Antispasmodic effect of *Dracocephalum kotschyi* hydroalcoholic extract on rat ileum contraction

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**Abstract**

*Dracocephalum kotschyi* Boiss. (Labiatae) is a traditional medicine which is believed to have antispasmodic and analgesic activities. The antispasmodic action of *D. kotschyi* essential oil has been shown in a previous report. The objective of this research was to study antispasmodic activity of hydroalcoholic extract of *D. kotschyi* on ileum contractions. Hydroalcoholic extract was obtained from aerial part of *D. kotschyi* using percolation method. For antispasmodic studies, a portion of rat ileum was suspended under 1g tension in Tyrode’s solution at 37 °C and gassed with O2. Effect of the *D. kotschyi* extract was assessed on ileum contractions induced by KCl (80 mM), acetylcholine (ACh, 500 nM) and electrical field stimulation (EFS). The *D. kotschyi* extract concentration dependently inhibited the response to KCl (IC50=36 ± 5.1 μg/ml), ACh (IC50=101 ± 9.5 μg/ml), EFS-1 (IC50=96 ± 7.1 μg/ml) and EFS-2 (IC50=53 ± 4.3 μg/ml). From this experiment it was concluded that *D. kotschyi* extract possessed potent antispasmodic activity. Therefore, identification of the active component(s) is (are) recommended in order to find the best lead compound for drug development.

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

**INTRODUCTION**

*Dracocephalum kotschyi* Boiss. (Labiatae) is an endemic herbaceous plant known in Iran as Badrandjboie-Dennaie and Zarrin-giah (1). *Dracocephalum* genus belongs to the Lamiaceae family (2). 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 (3). All these plant species in traditional medicine are used as carminative and tonic as well as for the treatment of aliment such as congestion, headache, stomachache and liver diseases (3,4).

*D. kotschyi* Boiss. is an aromatic and medicinal plant which grows in high and mountainous parts of Iran (5). It is a considerable plant for its high amount of essential oil (6). 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 (7,8). The essential oil *D. Kotschyi* has strong spasmylytic activities on isolated ileum (9). Boiled extract of this species is used as antispasmodic agent in Iranian traditional medicine (1). The constituents of the alcoholic extract has already separated and identified. These include, calycopterin, xanthomicrol, isokaempferide, luteolin, apigenin, luteolin 7-O-beta-D-glucopyranoside, luteolin 3'-O-beta-D-glucuronide, apigenin 4'-O-beta-D-glucopyranoside, acacetin 7-O-beta-D-glucopyranoside and rosmarinic acid (10).

Pharmacological studies have confirmed some medicinal properties of *D. kotschyi* including-antinociceptive (11)-antihyperlipidemic (12), immunomodulatory (13) and cytotoxic (14) effects.

Despite existence of scatter reports for the use of *D. Kotschyi* extract as antispasmodic herbal medicine there is no official
pharmacological report which could support the antispasmodic effect of its extract. Therefore, the aim of current study was to examine the inhibitory effect of *D. kotschyi* extract on rat ileum contraction using isolated tissue technique.

**MATERIALS AND METHODS**

**Drugs and solutions**

Tyrode's solution composed of (mM): NaCl, 136.9; KCl, 2.68; CaCl₂, 1.8; MgCl₂, 1.05; NaHCO₃, 11.9; NaH₂PO₄, 0.42 and glucose, 5.55, were made up in distilled water. Unless stated, all chemicals and drugs were from Merck.

Acetylcholine hydrochloride was purchased from Sigma Chemical Co. (Germany). The extract was made up as 20 mg/ml stock solution in dimethyl sulphoxide (DMSO), dilution being made in distilled water (2 mg/ml and 200 µg/ml). KCl (2 M) stock solutions were made up in distilled water. ACh was made up as 100 mM stock solution and acidified by 1% acetic acid, and further serial dilution was made in distilled water.

**Plant materials**

*D. kotschyi* aerial parts were collected from Chadegan (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 Pharmacognosy Department in 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 (15). The yield of dried extract was about 30%.

**Antispasmodic assessment**

Wistar rats obtained from School of Pharmacy and Pharmaceutical Sciences animal house in Isfahan. All animals were handled in accordance with the internationally accepted principles for laboratory animal use and care, as recommended by university authority (16). The animals were stunned to death, and the abdominal cavity was immediately opened with surgical scissors and 10-15 cm of ileum was clipped off. The intestine was placed in 250 ml warm Tyrode's solution and was transported to the laboratory, where it was immediately aerated with oxygen. Fresh ileum was cut into several 2-3 cm long sections. The intestinal was freed from the mesenteric and fat attachments. Each ileum section was mounted vertically in an organ bath (Harvard, England), one end was connected to the lower hook of the bath and the other end was connected to a force transducer. Ileum preparation was set up under 1g resting force in Tyrode's solution at 37 °C and gassed continuously with oxygen. Changes in length of preparation were recorded isotonically and printed on a Harvard Universal Oscillograph (England) pen recorder device. The tissue was washed several times every 15 min and allowed to relax to a stable baseline.

Contractions were induced by KCl (80 mM), acetylcholine (ACh, 500 nM) or electrical field stimulation (EFS, 6 V and 50 Hz for 1s duration in 10 min intervals) as described before (17). ACh was added into the tissue bath to give the final bath concentration requested. It was allowed to act for 30 s before it was washed with fresh Tyrode's solution. In the case of KCl drugs were added into the bath in a cumulatively manner. After reproducible contraction were established the extract or equal volume of the vehicle were added directly into organ bath at 10 min intervals. Initially a number of pilot experiments were carried out for determination of effective concentration ranges of the extract of *D. kotschyi*. Then full concentration response curves were obtained using 6-9 different concentrations of drugs. After maximum inhibitory effect was achieved, the tissue were washed with fresh Tyrode's solution and tested to see if the effect of extract was reversible.

**Measurements and statistical analysis**

Contractile response to KCl, ACh and EFS were measured as maximum amplitude from the baseline and expressed as the percentage of the initial response prior to addition of the extract or the vehicle. All the values are quoted as mean ± standard error of the mean (SEM).
Statistical significance were assessed using a one-way analysis of variance (ANOVA) for repeated measures and when appropriate were 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 response), was calculated. SigmaPlot computer program was used for statistical analysis and plotting the graphs.

**RESULTS**

Rat isolated ileum suspended in the fresh Tyrode’s solution gradually relaxed to a stable baseline over 10-20 min. Rat ileum contracted rapidly to EFS, reaching a peak (EFS-1) followed by partial relaxation which was then followed by a second peak (EFS-2) and then relaxed towards the baseline as described before (17,18).

In previous studies it has been shown that these parameters, mainly stimulate the neurones in the enteric nervous system (17,19). *D. kotschyi* extract (20 µg/ml–640 µg/ml) concentration dependently reduced both initial and secondary contractile responses to neuronal stimulation. At its highest used concentration, the extract totally removed the response to EFS (Fig. 1).

The inhibitory concentration causing 50% of maximum response were 96 ± 7.1 µg/ml and 53 ± 4.3 µg/ml for EFS-1 and EFS-2 respectively. After washing the tissue with fresh Tyrode’s solution, the contractile response to neuronal stimulation was gradually restored. There was no statistically difference in the vehicle treated time match control tissues over the course of studies (Fig. 1).

ACh caused a rapid contraction in rat ileum during 30 s of the contact time. The hyroalcoholic extract of *D. kotschyi* (20-320 µg/ml), concentration dependently inhibited ileum contraction induced by ACh (500 nM, Fig. 2). The extract at 320 µg/ml bath concentration removed the contractile response to ACh, while in the time matched vehicle treated control group (Fig. 2) there was a steady but statistically significant increase in contractile responses to ACh. The IC$_{50}$ value for the extract was 101 ± 9.5 µg/ml. The inhibitory effect of the extract on ACh responses was reversible following washing the tissue with fresh Tyrode's solution.

**Fig. 1.** Effect of *Dracocephalum kotschyi* extract on tension development to a; first and b; second contractile responses to electrical field stimulation (EFS, 6 V, 50 Hz, 1 s duration) in isolated ileum of rats. Ordinate scale: ileum contractions expressed as percent of 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 changes 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: $^*P<0.05$, $^{***}P<0.001$ (Student's t-test). Maximum concentration of vehicle (DMSO) in the bath was 1.6%.
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**Fig. 2.** Effect of *Dracocephalum kotschyi* extract on tension development to acetylcholine (ACh, 500 nM) in isolated ileum of rats. Ordinate scale: ileum contractions expressed as percent 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 increase 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*<0.001 (Student's *t*-test). Maximum concentration of vehicle (DMSO) in the bath was 0.63%.

**Fig. 3.** Cumulative effect of *Dracocephalum kotschyi* extract on tension development to potassium chloride (KCl, 80 mM), in isolated ileum of rats. Ordinate scale: ileum contraction 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). The oscillation in the control group 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 the vehicle (DMSO) in the bath was 0.36%.

KCl (80 mM) on the other hand, caused a tonic contraction which maintained for the duration of study. Relaxant effect of *D. kotschyi* extract were also examined on tonic contractions induced by KCl. *D. kotschyi* extract in a concentration dependent manner inhibited ileum contractions induced by KCl (Fig. 3). The inhibitory effect was started with 8 µg/ml of the extract in the bath and with 160 µg/ml extract in the bath the tissue returned to the initial baseline (i.e. removal of total contraction). The IC$_{50}$ value...
of hydroalcoholic extract of *D. kotschyi* on the contractile response of KCl was 36 ± 5.1 µg/ml. Following washing the tissue with fresh Tyrode's solution, the inhibitory effect of the extract was reversible. The oscillation in responses of vehicle treated time-matched control tissues (Fig. 3) was not statistically significant (*P* > 0.05).

**DISCUSSION**

On the basis of pharmacological and electrophysiological studies, it has been suggested that the non-adrenergic non-cholinergic (NANC) part of the nervous system in addition to classic adrenergic and cholinergic components plays an important role in the regulation of intestinal motility (20,21).

Addition of high concentration of KCl into the organ bath, cause a sustained contraction which is due to activation of L-type calcium channels and increase in intracellular Ca^{2+} concentration (22). This effect of KCl can be blocked by Ca^{2+} channel blockers including nifedipine (19). ACh is a natural neurotransmitter that is released during neuronal stimulation (20,21). It causes contraction by activating muscarinic receptors on illume smooth muscle cells (21,23). Contractions induced by ACh are competitively blocked by muscarinic antagonists such as atropine or propantoline (17,19). These M₃ muscarinic receptors are coupled to phospholipase C via a G protein (24-26). Therefore, activation of muscarinic receptors by ACh results in increase in inositol triphosphate production and release of Ca^{2+} from intracellular stores (24). However, there are several other neurotransmitters which are present in the enteric nerves system and are release during neuronal stimulation (20,21). Some of these neurotransmitters are also responsible for the contractile responses seen with EFS (18). For this reason, muscarinic antagonist only partially blocks the contractile responses to neural stimulation (17,19,21).

*D. kotschyi* extract totally remove the contractile responses to KCl, exogenous ACh as well as neuronal stimulation. Overactivities of enteric nervous system is considered to be responsible for a number of gastrointestinal disorders (27,28). Therefore, in this study we have shown that *D. kotschyi* extract can alleviate overactivity of gut motility and it would be a useful aliment for disease such is irritable bowl syndrome as well as diarrhoea. Of course in all pharmacological research drug concentration and selectivity is always an important issue. Comparison of inhibitory concentration of *D. kotschyi* extract with other reported plant extract with antispasmodic activity at IC₅₀ level indicate the *D. kotschyi* extract is about 10 times more potent than extracts of *Rosa damascene*, *Prangos ferulacea*, *Zataria multiflora*, *Satureja hortensis*, *Passiflora incarnata*, *Berberis integerrima*, *Crocus sativus*, *Ferula gummosa*, *Teucurium poliume*, *Melissa officinalis*, and *asa-foetida* (17,19,29-33) and it is relatively similar to inhibitory extract of *Pycnocycla spinosa* (34,35). In previous reports the antispasmodic effect of essential oil of *D. kotschyi* was also reported (9) but generally speaking, the essential oils have less specific action and are prone to various unwanted effect.

The extract, on the other hand, are more stable and give a high yield and can be produced economically. The components which have been identified in *D. kotschyi* extract are calycopterin, xanthomicrol, isokaempferide, luteolin, apigenin, luteolin 7-O-beta-D-glucopyranoside, luteolin 3'-O-beta-D-glucuronide, apigenin 4'-O-beta-D-glucopyranoside, acacetin 7-O-beta-D-glucopyranoside and rosmarinic acid (10). Trimethoxyflavone compounds are common substances found in several plant extract including *D. kotschyi* (10).

Trimethoxyflavone reported to cause concentration dependent vasorelaxation of isolated rat aorta (36) and therefore, same authors attributed the antispasmodic effect of extract to a synergistic activity among flavonoids and terpenoids (37). However, so far there is no direct report about antispasmodic activity of these agents. Therefore, identification of most active component in the *D. kotschyi* extract is recommended for further research.
CONCLUSION

In this research we have provided pharmacological evidence for effectiveness of *D. kotschyi* hydroalcoholic extract as an antispasmodic agent. As the extract of *D. kotschyi* is relatively more potent than some other known antispasmodic plant extracts, further drug design and development for identification of the lead compound is recommended.

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