640 μg/mL still 15% and 7% of initial response remained respectively (Figure 6). The inhibitory effect of the extracts on EFS response was reversible following washing the tissue. The IC$_{50}$ values are presented in Table 2 for comparison with KCl and ACh responses. There was no statistically significant change in the tissue response in the time matched control tissue treated with the vehicle (DMSO) (Figure 6).

Discussion

Bladder is a part of lower urinary tract and its function is storage and excretion of urine. The bladder is a hollow sac, the wall of which mainly consists of smooth muscles covered by several impermeable membrane layers (29-32). The bladder body comprise of detrusor and trigonal smooth muscles. The detrusor is innervated by parasympathetic nerves system and forms major muscular wall of the bladder. Detrusor muscle relaxes during bladder filling and contracts during micturition (33). The trigone structurally and functionally is different from detrusor and mainly innervated by sympathetic nerves system (34). Changes in the detrusor activity may result in urinary problem including incontinence or urine retention (35). The smooth muscle cells layers are oriented
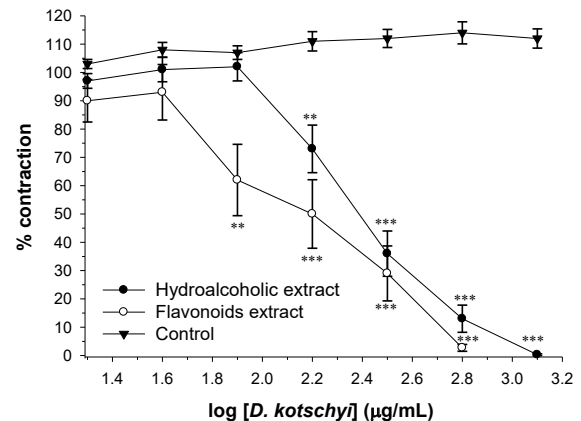

Figure 5. Inhibitory effect of *Dracocephalum kotschyi* extracts on acetylcholine (ACh) induced contraction in rabbit isolated bladder strips. Results are expressed as percentage of initial response prior to addition of extracts. Data are presented as mean ± SEM (n=6). Stars show statistically significant difference in comparison with the control group treated with the vehicle (**P < 0.01, ***P < 0.001, Student's *t* test). There was no reduction in the tissue response in the control group treated with equivalent volume of vehicle (DMSO). Maximum used concentration of DMSO was 0.8%.
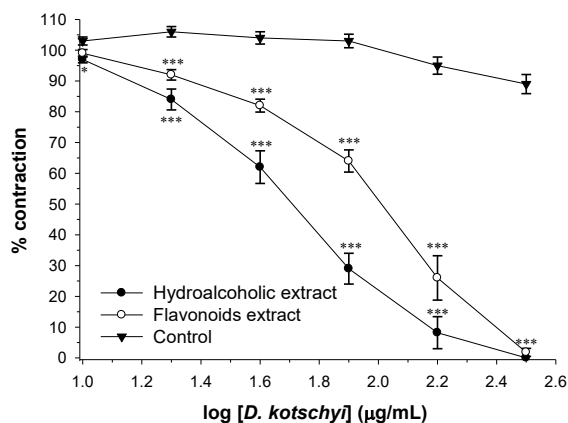

Figure 4. Inhibitory effect of *Dracocephalum kotschyi* extracts on KCl induced contraction in rabbit isolated bladder strips. Results are expressed as percentage of initial response prior to addition of the extracts. Data are presented as mean ± SEM (n = 6). Asterisk symbols show statistically significant difference in comparison with the control group treated with the vehicle (*P < 0.05, ***P < 0.001, Student's *t* test). The highest used concentration of vehicle (DMSO, 1.5%) slightly but significantly attenuated KCl induced response (ANOVA, *P < 0.001).
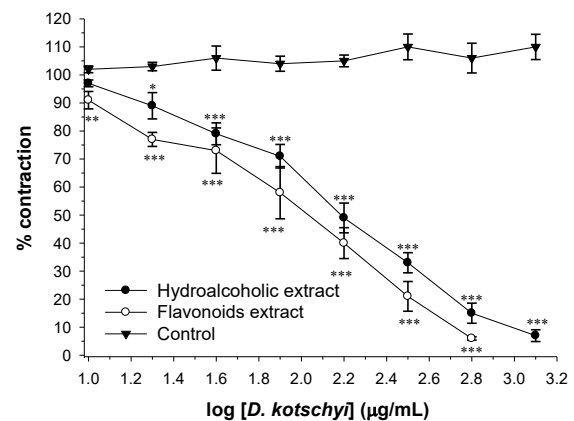

Figure 6. Inhibitory effect of *Dracocephalum kotschyi* extracts on electrical field stimulation (EFS) induced contraction in rabbit isolated bladder strips. Results are expressed as percentage of initial response prior to addition of extracts. Data are presented as mean ± SEM (n=6). Stars show statistically significant difference in comparison with the control group treated with the vehicle (*P < 0.05, **P < 0.01, ***P < 0.001, Student's *t* test). There was no reduction in the tissue response in the control group treated with equivalent volume of vehicle (DMSO). Maximum used concentration of DMSO was 1.6%.

both longitudinally and circularly (29). Although most smooth muscles have common pharmacologic properties nevertheless, significant variation has been seen in their responses. Detrusor smooth muscles are long, spindle-shaped cells like other typical smooth muscle cells (29-34). However, they have capability to relax to several hundred micrometers long (33, 34). This property was responsible for the relaxation of bladder strips in the organ bath. This elongation allows urine storage during filling period. Micturition is due to synchronized and fast muscle contraction which is due to parasympathetic activity initiated by stretch reflex from bladder wall (35,36). Acetylcholine released from parasympathetic neurons act on muscarinic receptors. Activation of muscarinic receptors increase phospholipase-C activity, production of inositol triphosphate (IP_3), release of intracellular Ca^{2+} ions, and smooth muscle contractions. In addition to autonomic nervous system, there are substantial reports that non-adrenergic non-cholinergic (NANC) neurotransmitters including ATP, nitric oxide, vasoactive intestinal peptide, neurokinines and prostaglandins also have functional roles in the bladder contraction and relaxation process (34,37). Furthermore, it has been reported that a minority of muscle fibers generates spontaneous action potentials in response to bladder stretches (36) which could explain spontaneous activity observed in the bladder strips suspended under 1 g tension in the organ bath. The main objective of this research was to investigate effect of *D. kotschyi* extract on isolated strips of detrusor smooth muscles. For this purpose each tissue was initially challenged with ACh. Bladder strips comprised of detrusor smooth muscles quickly responded to ACh with fast and sharp contraction. Smooth muscle contraction is initiated by an increase in the cytoplasmic Ca^{2+} ions concentration (38,39). ACh mainly acts on M_2 and M_3 subtypes receptors in the detrusor smooth muscle (40,41). These receptors are G-protein couple receptors associated with phospholipase C enzyme system and generation of intracellular second messenger IP_3 (42). IP_3 causes release of Ca^{2+} ions from intracellular stores (42). Like other smooth muscles, increase in free cytoplasmic Ca^{2+} ions activates myosin light chain kinase. Phosphorylation of myosin light chain, results in shortening of myofilaments and thus force is generated (43, 44). In the case of KCl induced contraction, enhancement of intracellular Ca^{2+} ion concentration is mainly due to Ca^{2+} influx through L-type calcium channels (45-47). Nifedipine, a selective blocker of L-type Ca^{2+} channels, (48) by blocking Ca^{2+} entry into the cell abolished KCl induced contraction of bladder strips. On the other hand, propantheline, a muscarinic receptor antagonist, did not affect KCl induced response but totally removed ACh induced contraction in the bladder strips suspended in the organ bath. This is because propantheline antagonizes muscarinic receptors on the cell membrane (49,50).

Nifedipine partially inhibited ACh response and this may indicate that following release of Ca^{2+} from sarcoplasmic reticulum, voltage gated Ca^{2+} channels are activated via Ca^{2+} induced calcium channel activation (46). Application of EFS stimulates all the neurons networks in the bladder wall and therefore directly and indirectly stimulates smooth contraction. The nature of contraction depends of contribution of types of neurotransmitters released during stimulation periods. Partial inhibition of EFS response by propantheline indicates that cholinergic transmission has partial role in the process of EFS stimulation. This is consistent with other reports which investigated effect of muscarinic antagonist on bladder EFS stimulation (51). This shows that NANC neurotransmission has major role in contraction induced by application of EFS on bladder smooth muscles. Partial inhibition of EFS response by nifedipine indicates that activation of voltage gated Ca^{2+} channels has major contribution in the EFS induced contraction.

Both hydroalcoholic and flavonoids extracts of *D. kotschyi* inhibited bladder contraction induced by KCl, ACh and EFS in bladder strip suspended in the organ bath. These findings indicate that active substance in the extracts may have affected the final stage of contraction process. However, another possibility is existence of several active ingredients in the extract with different mechanism of action which of course needs to be investigated. Comparison of total and flavonoids rich extracts also indicates that *D. kotschyi* extract is enrich in active components. For instance, it can be seen that total hydro-alcoholic extract is more effective than flavonoids rich extract in inhibiting KCl induced responses (see Figure 4). This shows existence of active ingredients in the total extract which probably are not flavonoids and possibly dissolved in the chloroform phase and needs proper investigation. Among the flavonoids identified in the *D. kotschyi* extract apigenin and luteolin have antispasmodic activity on isolated ileum and uterus smooth muscles (20,23, 52).

These findings confirm spasmolytic action of *D. kotschyi* extract on isolated rabbit bladder and furthermore indicate that active flavonoids exist that have substantial roles in inhibiting bladder contraction. Although *D. kotschyi* is used as food flavoring agent and herbal medicine, our results indicate that potent pharmacologically active compounds exist in the plant extracts.

Inhibition of bladder contraction means that over consumption might result in urinary retention. Urinary retention is major problem with muscarinic antagonist and contraindicated for patients with prostate hypertrophy. As *D. kotschyi* extract is more potent than propantheline, it should be warned that it may aggravate micturition difficulty in people with prostate hypertrophy. On the other hand, in patients with bladder incontinence, *D. kotschyi* could inhibit uncontrolled spasm of bladder and

could have beneficiary effect for these patients. However, its effectiveness must be determined in clinical situation.

Conclusion
This study confirms spasmolytic activity of *D. kotschyi* extract on isolated rabbit bladder *in vitro*. This means that although *D. kotschyi* extract may have beneficiary effect for control of bladdery incontinence due to involuntary bladder spasm. However, it should be avoided in patients with prostate hypertrophy.

Authors' contributions
HS was project manager and was responsible for writing the paper. SES was supervised extract preparation. HS supervised the pharmacological studies. AT was responsible for the experimental work and analysis of data. All authors approved the final manuscript.

Conflict of interests
The authors declare no conflict of interests.

Ethical considerations
Animal care and experiments were performed in accordance with the guidelines for the care and use of laboratory animal of the Isfahan University of Medical Sciences. The project was confirmed by the ethical committee of the university (IR.MUI.RESEARCH. REC.1398.047).

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