

Alteration in Hematological and Biochemical Parameters Following the Long-Term Consumption of *Zataria multiflora* Essential Oil in Patients Infected with Liver Hydatid Disease

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Abstract

Background: *Zataria multiflora* Boiss is a well-known medicinal plant and its essential oil (EO) is traditionally used for treatment of respiratory and digestive disorders as well as bacterial, viral, fungal and parasitic infections. This study was undertaken to evaluate the effect of the long-term consumption of *Z. multiflora* EO on the hematological and biochemical parameters of patients infected with liver hydatid disease.

Methods: Fifteen patients were administered orally with *Z. multiflora* EO (60 mg daily) for six months. Hematological and biochemical analyses were performed on the blood samples of patients before the start of treatment and after the completion of the treatment course. In the hematological analysis, WBC, RBC, Hb, hematocrit, MCV, MCH, MCHC and platelet levels were measured. In the biochemical analysis, FBS, BUN, creatinine, cholesterol, triglycerides, HDL, LDL, Na, K, Cl, AST, ALT, and ALK were assessed.

Results: No significant changes were observed in the values of the patients' hematological parameters after treatment. No significant changes also occurred in the values of FBS, BUN, creatinine, cholesterol, triglycerides, HDL, LDL, Na, and K. The mean values of AST, ALT and ALK enzymes decreased significantly after treatment ($P<0.05$). No adverse clinical event was noticed in the patients during the course of the study.

Conclusion: Long-term consumption of *Z. multiflora* EO induced no adverse effect on the hematological and biochemical parameters of patients infected with liver hydatid disease. Since *Z. multiflora* EO reduced the level of liver transaminases (AST, ALT and ALK), this herbal product may be considered as a hepatoprotective agent in human beings.

Keywords: *Zataria multiflora*, Essential oil, Hematological parameters, Biochemical parameters, Clinical study

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Introduction

Zataria multiflora Boiss ("Avishan-e-Shirazi" in Persian language), belonging to the Lamiaceae family, grows wildly in the central and southern parts of Iran, Pakistan and Afghanistan. This plant is well-known in Iranian traditional medicine and is widely used as a flavoring ingredient in a wide range of foods. Historical evidence show that *Z. multiflora* has been used from ancient times till date to cure parasitic and fungal infections, gastrointestinal ailments, dyspepsia, cough, joint pain, tooth pain, sore throat, children's enuresis, hysteria and tonsillitis (1-3).

The chemical composition of *Z. multiflora* EO has previously been analyzed in detail by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS); thymol and carvacrol were found to be the main components of the oil (1, 3-10).

Prior studies have shown that *Z. multiflora* EO has anti-nociceptive (11), antioxidant (12), immunostimulatory (13, 14), antidiabetic (15) and anticonvulsant (16) properties. Furthermore, it has been revealed that *Z. multiflora* EO has antimicrobial (12), antibacterial (17), antileishmanial (8), scolicidal (6, 9, 18), antifungal (19, 20) and insecticidal (21) properties. In addition, *Z. multiflora* EO has been used for treatment of different human diseases such as Alzheimer's disease (22), recurrent aphthous stomatitis (23), denture stomatitis (24), recurrent oral aphthous lesions (25), irritable bowel syndrome (1,3) and vaginal infections including bacterial vaginosis, candidiasis and trichomoniasis (26).

Due to the wide-range of biological properties that *Z. multiflora* EO possesses, this herbal product could be considered as a valuable source for drug development against different human diseases. Presently, some medicinal forms of this plant, such as vaginal creams, soft capsules, syrups and oral drops are used as traditional treatments for different diseases. Despite this fact, the safety of *Z. multiflora* EO for human beings is an important issue.

Objectives

The present work was performed to evaluate the effect of *Z. multiflora* EO on the hematological and biochemical parameters of patients infected with liver hydatid disease.

Materials and Methods

Zataria Multiflora Essential Oil

The herbal medicine used in this trial was the essential oil of the aerial parts of *Zataria multiflora* Boiss (Lamiaceae). The oil was obtained from Barij Essence Pharmaceutical Company, Kashan, Iran (with plant voucher specimen BEC189, deposited at the Herbarium of Agriculture Department, Research Center of Barij Essence Pharmaceutical Company,

Kashan, Iran). This company manufactures *Z. multiflora* EO in the form of 20 mg soft capsules (each containing 5.5- 6.5 mg thymol) with the commercial name of Gastrolit. This product is registered for use as a natural health product in Iran, commonly administered for the treatment of irritable bowel syndrome (IBS).

Study Design and Participants

This non-randomized clinical study was carried out from 2016 to 2018 at Shiraz University and Shiraz University of Medical Sciences (Shiraz, Iran). A total of 15 patients (7 male and 8 female) who were infected with hepatic hydatid disease were included in this study. The patients were 31-59 years old (mean: 49.6 ± 8.09) and were administered orally with *Z. multiflora* EO for six months (180 consecutive days). The oil was prescribed three times a day (morning/afternoon/evening) at the dose of 20 mg (60 mg daily). Hematological and biochemical tests were performed before the start of treatment and after completion of the course of treatment. To evaluate the probable adverse effects, the patients were under the care of the physician team during the course of treatment.

Biochemical and Hematological Tests

In the hematological analysis, white blood cells (WBC), red blood cells (RBC), hemoglobin (Hb), hematocrit, mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC) and platelet levels were measured before and after six months of treatment. In the biochemical analysis, measurement of fasting blood sugar (FBS), blood urea nitrogen (BUN), creatinine, cholesterol, triglycerides, high density lipoprotein (HDL), low density lipoprotein (LDL), sodium (Na), potassium (K), chlorine (Cl), aspartate aminotransferase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALK) was done before and after six months of treatment. Hematological tests were performed by an automated hematology analyzer (Sysmex kx-21n, USA), while biochemical measurements were made using a BS-200 Chemistry Analyzer (Mindray, China).

Statistical Analysis

The results are presented as mean \pm standard deviation (SD). Normality of the data was checked by the one-sample Shapiro-Wilk test. Accordingly, Cl, MCV, MCH and MCHC were analyzed by the Wilcoxon signed-rank test (a non-parametric test), while the paired-samples T test was used to compare the values of all other hematological and biochemical parameters before and after treatment. To compare the values of hematological and biochemical parameters between male and female patients before and after treatment, the Mann-Whitney test (a non-parametric test) and independent samples t-test (a parametric test) were used based on the normality

of data distribution. All statistical analyses were done with SPSS 23. *P*-values less than 0.05 were considered statistically significant.

Ethical Considerations

This study was approved by the Human Ethic Committee of Shiraz University of Medical Sciences (permit number: IR. SUMS. Rec. 1396.335) and was conducted according to the ethical guidelines and principles of the International Declaration of Helsinki for Good Clinical Practice and the Medical Research Council (MRC) Ethical Guidelines for Research. Informed consent was obtained from the patients.

Results

The baseline demographic characteristics (sex and age) of the patients are shown in Table 1. The mean values of the hematological and biochemical parameters before and after 6 months of treatment with *Z. multiflora* EO are shown in Tables 2 and 3, respectively. As shown in Table 2, no significant changes were observed in the values of hematological parameters six months after treatment. Notably, these values remained within the normal range after treatment with *Z. multiflora* EO.

In the biochemical analysis, the values of FBS, BUN, creatinine, cholesterol, triglycerides, HDL,

LDL, Na, and K were in the normal range before commencing treatment. These values remained in the same range after six months of treatment with *Z. multiflora* EO (Table 3). The only electrolyte that increased significantly (*P*<0.05) in level six months after treatment with *Z. multiflora* EO was Cl, though its value also remained within the normal range (Table 3).

As shown in Table 3 the mean levels of the AST, ALT and ALK enzymes were higher than the normal range before the start of treatment. Interestingly, six months after treatment, the mentioned values decreased significantly (*P*<0.05) (Figure 1), despite remaining above the normal range.

There was no significant difference between male and female patients in terms of the effect of *Z. multiflora* EO on hematological or biochemical parameters, except in the case of triglycerides. The effect of *Z. multiflora* EO on the level of triglycerides was significantly different between men and women patients (*P*<0.05). In men, the consumption of *Z. multiflora* EO reduced the level of serum triglycerides; conversely, it increased the serum level of triglycerides in women. Nevertheless, the value of triglycerides remained within the normal range in both men and women after treatment.

Overall, the long term consumption of *Z. multiflora* EO had no adverse effects on the hematological and biochemical parameters of patients who were

Table 1: Baseline demographic characteristics of the patients treated by *Zataria multiflora* essential oil (ZMEO)

Patient Number	Sex	Age
1	Female	41
2	Male	52
3	Male	59
4	Male	45
5	Female	57
6	Male	57
7	Female	57
8	Female	48
9	Male	49
10	Female	52
11	Male	41
12	Female	43
13	Female	54
14	Male	58
15	Female	31

Table 2: Hematological analysis on the blood samples of patients infected with hydatid disease before and after six months of treatment with *Z. multiflora* essential oil (Mean±SE)

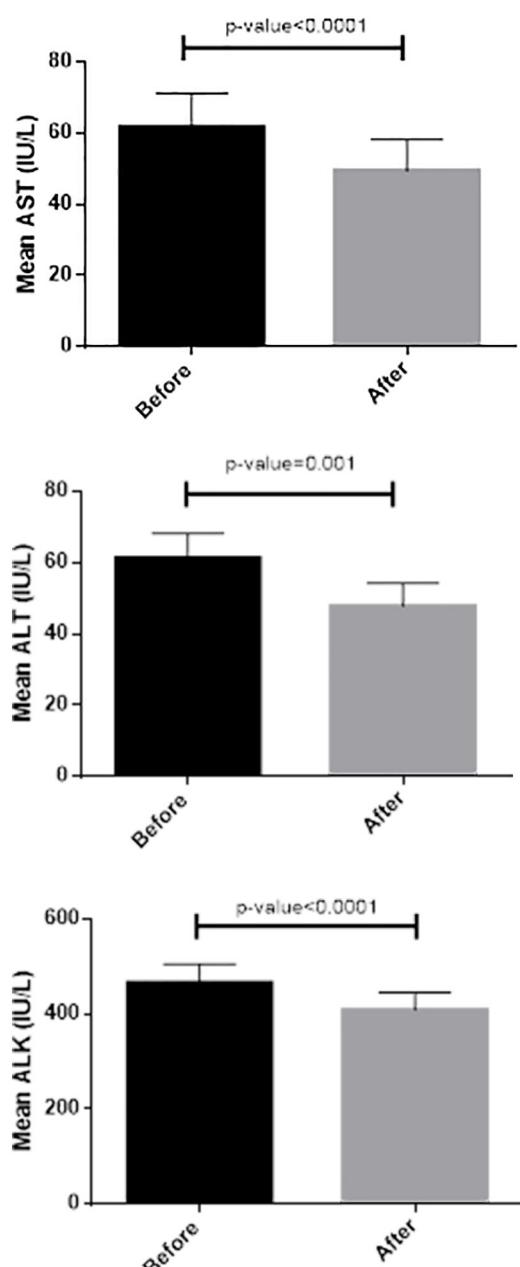
Factor	Before treatment	Six months after treatment	P value	Normal range
WBC (/uL)	6436±708	6760±553	0.168	4400-11000
RBC (Mil/uL)	5.07±0.23	4.97±0.212	0.159	4.5-5.1 (M:4.3-5.6)
Hemoglobin (g/dL)	14.16±1.22	14.17±1.07	0.973	12.3-15.3 (M*:13.2-17.2)
Hematocrit (%)	42.14±3.47	41.78±2.84	0.806	35.9-44.6 (M*:39-50)
MCV (fL)	82.89±6.97	83.82±5.73	0.443	80-96
MCH (Pg)	28.48±1.92	28.61±1.64	0.410	27-33
MCHC (g/dL)	34.51±1.79	34.41±1.29	0.527	33-36
Platelets (1000/uL)	244±44.72	236±51.51	0.621	150-450

M*: male

Table 3: Biochemical analysis on the blood samples of patients infected with hydatid disease before and after six months of treatment with *Z. multiflora* essential oil (Mean \pm SE)

Factor	Before treatment	Six months after treatment	P value	Normal range
FBS (mg/dl)	98.4 \pm 7/77	102.66 \pm 4.89	0.068	70-110
BUN (mg/dl)	15.2 \pm 6.18	14.73 \pm 4.07	0.733	7-24
Creatinine (mg/dl)	0.999 \pm 0.27	1.01 \pm 0.23	0.876	0.7-1.4
Cholesterol (mg/dl)	176.60 \pm 26.79	172.80 \pm 24.92	0.572	130-200
Triglycerides (mg/dl)	183.20 \pm 8.66	182.46 \pm 5.23	0.745	40-200
HDL (mg/dl)	57.73 \pm 6.95	59.20 \pm 5.12	0.505	35-68
LDL (mg/dl)	63.60 \pm 24.39	59.13 \pm 17.27	0.574	\leq 130
Sodium (mEq/dl)	138 \pm 5.95	138.73 \pm 5.4	0.969	135-148
Potassium (mEq/dl)	4.27 \pm 0.59	4.42 \pm 0.63	0.356	3.5-5
Cl (mEq/dl)	88.27 \pm 24.60	102.06 \pm 6.89	0.004	90-110
AST (IU/L)	61.73 \pm 9.46	49.53 \pm 8.70	0.001	1-31 (M*:1-37)
ALT (IU/L)	61.20 \pm 7.00	47.73 \pm 6.44	0.000	1-31 (M*:1-41)
ALK (IU/L)	466.66 \pm 38.89	409.07 \pm 36.69	0.000	80-306

M*: male

**Figure 1:** Alteration in liver enzymes in patients infected with hydatid disease, before and after six months of treatment with *Z. multiflora* EO (Error bars: 95% CI).

infected with liver hydatid disease. In addition, we observed no adverse clinical events in the patients during the course of this study.

Discussion

The results of the present study show that six months after treatment, the mean values of hematological parameters of the patients remained almost as they were before the start of treatment. Therefore, it can be concluded that long-term consumption of *Z. multiflora* EO had no adverse effect on hematological parameters, which could be considered as an advantage for this herbal product.

The mean values of most biochemical parameters remained almost the same as they were prior to the start of treatment. As a result, it can be stated that *Z. multiflora* EO had no adverse effects on biochemical parameters. Since BUN and creatinine are usually measured to assess kidney function, it can be concluded that long-term consumption of *Z. multiflora* EO has no adverse effect on kidney function. This also could be considered as a positive point for the recommendation of *Z. multiflora* EO as human herbal medicine. Overall, the results of this study revealed that long-term administration of *Z. multiflora* EO does not alter electrolyte values except for Cl, which significantly increased after treatment. Even though the Cl value remained within the normal range after treatment, the reason for enhancement of this electrolyte after treatment of patients with *Z. multiflora* EO remains to be explored in future studies.

In this study, the mean values of liver enzymes including AST, ALT and ALK were extremely higher than the normal ranges before the start of treatment. Liver damage is usually accompanied by elevation of liver enzymes (27), and this could be observed in the biochemical analysis of blood samples obtained from the patients with liver hydatid disease (28). On the other hand, hepatic hydatid disease may induce oxidative stress and liver toxicity in different animal species and humans (29). The AST, ALT, and ALK

enzymes are usually assessed as the liver function test (27). In the present study, the mean values of AST, ALT and ALK not only did not rise, but also significantly decreased six months after treatment, and this could be considered as another positive point regarding *Z. multiflora* EO.

As previously mentioned, thymol and carvacrol are the main components of *Z. multiflora* EO. Previous studies have shown that thymol and carvacrol are safe compounds for human use (1, 30-32). Furthermore, *Z. multiflora* EO contains compounds that act as scavengers of free radicals (33). In addition, the protective effects of the methanolic and ethanolic extracts of *Z. multiflora* on the liver and lung have previously been documented (34, 35). Furthermore, mice infected with cystic echinococcosis showed less oxidative stress and hepatic injury when orally administered with the aromatic water of *Z. multiflora* relative to the control (28).

In accordance with the results of the referenced studies, this study revealed that six months of treatment with *Z. multiflora* EO could reduce the level of liver transaminases in patients with liver hydatid disease, confirming the hepatoprotective property of this herbal product in human beings. Even though the results of the present study are promising, further studies with more human cases should be performed to determine the exact margin

of safety of this herbal product.

The contrasting effects of *Z. multiflora* EO on the serum triglycerides of men and women may be related to sex hormones or other physiological conditions. However, this issue also needs to be explored in future studies.

In conclusion, to the best of our knowledge, this is the first study that investigated the hematological and biochemical consequences of long-term consumption of *Z. multiflora* EO in human beings. The results of the present study revealed that long-term consumption of *Z. multiflora* EO with conventional doses had no adverse effects on hematological and biochemical parameters in humans. In fact, *Z. multiflora* EO induced no adverse effect on kidney function, and demonstrated hepatoprotective activity in patients who were infected with liver hydatid disease. Accordingly, *Z. multiflora* EO may be consumed safely in human beings for medical purposes.

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Conflict of Interests: None declared.

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