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Original article

Evaluating the effect of *Dracocephalum kotschyi* methanol extract on *Mycobacterium tuberculosis*

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Abstract

Background and objectives: Tuberculosis (TB) is the major public health problem in the world. Each year there are 2-3 million deaths worldwide caused by TB. The increasing incidence of Multi Drug Resistance tuberculosis (MDR-TB) worldwide highlights the urgent need to search for new anti-tuberculosis compounds. It has been reported that medicinal plant, *Dracocephalum kotschyi*, possesses some antibacterial effect, thus in the present study its anti-mycobacterial property was evaluated. **Methods:** The sensitivity and resistance of *M. tuberculosis* strains at concentration of 0.2 µg/mL isoniazid was determined by proportion method. Methanol extract of *D. kotschyi* was prepared using maceration method. Six concentrations of *D. kotschyi*, including 20, 40, 80, 160, 320 and 640µg/mL were prepared and its anti-mycobacterial effect on four groups of *M. tuberculosis* including *M. tuberculosis* H37Rv (ATCC 27294), isoniazid susceptible and resistance and MDR strains was determined. **Results:** The methanol extract of *D. kotschyi* was significantly against *M. tuberculosis*. The percent of growth was decreased from 100% to 0% in *M. tuberculosis* H37Rv (ATCC 27294), isoniazid resistant and isoniazid susceptible strains but from 100% to 50% in MDR strain in 640 µg/mL concentration. **Conclusion:** The results showed that different concentrations of *D. kotschyi* methanol extracts showed a remarkable inhibitory effect on *M. tuberculosis*. Identification of the effective fraction of *D. kotschyi* against *M. tuberculosis* is a further step to be studied.

Keywords: *Dracocephalum kotschyi*, isoniazid, methanol extract, *Mycobacterium tuberculosis*

Introduction

Tuberculosis is a major public health problem in the world. Each year there are 2-3 million deaths worl-dwide caused by TB. The increasing incidence of Multi Drug Resistance Tuberculosis (MDR-TB) highlights the urgent need to search for new anti-tuberculosis compounds [1]. The rate of mortality has been predicted to be 1.4 million annually [2]. MTB strains can lead to pulmonary and extra pulmonary tuberculosis infections and can remain in infected tissues of body as dormant bacilli. The bacilli can be reactivated in immune-compromised patients [3-5]. Rifampin has been used in combination withisoniazid, ethambutol, and pyrazinamide as a

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multidrug regimen for TB treatment in a period of six months [6,7].

With respect to the different types of mechanisms against anti-mycobacterial agents, the emergence of *Mycobacterium tuberculosis* (MTB) resistance strains has become an important health care issue particularly in developing countries. Due to the poor patient compliance and long treatment schedule which may lead to accumulated spontaneous mutations in the genome, the emergence of multidrug-resistant (MDR) and extremely drug resistant (XDR) strains in patients is not unexpected [8].

273,000 of new MDR-TB cases have been accrued worldwide [9,10]; hence, the treatment of MDR/XDR-MTB due to the limitation of therapeutic choices is difficult [11]. Research studies have made an attempt to find the new antimicrobial agents with herbal origin to develop treatments for TB resistance strains [12]. In many developed countries an interest has been shifted toward utilizing the traditional medicine as their major primary health care requirements, thus a fundamental exploring of alternative anti-TB agents is demanded [13]. For this purpose evaluating plants and herbal agents should be conducted to find their biological properties and also their safety. For example garlic (*Allium sativum*) is a medicinal plant with variety of biological properties like anti-tumor, anti-hyperlipedemic and anti-mycobacterial activities [14]. Regarding the anti-tuberculosis properties, extracts of *Acalypha indica*, *Adhato davasica*, *Allium cepa*, *Allium sativum* and *Aloe vera* have revealed anti-tuberculosis activity [15]. A traditional herbal agent, *Dracocephalum kotschyi*, a member of Lamiaceae family, which is a wild-growing plant is known for biological activities of its oil [16]. *D.kotschyi* called "Zarringiah" in Persian is endemic to Iran. It grows in various regions such as Alborz Mountains and North of Khorasan. It was primarily used as anti-spasmodic, analgesic (anti-visceral) and anti- hyperlipidemic [17], treatment of rheumatoid disorders, and cancer therapy such as leukemia and GI tract malignancy [18,19]. It should be mentioned that some parts of the plant such as its root possess more anti-bacterial effects in comparison to other plant parts [20]. The anti-mycobacterial effects of *D. kotschyi* have not yet been reported and in the present study the anti-mycobacterial activity of the methanol extract *D. kotschyi* were evaluated against different strains of MTB.

Experimental

Plant material

D. kotschyi was collected from a village located in the west regions of Isfahan (Hojat Abad, April 2013). It was authenticated at the Botany Department of the Faculty of Science, Isfahan University, Iran. The samples were ultimately dried in shade at 25-30°C. A voucher specimen (NO1448) was deposited at the herbarium of School of Pharmacy and Pharmaceutical Science, Isfahan University of Medical Sciences, Isfahan, Iran.

Preparation of the plant extract

Air-dried leaves powder (500 g) was macerated in 4 liters of methanol 70% at room temperature for 48 h. Then the extract was obtained and concentrated using rotary vacuum. The stock of extract in concentration of 1mg/mL in water was prepared. The concentrations 20, 40, 80, 160, 320 and 640µg/mL of the extract were prepared in water from the stock.

M. tuberculosis strains

Four groups of *M. tuberculosis* strains including *M. tuberculosis* H37Rv (ATCC 27294), isoniazide resistance, isoniazide susceptible and MDR strains were provided from Tuberculosis Center of Isfahan, Iran. All strains were subcultured on Lowenstein–Jensen and Middle-brook 7H9. The strains were investigated by primary conventional methods consisting of colony characteristics, pigmentation, growth temperature, rate of growth, Ziehl–Neelsen staining and other phenotypic tests.

Sensitivity evaluation of Mycobacterium tuberculosis strains to isoniazid

Sensitivity and resistance of *M. tuberculosis* strains to isoniazid (0.2µg/mL) and *D. kotschyi* methanol extract was determined separately by proportion method [21, 22]. A standard suspension of 10^7 (CFU)/mL of MTB strains was prepared and 3 to 5 colonies of *Mycobacteria* was suspended in distilled water. L-J mediums contained different concentration of *D. kotschyi* methanol extract were prepared and inoculated with 0.2 mL of 10^{-2} and 10^{-4} dilutions of a McFarland 1.0 standard of each strain and incubated at 37°C for 42 days. Finally the percent of growth was determined in different groups of strains [23].

Results and Discussion

M. tuberculosis is one of the most significant pathogens which may lead to mortality and morbidity of human being and may affect various organs such as lungs, bones, CNS, etc. [24]. Regarding the importance of tuberculosis and related diseases, the emergence of (MDR-XDR)-TB, lack of response to medical treatment and high rate of dissemination and rapid proliferation of mycobacteria, the researchers are interested in new ways to treat tuberculosis with new efficient drugs. Medicinal plants such as *Allium ascalonicum* [25], *Alstonia scholaris* [26], *Pavetta corymbosa*, *Canarium schwein furthii*, *Piliostigmat honningii*, *Syzygium guineense*, *Vitex dononia*, *Erythrina senegalensis*, *Pavetta owariensis*, *Terminalia glaucescens Planch. Ex Benth*, *Cassia mimosides* [27] and *Myrtus communis* [28] have been reported as anti-mycobacterial agents. The antimycobacterial potential of laurel oil, its fractions and its two sesquiterpene lactones against several mycobacterial strains and clinical isolates, has been shown by J. Luna-Herrera *et. al*. [29]. Other researchers have investigated the effect of the oil from S*enna alata* and *Salvia glutinosa* have, a potential phytotherapeutic agent, for human tuberculosis control. Cantrell CL *et al*. have illustrated that mono-sesqui, di and triterpenes, sterols, analog structures and semisynthetic derivatives may have anti-mycobacterial activity against *M. tuberculosis* [30]. The essential oil of *D. kotschyi* which contains α-bisabolene, caryophyllene, cuminyl aldehyde, and carvone oxide are known for their antimicrobial properties [31]. The anti-mycobacterial activity of different concentrations of *D. kotschyi* hyrdroalcoholic extract against four groups of *M. tuberculosis* strains including *M. tuberculosis* H37Rv (ATCC 27294), isoniazid susceptible, isoniazid resistant and MDR were evaluated in the present study (table1).

The percent of growth was decreased from 100% to 0% in *M. tuberculosis H37Rv* (ATCC 27294), isoniazid resistant and isoniazid susceptible strains but from 100% to 50% in MDR strain in 640 µg/mL concentration. There was no

Table 1. The effect of 6 different concentrations of *D. kotschyi* methanol exracts on *M. tuberculosis* strains

Strains	Percentage of colony growth at different concentrations of *D.kotschyi* extract and control media							
	Control media	**Isoniazid containing media**	**20 µg/mL**	**40 µg/mL**	**80 µg/mL**	**160 µg/mL**	**320 µg/mL**	**640 µg/mL**
Isoniazid Resistant	100%	100%	100%	100%	50%	15%	10%	0%
Isoniazid Susceptible	100%	0%	100%	50%	50%	50%	25%	0%
M. tuberculosis H37Rv (ATCC 27294)	100%	0%	100%	100%	100%	100%	20%	0%
MDR strain	100%	100%	100%	100%	100%	100%	50%	50%

significant effect against strains in 20 μg/mL of methanol extract of *D.kotschyi* plant. By raising the concentration, the total colonies were decreased and in 640μg/mL no colonies of *M. tuberculosis* were observed (table 1). The results showed a significant activity of *D. kotschyi* methanol extract against *M. tuberculosis* strains ($p \leq 0.001$).

The results suggested that *D. kotschyi* could be regarded as a source of new antimicrobial agents which can affect problematic drug-resistant infection. These results are correlated with the traditional reports [32].

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Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

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