



# Bioassay standardization of drug dosage form prepared from hydroalcoholic extract of *Dracocephalum kotschy*

Hassan Sadraei<sup>1\*</sup>, Amir Hossein Rasouli-Amirabadi<sup>1</sup>, Afsaneh Yegdaneh<sup>2</sup>, Naser Tavakoli<sup>3</sup>

<sup>1</sup>Department of Pharmacology and Toxicology, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran

<sup>2</sup>Department of Pharmacognosy, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran

<sup>3</sup>Department of Pharmaceutics, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran

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## ABSTRACT

**Introduction:** Zaringiah (*Dracocephalum kotschy*) is a famous Iranian herbal plant with anti-inflammatory and spasmolytic activities. There is no standard drug dosage form for the *D. kotschy* extract on the market. The objective of this project was to design a suitable oral dosage form for the hydroalcoholic extract of *D. kotschy*.

**Methods:** Standard granules were prepared using the moist granulation technique. Physical properties of the granules were determined before filling the capsule with fixed doses of the drug (25 mg and 50 mg). Syrup was prepared in sucrose solution at 5 mg/mL concentration. Bioactivity and phytochemical assays were used for dosage form stability and uniformity evaluations before and after 3- and 6- months incubation. Pharmacological bioassay method was designed to determine the bioactivity of the products before and after incubation. Pharmacological effects of the prepared capsule and syrup were determined on rat isolated ileum and intestinal meal transit, respectively.

**Results:** In this study, *D. kotschy* extract was effectively formulated as capsule and syrup for oral consumption. Environmental and aging factors had no significant effect on the total flavonoid or phenolic contents or bioactivity of the manufactured products. Furthermore, the ingredients used in the formulation had no effect on the bioactivity of the active substances in the extract.

**Conclusion:** The standard oral dosage forms prepared from *D. kotschy* extract can be used for clinical trials. In addition, we introduced a reliable bioassay technique, which might be applied for the evaluation of herbal medicines with antispasmodic activities.

### Implication for health policy/practice/research/medical education:

This paper provides information for drug granule preparation in order to make standard solid dosage forms of *D. kotschy* extract. Furthermore, this paper provides a bioactivity assessment designed for *D. kotschy* extract based on the antispasmodic activity. This bioassay technique could effectively be used for the standardization of the product. The prepared standard capsule and syrup can be used in further investigations, including clinical trials for the treatment of asthma.

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## Introduction

Natural products derived from herbal plants are important source for the identification of new therapeutic agents (1). The main disadvantage of natural products is that they often contain numerous active and inactive substances with great biochemical diversity (2). Therefore, it is more convenient to identify and purify lead compounds for medicinal use. However, in many cases purification of active compounds turned out to be difficult and

commercially expensive. Furthermore, the active substances could be complex molecules that are difficult to synthesize or modify by conventional synthetic chemistry (3,4). Therefore, utilization of crude extract is sometimes a more feasible and convenient way for the preparation of effective herbal medicine.

Zaringiah (*Dracocephalum kotschy* Boiss.) is a perennial plant belonging to Labiatae family, which grows in relatively cold and high altitude locations (5). Zaringiah

\*Corresponding author: Hassan Sadraei,  
Email: sadraei@pharm.mui.ac.ir

is famous as a natural food flavoring agent. However, it also is traditionally used by local people for the treatment of rheumatism, gastrointestinal disorders and asthma (6,7). Advanced pharmacological studies have confirmed the anti-inflammatory, immunomodulatory, and anti-spasmodic actions of the *D. kotschy* extract (8-14). For instance, the hydroalcoholic extract of *D. kotschy* has been shown to inhibit ileum contractions both *in vitro* and *in vivo* situations (8-11). In addition, it has anti-inflammatory effects on the stomach ulcer and induced colitis in animal models (15,16). Furthermore, *D. kotschy* extract inhibits bronchial smooth muscle contractions and has shown both anti-inflammatory and anti-fibrotic properties in bleomycin induced bronchitis (12,14). In order to find a clue for the possible mechanism of action, the effect of *D. kotschy* extract was examined on the expression of key inflammatory mediators and main signaling molecules involved in the regulation of inflammation. *D. kotschy* extract inhibited gene expressions of nuclear factor (NF- $\kappa$ B), cytokine levels, and phosphorylated forms of stress activated protein kinases/c-Jun N-terminal kinase (SAPK/JNK), as well as signal transducer and activator of transcription (STAT-3 and p-38). Therefore. The anti-inflammatory effect of hydroalcoholic extract of *D. kotschy* might be through reduction in the expression of key mediators of inflammation (17).

A combination of anti-spasmodic and anti-inflammatory actions makes *D. kotschy* extract an ideal remedy for the treatment of bronchoconstriction associated diseases, including asthma. Traditional application and preliminary pharmacological investigations are also in consistence with the anti-asthmatic effect of *D. kotschy* extract.

Following several investigations and optimization of *D. kotschy* extract on wide ranges of preclinical assays, the next stage was demonstration of effectiveness and stability of the product following suitable formulation (18). Most drugs that are administered as medicine have been formulated along with other materials known as excipients, which are pharmacologically inactive (19). The formulation serves the following purposes: The drug is presented in a convenient form for administration, the rate of disintegration and/or solution of medicine is regulated, and drug stability is improved, and an accurately measured dose can be administered (20). Most drugs are given orally as tablets or capsules (21). Advantages of these formulation include precise control of dose and chemical stability of drugs as dry solids (22). Two processes precede absorption, disintegration, and dissolution. The rate of disintegration is determined by pharmaceutical formulation. Minimum standards are specified in the pharmacopoeias (23). Disintegration may be deliberately delayed by an 'enteric coating' which is soluble only at the higher pH of intestine. The dissolution rate depends on the particle size, ambient pH, and drug type (24,25). In solutions, the drug is available for absorption through the appropriate mucosa membrane.

Drug formulations usually are quantified by dissolution test (23). However, herbal extracts contain numerous active and inactive substances with different solubility, which make it so difficult to prepare optimum dissolution medium. Furthermore, the amount of a single substance dissolved in the medium is often so small that its quantification turns out to be very costly. That's why herbal medicinal products are routinely standardized according to their total flavonoid or phenolic contents (26). Therefore, an alternative approach of product quantification needs to be considered. Bioassay plays a key role in purification and identification of bioactive substances (27,28). Bioassay is also a method of measurement in pharmacology (28). The bioassay technique provides requirements for measuring drug effect of the same substances under different circumstance. It is defined as the estimation of the concentration or potency of a substance by measuring the biological response that it produces (28,29). Bioassay is useful in the study of new chemically mediated control system. Mediators in such system are often first recognized by the biological effects that they produce. The first clue may be the finding that herbal extracts produce effects on assay systems. For example, the ability of *D. kotschy* extract to inhibit contraction of the ileum can be developed as quantitative an assay procedure for the standardization of the extract. Therefore, in this research a suitable biological assay was designed to evaluate the pharmacological efficacy of *D. kotschy* extract before and after the preparation of standard dosage form.

## Methods and Materials

### Chemicals and solutions

The following substances were used in this project. Ethanol (Iran),  $\text{Ca}_3(\text{PO}_4)_2$ , KCl, NaCl,  $\text{CaCl}_2$ ,  $\text{NaH}_2\text{PO}_4$ ,  $\text{MgCl}_2$ ,  $\text{AlCl}_3$ , glucose, lactose, sucrose, corn starch, sodium acetate trihydrate, charcoal, tragacanth, acetic acid, gallic acid, quercetin, Folin-Ciocalteu's reagent, capsule shell (Iran). Starch-base adhesive was prepared by adding water to the starch (8%) with mild heating. Tyrode's solution was prepared with the following composition (mM): 136.9 mM NaCl, 1.8 mM  $\text{CaCl}_2$ , 2.68 mM KCl, 11.9 mM  $\text{NaHCO}_3$ , 1.05 mM  $\text{MgCl}_2$ , 0.42 mM  $\text{NaH}_2\text{PO}_4$ , 2.0 mM and 5.55 mM glucose. Unless stated, all the chemicals were from Merck.

### Plant materials

During the flowering time (June 2020), the aerial parts of Zaringiah were collected from Pertican farm located in the Shahankoh of Fareydunshar (Isfahan province, Iran). Zaringiah was identified as *Dracocephalum kotschy* by botanist Mohamad Asfa from the Department of Natural Resources in Isfahan province. A sample voucher was deposited in the Department of Pharmacognosy herbarium (1519) at the School of Pharmacy and Pharmaceutical Sciences. The collected plant materials were dried in the shade with free ventilation. The dried

materials were pulverized into a fine powder by a miller (Keep, Korea). This powder was moisturized with 70% ethanol for 2 hours before it was packed into a percolator. The percolator was filled with 70% ethanol using 1 to 8 ratio (powder/solvent). Special care was made to ensure that entire materials were soaked and covered with the ethanol. The maceration was continued for three days with regular agitation, before the extract was collected. The reservoir was then refilled with fresh ethanol and the maceration procedure was repeated thrice. The eluted extracts were filtered through Whatman filter papers. The extracted fluid was then concentrated under vacuum using Heidolph rotary evaporator (Germany) at 40°C until a viscous residue obtained. The yield of the dried extract was calculated (W/W), and the extract was stored in a refrigerator.

#### Drug dosage form preparation

The concentrated hydroalcoholic extract contained 30% moisture; therefore, the moist granulation technique was used for the preparation of granules (30). Calcium triphosphate, lactose, and starch were mixed with 2:1:1 ratio, respectively. The hydroalcoholic extract was then added in a ratio of 1 to 20 for 25 mg capsules and 1 to 10 for 50 mg capsules, and mixed for 30 minutes in a mixing machine. Starch-based adhesive was then added step by step until suitable granules were formed. The product was sieved with mesh No 18 and dried in a cabinet granule dryer (Dott. Bonapace & C, Italy) at 50°C for 6-7 hours. The capsules were prepared by punched method (31,32). The capsule shells were filled with 500mg of the prepared granules using a manual capsule filling machine (31,32). The average capsules (empty shell and filled capsules) weight was also determined.

#### Granule physical controls

First of all, the granules densities were determined. The volume of the granules were determined by pouring 100 g granules into a measuring cylinder for determination of primary density. The cylinder then dropped three time from 5cm altitude on a wooden bench for determination of bulk density. The tapping continued until volume was fixed and tapped bulk density was determined. Fixed funnel method was used for determination of the granules flow ability and its angle of repose (33). The prepared granules (100 g) were placed in a funnel with 0.5 cm orifice and the emptying rate was assessed. The funnel, filled with 100 g granule, was held 8 cm above the bench and the granule was released into the surface to form a pyramidal shape. The diameter of the pyramid and its height were measured in order to determine the angle of repose.

Capsule disintegration time was determined using the United States Pharmacopeia (USP) method (23,34). Six capsules were placed in the disintegrating basket (Pharma Test, Germany), filled with a litter acetate buffer (2.99 g sodium acetate trihydrate+1.66 mL acetic acid+distilled

water), and allowed to disintegrate for 30 minutes.

#### Syrup preparation

One hundred and two grams sucrose was placed in a suitable glass container, moistening with warmed distilled water. After cooling down, the extract (600 mg) was added and mixed regularly. Sufficient amount of distilled water was added to bring the volume to 120 mL. The prepared syrup was stored in a non-transparent bottle in the fridge.

#### Stability study tests

According to the USP, drug stability refers to “the extent to which a drug substance or product retains within the specified conditions, the same properties and characteristics that it possessed at the time of its manufacture” (23). Accelerated stability test was used for capsule and syrup shelf-life stability (35). In this test, the products were stored at elevated temperature (40°C) and humidity (75%) for 3 and 6 months.

The principle of standardization, as applied to a crude preparation, is a required standard such as physical, chemical, or physiological properties. The objective of standardization is to ensure the uniformity of the product, especially with respect to biological activity. Herbal extracts are normally standardized with their total flavonoids (36,37). Standardization of herbal medicine refers to confirmation of its identity and determination of its quality. Therefore, the quality control of prepared capsules and syrup was assessed using biochemical and bioassay techniques.

#### Phytochemical standardization

Folin-Ciocalteu's phenol reagent test (36) was used for the determination of total phenolic compounds in the crude extract and prepared drugs before and after 3- and 6-month of incubation. In addition, total flavonoids were also measured using a colorimetric aluminum chloride technique (37).

#### Bioassay standardization

Bioassay is a standard method that can be used to assess the amounts of biologically active substances present in a complex mixture. Establishment of concentration-response curve was used for comparing biological activity of the crude extract with the drug preparations before and after incubation for 3 and 6 months. For this purpose, relative inhibition of pre-contracted ileum by *D. kotschyi* extract was determined in the crude form and the prepared drug formulations. Twelve capsules (25 or 50 mg) were opened up and dissolved in 30 mL DMSO to make 50 mg/mL stock solutions. After solubilizing the extract, precipitants were removed by paper filtration using a Buchner flask.

The pharmacological bioassay study was approved by the university ethical committee for laboratory animal care and welfare (IR.MUI.RESEARCH.REC.1399.441) (38).

Male Wistar rats (180-220 g) were purchased from the School of Pharmacy and Pharmaceutical Sciences animal house and killed by carbon dioxide asphyxiation. A piece of ileum was dissected out and placed in an oxygenated Tyrode's solution. A strip of ileum was cut and placed in a petri dish filled with Tyrode's solution. The connective tissues were gently trimmed off and both ends of the strip were tied with two separate threads. One thread was tied to a tissue holder. The tissue holder was then secured into an organ bath (Palmer, England). The string at the top of the ileum was then attached to the displacement transducer, which converted the contractions into electrical signals for recording on Harvard Universal Oscillograph device (England). Isotonic contractions of the tissues were recorded under 1 g tension. All the tests were conducted on at least six different tissues.

Sustained contraction was induced with the addition of KCl (80mM) into the organ bath. Following establishment of stable contraction, the extract solution was added in a cumulated manner using two folds increment in the concentration until a full concentration-response curve was constructed. Drug concentration causing 50% of maximum response ( $IC_{50}$ ) was calculated for the crude extract and the prepared capsules before and after 3- and 6-month of incubation.

For the assessment of the syrup biological activity, the charcoal meal transit technique was used (11). The mice were fasted overnight with free access to water. The animals were kept in separate cage with free movement and access to fresh air in the School of Pharmacy and Pharmaceutical Sciences animal house. The charcoal meal was prepared in distilled water by mixing an equal volume of charcoal (3%) with tragacanth (5%). *D. kotschyi* extract (80 mg/kg) or the syrup (80 mg/kg) were given orally to the mice. Half an hour later, 0.5 mL of charcoal meal suspension was administered. The mice were killed 45 minutes later by  $CO_2$  asphyxiation, and small intestine was dissected out and the charcoal meal travelled down

the intestine was measured. Ten mice were used for each group of experiments.

#### Statistical and data analysis

Ileum contractions were expressed as a percentage of the height of the initial contraction induced by KCl from the baseline. The  $IC_{50}$  value was calculated to form the plotted concentrations-response curve for each tissue. The percentage of intestinal meal transit was assessed by measuring the distance movement from the pylorus toward the caecum relative to the whole length of the small intestine. All the results were presented as mean  $\pm$  standard error of the mean (SEM). Data were compared statistically using unpaired Student's t-test or one way analysis of variance (ANOVA) as appropriate.

For the determination of total phenolic and flavonoid constituents, standard linear curve was constructed for gallic acid and quercetin, respectively. Phenolic or flavonoid contents were determined from the standard curves using corresponding light density values. Each sample was examined three time.

## Results

### Phytochemical analysis

The yield of dried extract was calculated as 33.5% (W/W). Total phenolic compounds determined by Folin-Ciocalteu's phenol reagent test was 90.5 mg/g extract. The total flavonoid content of the extract determined by colorimetric technique was calculated as 12.6  $\mu$ g/g extract. The amount of phenolic and flavonoid contents in the capsules and the syrup before and after incubation are compared in Table 1.

### Physical analysis

The applied method of granulation produced fine particles with irregular spherical shapes. The prepared granule (primary, bulk and the tapped bulk) densities were determined as 25 g/mL, 0.27 g/mL, and 0.33 g/mL,

**Table 1.** Assessment of total flavonoid and phenolic contents of *Dracocephalum kotschyi* extract and its dosage forms

Dosage form	Total flavonoids (Equivalent to ng quercetin)	Total phenolic content (Equivalent to $\mu$ g gallic acid)
25 mg capsule (0)	64 $\pm$ 3	82 $\pm$ 4
25 mg capsule (after 3 months)	59 $\pm$ 4	126 $\pm$ 15
25 mg capsule (after 6 months)	70 $\pm$ 6	40 $\pm$ 3
50 mg capsule (0)	202 $\pm$ 10	925 $\pm$ 11
50 mg capsule (after 3 months)	226 $\pm$ 12	900 $\pm$ 9
50 mg capsule (after 6 months)	224 $\pm$ 12	925 $\pm$ 13
Syrup (0)	19 $\pm$ 2	403 $\pm$ 7
Syrup (after 3 month)	10 $\pm$ 1	341 $\pm$ 5
Syrup (after 6 month)	13 $\pm$ 1	311 $\pm$ 5

Folin-Ciocalteu's phenol reagent test and colorimetric aluminum chloride test were used for the assessment of phenolic and flavonoid contents of the prepared capsule and syrup, respectively. Each measurement was repeated three times. The data are presented as mean  $\pm$  SEM before and after 3- and 6-month incubation of capsules or syrup at 40°C with 75% humidity.

respectively. The flow ability of the granules was 4.58 g/s, and the angle of repose was  $5.76 \pm 0.016$  ( $n=3$ ). The capsules were uniformly filled, and the samples had very close weight. In the disintegration test, all the gelatin capsules were dissolved within 30 minutes of the testing time. After 3 months of incubation, no changes in capsules appearance were observed. However, after 6 months of incubation, about 10% of the capsules' color was faded or punctured.

The *D. kotschy* extract comfortably spread into sucrose solution to form the syrup. Following 3- and 6-months of incubation, there was no change in the color or smell, and no precipitation occurred in the bottle filled with the syrup.

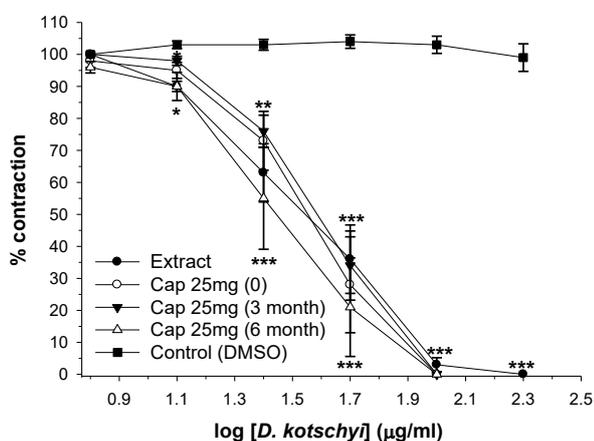
### Bioassay evaluation

The hydroalcoholic extract of *D. kotschy* in a concentration-dependent manner inhibited sustained contraction induced by KCl (80mM) in the rat isolated ileum (Figure 1). This finding was consistent with the previous reports (5-7). Equivolume amount of the vehicle (DMSO + water) had no effect on the induced contractions. When capsule contents were spread into DMSO, with magnetic stirring, the solution quickly got dark yellowish color, indicating the dissolution of the extract. The remaining precipitant on the filter paper had a white plane color, indicating that the precipitant was mainly the excipients. Examination of individual excipient solubility in the DMSO proved that only lactose was dissolvable in DMSO. The rest of the excipient was insoluble in DMSO. In order to exclude

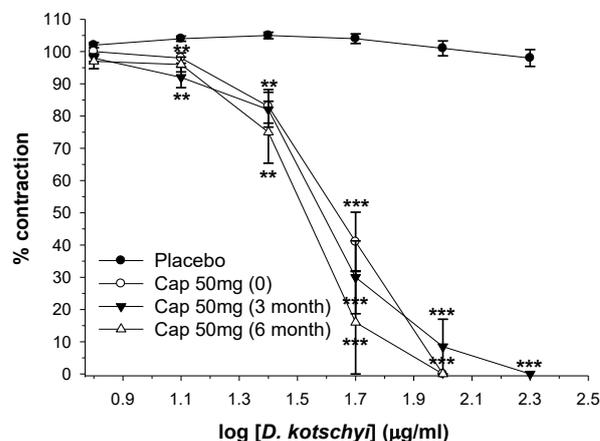
excipients intervention, under a similar condition, placebo capsules containing the excipients were also prepared and their biological responses were examined on ileum contractions under similar experimental conditions. The soluble component of the placebo in the DMSO had no effect on the rat ileum contractions induced by KCl. In fact, there was no statistically significant difference between the vehicle-treated control and the placebo-treated groups.

The hydroalcoholic extract solubilized from the capsules content, concentration-dependently inhibited rat ileum contractions induced by KCl (Figures 1 and 2). The concentrations-response curve constructed for the crude extract and the capsules, before and after 3- and 6-month of incubation, almost overlapped each other (Figures 1 and 2). Comparison of  $IC_{50}$  values showed no statistically significant difference between the crude extract and the prepared capsule dosage forms values. Furthermore, after 3- and 6-months of incubation under harsh conditions, no significant changes occurred in the antispasmodic activities of the preparations. The  $IC_{50}$  values are presented in Table 2.

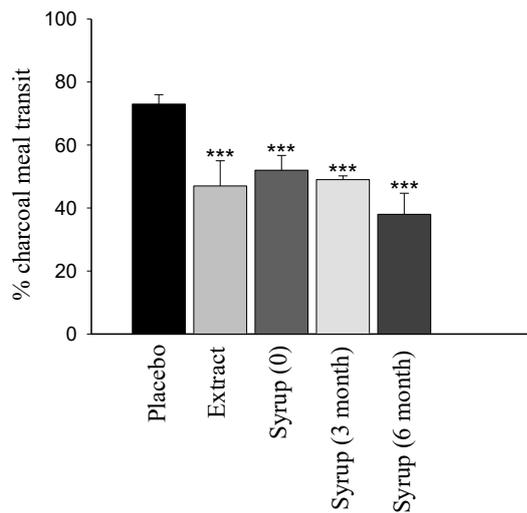
Oral administration of the hydroalcoholic extract of *D. kotschy* (80 mg/kg) inhibited the intestinal charcoal meal transit by 36% in comparison to the vehicle-treated control group (Figure 3). Oral administration of the syrup dosage form of *D. kotschy* extract (80 mg/kg) inhibited the intestinal meal transit by about 29% (Figure 3). There was no statistically significant difference in meal retardation induced by either the crude extract or the



**Figure 1.** Inhibitory concentration-response curve for the crude extract and the capsule dosage forms prepared from *Dracocephalum kotschy* extract (Cap 25 mg) before and after 3- and 6-month incubation time. Contraction was induced in rat isolated ileum by the addition of KCl (80mM) into the organ bath. Ordinate scale: tissues contractions expressed as % of initial KCl response. Abscissa scale:  $\log_{10}$  concentration of *D. kotschy*. Lines were drawn through the points using two-fold increments in the concentration. The points are the means, and the vertical bars show the SEM ( $n=6$ ). Stars show statistical differences between each extract 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 1%.



**Figure 2.** Inhibitory concentration-response curve for the crude extract and the capsule dosage form prepared from *Dracocephalum kotschy* extract (Cap 50 mg) before and after 3- and 6-month incubation time. Contraction was induced in rat isolated ileum by the addition of KCl (80mM) into the organ bath. Ordinate scale: tissues contractions expressed as % of initial KCl response. Abscissa scale:  $\log_{10}$  concentration of *D. kotschy*. Lines have been drawn through the points, using two-fold increments in the concentration. The points are the means, and the vertical bars show the SEM ( $n=6$ ). Stars show statistical differences between each extract concentration with its corresponding placebo treated control. Keys: \*\* $P < 0.01$ , \*\*\* $P < 0.001$  (Student's *t* test). Maximum concentration of the vehicle (DMSO) in the bath was 1%.



**Figure 3.** Biological quantitative comparison of the syrup dosage form prepared from the hydroalcoholic extract of *Dracocephalum kotschy* (80 mg/kg) on intestinal meal transit. Intestinal charcoal meal (0.5 mL; per orum) travelled within 45 minutes was compared with the syrup oral dose before and after 3- and 6-month incubation. Data indicating the percentage of meal moved down the small intestine are expressed as mean $\pm$ SEM (n=10). Stars show the degree of statistically significant difference in comparison with the appropriate placebo group (\*\* $P < 0.001$ , Student's *t* test). There was no difference between the crude extract or syrup dosage form before and after 3- and 6-month incubation.

syrup after 3- and 6- months of incubation in the standard pharmaceutical incubator.

## Discussion

Zaringiah is an Iranian herbal plant that is commercially cultivated for herbal medicinal utilization. Pharmacological investigations have revealed that in comparison with the other recognized herbal medicines possessing antispasmodic activity, *D. kotschy* extract has a higher potency, efficacy, and relative selectivity (8-17). Therefore, it was selected as suitable candidate for further pharmacological evaluations. The hydroalcoholic extract of *D. kotschy* is a potent relaxant of the ileum, bladder, uterus, and tracheal smooth muscles (8-13). In addition, it has anti-inflammatory and anti-fibrotic effects in pulmonary induced fibrosis (14). The anti-inflammatory and anti-spasmodic effects of *D. kotschy* extract have been reported to be due to active components exist in the essential oils and the flavonoid's contents (10,15,39). The following components exist in the essential oil:  $\alpha$ -terpineol,  $\alpha$ -pinene,  $\alpha$ -citral, carveol, cyclonadiene, geraniol, neral, linalool, germacrene-D, isopinocarveol, and limonene (39). The following flavonoids have been identified in the Zaringaih extract: luteolin, luteolin 3'-O-beta-D-glucuronide, luteolin 7-O-beta-D-glucopyranoside, apigenin, apigenin 4'-O-beta-D-glucopyranoside, calycopterin, isokaempferide, xanthomicrol, acacetin 7-O-beta-D-glucopyranoside, and rosmarinic acid (40,41). However, as the aqueous

**Table 2.** The inhibitory concentration of *Dracocephalum kotschy* extract causing 50% of the maximum response ( $IC_{50}$ ) on rat ileum *in vitro*

Product	$IC_{50}$ ( $\mu$ g/mL)
Extract	50.8 $\pm$ 6.2
25 mg capsule (0)	45.2 $\pm$ 9.8
25 mg capsule (after 3 months)	50.3 $\pm$ 5.9
25 mg capsule (after 6 months)	35.5 $\pm$ 8
50 mg capsule (0)	46 $\pm$ 5
50 mg capsule (after 3 months)	55.3 $\pm$ 13.3
50 mg capsule (after 6 months)	41 $\pm$ 8

A full inhibitory concentration-response curve was constructed from a stock solution prepared from the curd extract or prepared capsule dosage forms before and after 3- and 6-month incubation at 40°C with 75% humidity. The data are presented as mean  $\pm$  SEM (n = 6). There was no statistically significant difference between the  $IC_{50}$  values of the *D. kotschy* extracts before and after formulation or after the incubation time (Student's *t* test).

and chloroform fractions of *D. kotschy* extract also have potent anti-spasmodic and anti-inflammatory properties, it is likely that other active substances exist that need to be identified (42).

Plant extracts are regarded as an enriching source of active chemical compounds for the development of novel drugs (1). However, herbal medicines also have to go through the drug design and development process before they can be introduced as effective medicinal products. Chemical profile and secondary metabolites of herbal plants depend on cultivation condition, soil nutrition, and other environment parameters (2). Time and method of harvesting, storing, extraction, and formulation have also great impacts on the effects of the final product. Therefore, standardization of the final herbal product is required. Following screening for biological activity, the product must be administered in a convenient form. Oral dosage forms (tablet, capsule, syrup) are the most popular figures for drug administration. Capsules are suitable solid dosage form of drugs in a dissolvable gelatin shell (43). Release of drug from capsule is more convenient than tablets (44). The gelatin shell can be filled with fixed amount of formulated drug granules for oral consumption. In contrast to herbal decoction and other forms of herbal medicine (for instance capsules filled with the raw materials), extract formulation in a capsule form has many advantages. Parameters used for assessment of prepared drug granule such as powder flowability, angle of repose, and bulk densities determine the physical properties of the prepared granules. These parameters are dependent on the solvent, particle size, and surface roughness of the granules. These give a good indication of granules flow through the filler facilities and capsule shell without sticking to the wall. Bulk density is a parameter used for the assessment of how much powder granule can be packed into a capsule shell. Weight uniformity between

the capsules confirms that capsule filling has been done in a standard fashion. Formulating the extract as a syrup also provides a uniform dose and therefore, by using different volumes, the dosage of the drug can be adjusted, especially for children or the elderly.

During the drug formulation process, there has to be compatibility between the chemical ingredient of the extract and the excipient used in the formulation. Any incompatibility between the excipient and the extract constituents may alter the stability and efficacy of the product. Therefore, in this research, the stability and efficacy of the capsules and the syrup dosage forms prepared for *D. kotschy* were examined to make sure that there were no changes in the effects of the extract during process of formulation or storing shelf life.

Conventional drug formulation usually is quantified by dissolution test because they contain pure active substances. Herbal medicine dissolution test is often not feasible because, first of all, the active ingredients are unknown or exist in a tiny small amount that is out of the range of the conventional analytical techniques, and HPLC analysis is costly. Secondly, it is possible that the combination of numerous substances is responsible for the observed biological response and therefore, evaluation of all effective constituents is not an easy task. Furthermore, herbal products have a complex chemical nature with different solubility, and that makes it difficult to design an optimum dissolution medium. Currently dissolution tests have not been required for herbal medicinal products (USP 32/NF 27, 2010), and herbal medicinal products are routinely standardized according to their total flavonoid or phenolic contents (36,37).

In this research, the contents of the extract in the dosage forms were analyzed by phytochemical and bioassay techniques. Folin-Ciocalteu's phenol reagent test and colorimetric aluminum chloride technique are known analyzing methods, which are routinely used for the standardization of the herbal extracts (36,37). In these techniques a phenolic compound (gallic acid) or a flavonoid (quercetin) have been used as reference agents and the extract contents were assessed according to their standard curves. Although, these types of phytochemical analysis are routinely used for the herbal medicine; however, it only gives an estimation of total phenolic or flavonoid contents of the extract regardless of being active substances or secondary plant metabolites. Therefore, a more convenient method of quantification is required. For herbal medicines with antispasmodic activity, bioassay quantification is possible. In this way, the effectiveness of herbal medicine with numerous chemical constituents can be easily assessed and compared without extensive studies.

The magnitude of the effect of a drug is usually related to its concentration at its site of action. For instance, *D. kotschy* extracts progressively inhibited the ileum contraction as the concentration of the extract increased

in the organ bath (Figures 1 and 2). Therefore, the construction of a standard concentrations-response curve for the extract was achievable. Most commonly, the specified effect used in assessment for comparison of the drug properties is the half-maximal effect (i.e.,  $IC_{50}$ ). Comparison of  $IC_{50}$  values of the crude extract on rat ileum contraction was consistent with the previous reports (8-10). Following the formulation of the extract as capsule or syrup dosage forms, the effectiveness of formulated extract was not changed, and there was no statistically significant difference compared with the crude extract at  $IC_{50}$  levels. Furthermore, following the incubation of the capsules for 3 and 6 months, despite minor shell deformation, there were no significant changes in the potency of the granulated extract (Table 2). Effectiveness of a single dose of the extract on inhibiting intestinal meal transit, in crude form or as in the syrup, also remained the same before or following incubation for 3 and 6 months. This finding confirmed that neither the designed formulation nor the shelf-life time elapse did not affect the integrity of the active chemical constituents of *D. kotschy* extract. Therefore, the bioassay technique used in this research is recommended for standardization or drug dosage forms preparation for *D. kotschy* extract.

### Conclusion

In this research, we have introduced a preparation method for making standard granules for *D. kotschy* extract. The results of phytochemical analysis and bioassay tests showed that formulation of *D. kotschy* extract in capsule or syrup dosage forms had no effect on the pharmacological activity of the extract. In addition, keeping capsules or syrup for 3 or 6 months under a harsh condition caused no significant changes in the total phenolic or flavonoid contents. More importantly, there was no change in the antispasmodic action of the extract following formulation or incubation for at least six months.

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### Authors' contribution

HS was project manager and supervised the pharmacological studies. AY supervised the extract preparation. NT supervised dosage form preparation. AHR was responsible for the experimental work and analysis of the data. HS was responsible for writing the paper. All authors approved the final manuscript for publication.

### 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 animals of the Isfahan University of Medical Sciences. The project was confirmed by the ethical committee of the university (IR.MUI.RESEARCH.REC.1399.441).

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