Jundishapur J Nat Pharm Prod. 2016 November; 11(4):e29911. doi: 10.17795/jjnpp-29911.

Published online 2016 October 2. **Brief Report**

The Effect of Hydroalcoholic Extract of *Artemisia dracunculus* L. (Tarragon) on *Candida albicans* Infection in Mice

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Received 2015 May 22; **Revised** 2016 February 20; **Accepted** 2016 February 27.

Abstract

Background: *Candida albicans*, an opportunistic pathogen, causes disseminated infection in immunocompromised host. *Artemisia dracunculus* L. (Tarragon) is a plant used as meal and remedy for a long time, and possesses some medicinal characteristics. Its pharmacological properties have been shown in a large number of in-vitro and in-vivo studies.

Objectives: Regarding the antifungal activity, widespread secondary metabolites, and proved biological activities of tarragon, this study aimed to investigate the effect of hydroalcoholic extract of *A. dracunculus* L. on *C. albicans* infection in mice.

Methods: The mice were divided into six groups including a normal control group, a placebo group, and three treatment groups receiving 50, 100, and 200 mg/kg doses of hydroalcoholic extract via intraperitoneal (ip) injection followed by inoculation with *C. albicans*. A control[+] group, inoculated only with *C. albicans*, was also employed. After all treatments, the mice were killed by cervical decapitation and the concentration of *C. albicans* was measured in the liver and kidney homogenates.

Results: The results showed a significant decrease in *C. albicans* concentration in the liver and kidney homogenates in the treatment groups ($P < 0.05$).

Conclusions: It can be concluded that hydroalcoholic extract of *A. dracunculus* L. improves *C. albicans* infection in mice as a result of possessing antifungal effects.

Keywords: Hydroalcoholic Extract, Mice, *Artemisia dracunculus* L. (Tarragon), *Candida albicans*

1. Background

Candida albicans is an opportunistic pathogen that causes serious diseases in immunocompromised patients. It was reported that *C. albicans* is the fourth cause of nosocomial infections, having a mortality rate of up to 40% (1). There is much evidence that show *C. albicans* proteins have immunosuppression property (2). Pathogenesis of *C. albicans* is related to virulence factors such as kinds of adhesins, phospholipases, and aspartyl proteases enzyme (3). Also, *C. albicans* is considered as a dimorphic pathogen because of its ability to transition from yeast to hyphae (4). The risk factors for candidemia include lesion of mucosal surface (due to chemotherapy), surgical intervention, neutropenia, change of digestion system (due to the use of broad-spectrum antibiotics), acquired immune deficiency syndrome (AIDS), neoplastic disorders, and breaking skin. Innate immunity is the dominant protective mechanism against candidiasis (4, 5). Amphotericin B is the common drug applied to treat disseminated candidiasis in spite of its reported toxicity (6). Cell-mediated immunity is essential for the host defense against *C. albicans* infections. Neutrophils and monocytes can damage and kill yeast cells, hyphae, and pseudohyphae (5). Despite the development of new antifungal drugs, mortality associated with candidiasis remains high (7).

Recently, treating many diseases with medicinal plants has been increased (8). The low efficiency, high doses, and increasing side effects of synthetic medicine has led human to use natural products (9, 10). Also, plant drugs are more economic than synthetic ones (9). *Artemisia dracunculus* L. (tarragon) is a perennial plant that is classified in the *Asteraceae* family. French tarragon (also named German tarragon) and Russian tarragon are two main varieties of this plant (11). It is used as an herbal medicine because it provides health benefits. In traditional medicine, it had been used to improve the function of digestive system, flush toxins from the body, treat insomnia, and act as both anesthetic and antiepileptic agent. In the current therapeutics, it is used to alleviate the nervous system (as an antiepileptic agent) and improve the function of digestive and renal systems; it also possesses anti-inflammatory, anticancer, and antibacterial effects (11). The most important biologically active secondary metabolites in tarragon are essential oils, coumarins, flavonoids, and phenolcarbonic acids (12). Considering the therapeutic effects of tarragon, this study was conducted to investigate the effects

of hydroalcoholic extract of *A. dracunculus* L. on *C. albicans* infection in mice.

2. Methods

2.1. Animals

In this study, 48 male mice (30 ± 5 g) were provided from Pasteur institute of Iran and divided into six groups. They were housed in cages at 22 ± 2°C in a cycle of 12:12 hours light / dark while they had free access to water and food. This study was ethically approved by the Ethical committee of Islamic Azad University, Falavarjan Branch.

2.2. Preparation of Tarragon Extract

The plant was identified by Isfahan center for research of agricultural science and natural resources. The leaves were separated, dried in the shade, and powdered using an electric grinder. Fifty grams of the powder was moved to a sterile Erlenmeyer and mixed with ethyl alcohol until the powder suspended. The mix was shaken for 48 hours and after that, it was filtered using filter paper. The obtained liquid was heated in an oven at 40°C until the alcohol evaporated. The residual was the pure extract of the plant. By adding a given amount of normal saline, three doses of extract (50, 100, and 200 mg/kg) were prepared and refrigerated at 4°C for injections.

2.3. Microorganism

C. albicans (ATCC 1677) was cultured on sabouraud dextrose agar (SDA) media and put in incubator at 37°C for 48 hours. The characteristics of yeast cells were scrutinize by microscopic examination, colony shape on SDA, and germ tubes forming in serum and then culturing on BBL™ CHROMagar™ Candida (a selective medium for the isolation and presumptive identification of yeast and filamentous fungi and differentiation of Candida species).

2.4. Experiment

48 mice were divided into six groups of eight members: a normal control group, a placebo group that received 0.5 mL of normal saline, three treatment groups that received 50, 100, and 200 mg/kg doses of each hydroalcoholic extract by intraperitoneal (ip) injection followed by inoculation with *C. albicans* (after the fifth injection) to get experimental infection, and a control^{+} group that was only inoculated with *C. albicans*. The extract injections were administered in the next 20 days (10 injections). *C. albicans* was inoculated intraperitoneally with 0.2 mL of 10^5 CFU/mL suspension of organism in a sterile saline (6). It was confirmed by quantitative culture technique and counting by a hemocytometer.

2.5 Quantification of Microorganism in Organs

After the final injection, the mice were anaesthetized and killed by cervical decapitation. The animals were autopsied aseptically immediately after the death. To measure *C. albicans*, the liver and kidney were removed, weighed, rinsed, and homogenized in 3 mL normal saline. Afterwards, 1 mL of the dilutions were plated on Petri dishes containing SDA and incubated for 48 hours at 37°C. The CFU/g was determined in both organs.

2.6. Statistical Evaluation

All data were expressed as mean ± SEM for the eight animals in each group. Statistical analysis was conducted using analysis of variance (ANOVA) followed by Tukey's Post Hoc in SPSS software. A $P < 0.05$ was considered as significance criterion.

3. Results

Table 1 indicates *C. albicans* concentrations in the liver and kidney homogenates in all of the groups. It is deduced that there is a more significant decrease ($P < 0.05$) in the concentration of *C. albicans* in the two treatment groups receiving 100 and 200 mg/kg doses than the control^{+} group.

4. Discussion

Plants have the ability to synthesize secondary metabolites like proteins, flavonoids, alkaloids, steroids, and phenolic substances that have medicinal properties. The medicinal plants can enhance the resistance of the body against many infections and disorders because of their immunomodulatory activities (13). Many studies have shown that *A. dracunculus* L. has therapeutic uses, and a number of studies confirm its beneficial medicinal properties (11). In the present research, the effect of hydroalcoholic extract of *A. dracunculus* L. was studied on *C. albicans* infection in mice. The results showed *C. albicans* cells decreased in the treatment groups in comparison with the control^{+} group. Experimental candidiasis in this study was a sub-acute systemic infection which was tolerated by the mice (14). Neutrophils and macrophages are responsible for the host defense against a *C. albicans* infection (7). These defensive cells express surface receptors that recognize foreign substances in blood or tissues and stimulate the phagocytosis procedure. Neutrophils and macrophages also have receptors that activate the cells to produce cytokines (groups of proteins that mediate many of responses in innate and adaptive immunity). *C. albicans* is killed by oxidative mechanisms, including generation of reactive oxygen and nitrogen intermediate and non-oxidative mechanisms, as well

Table 1. *C. albicans* Concentration in the Liver and Kidney in the Defined Groups

Groups	Liver Mean CFU, $g^{-1} \pm$ SEM	Kidney Mean CFU, $g^{-1} \pm$ SEM
Normal Control	0.0 ± 0.0	0.0 ± 0.0
Placebo	0.0 ± 0.0	0.0 ± 0.0
Treatment, 50 mg/kg	34.42 ± 6.85^{b}	15.51 ± 2.56^{b}
Treatment, 100 mg/kg	2.71 ± 2.21^{a}	0.0 ± 0.0^{a}
Treatment, 200 mg/kg	16.08 ± 9.32b	0.0 ± 0.0^{a}
Control^{+}	36.28 ± 7.80	53.31 ± 25.70
Between groups Sig.	0.006	0.021

[a] $P < 0.05$ compared to control^{+} group
[b] $P < 0.01$ compared to control^{+} group

(5). Neutrophils, also called polymorphonuclear (PMN) leucocytes, are recruited by granulocyte-colony stimulating factor (G-CSF). Treatment of mice with recombinant (rG-CSF) can lead to the significant reduction of mortality during disseminated candidiasis (15). As described previously, *A. dracunculus* L. has secondary metabolites such as essential oils, flavonoids, etc. The extensive researches have shown that the essential oils in Artemisia spp. are effective against *C. albicans* and volatile fractions of Artemisia plants exhibit antifungal in vitro activity (16, 17). *A. dracunculus* has been reported to act as antifungal agent against *Phythium ultimum*, *Sclerotinia sclerotiorum*, *Botrytis* spp., *Fusarium seminectum*, *Colletrotichum fragariae*, *Collectrotichum gloeosporioides*, and *Collectrotichum acutatum* (11). It has been shown that some flavonoid compounds have antifungal and anti-*Candida* spp. activities (18). In addition, antimicrobial and antioxidant activity of flavonoid components have been confirmed previously (19, 20). As the innate immunity plays the dominant role in candidiasis (5), it seems that *A. dracunculus* L. might boost the mentioned mechanism, too. The anti-candidiasis effects of other medicinal plants were reported in some researches. It has been shown that the treatment with garlic extract can decrease *C. albicans* cells in the liver and kidney in the diabetic rats (14). Effects of Aloe vera gel extract on the response of macrophages to *C. albicans* indicated that R100 fraction is the most effective fraction of *A. vera* (21). In conclusion, our results suggest that *A. dracunculus* L. can exhibit anti-fungal activity, and improve *C. albicans* infection in mice due to possessing effective anti-candida metabolites. To find out the effective component(s) and anti-fungal mechanism(s) of the plant in this approach, we need to isolate and purify tarragon components. Also, investigation on the effect of other extracts (aquatic, acetonic, etc.) of *A. dracunculus* L. on other infections and diseases is also suggested.

Acknowledgments

This paper is derived from a postgraduate thesis for the degree of M.Sc in microbiology at Falavarjan branch, Islamic Azad University. The research was performed at the research laboratory of Islamic Azad University, Falavarjan branch. The authors are grateful for adequate facilities and supports of the university.

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