The Effects of Zataria Multiflora Hydroalcoholic Extract on Some Liver Enzymes, Cholesterol, Triglyceride, HDL-Cholesterol, LDL-Cholesterol, Albumin and Total Protein in Rat

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Abstract: *Zataria multiflora* is a valuable medicinal plant grown extensively in Iran, Pakistan and Afghanistan. The chemical compositions of their extracts have been extensively characterized in Iran and Pakistan. The present study was undertaken to investigate the effects of *Zataria multiflora* on some liver enzymes, triglyceride, cholesterol, HDL-cholesterol, LDL-cholesterol, albumin and total protein in rat. Sixty adult male Wistar rats weighing about 200 to 220 g were divided into six groups of ten. The control group (group 1) did not receive any drug. The sham group (group 2) received 2 cc of distilled water. The other four experimental groups (groups 3 to 6) including very low (100 mg/kg BW), low (200 mg/kg BW), medium (300 mg/kg BW) and maximum dose (400 mg/kg BW) received *Zataria multiflora* hydroalcoholic extract intraperitoneally daily for 28 days. After 28 days all animals in the different groups were weighed and blood samples were collected from heart vein. Serum biochemical parameters were measured using validated standard methods. The results of this study showed *Zattaria multiflora* hydroalcoholic extract analyses various lipids in lipid tissues and transfer to blood for elimination from body.

Keywords: *Zataria multiflora* hydroalcoholic extract, Liver enzymes, Lipids, Proteins, Rat.

INTRODUCTION

The use of herbal medicine has become more prevalent, and the past few decades have witnessed a rapidly increasing demand worldwide. The range of medicinal plants is very diverse and it has been estimated that around 70000 different plant species have been used at least once during the history of traditional medicine [1].According to a WHO report, around four billion people (80% of the world´s population) use herbal medicine [1] , with eleven different bioclimatic regions and around 7500 different plant species.

Herbal medicines have been studied by some investigators [2-5].

Zataria multiflora (Avishan-e-Shirazi in Persian and Sa'atar or Zaatar in the old Iranian medical books) is a thyme-like plant and a member of Labiata family that grows wild in central and southern Iran [6]. In Iran, *Z. multiflora* is used in traditional folk remedies for its antiseptic, analgesic (pain relieving), carminative (anti-flatulence and intestine soothing) and elimination of liver and kidney failure properties [7]. The essence is obtained by distilling or pressing the plant's leaves, roots, fruits, seeds, stems, or flowers. The essential oil contains the plant's essence, a complex chemical that provides its smell and other properties [8]. In general, the essence was found to contain 26 types of different substances such as thymol (48.4 percent) and carvacrol (12.6 percent), linalool and para-cymene [9, 10].

In recent years, cardiovascular diseases such as arteriosclerosis that are caused as a result of hyperlipidemia elevate mortality percent, and the age of death has reduced [11], so reducing serum hyperlipidemia is very important; a 1% reduction in serum cholesterol concentration results in a 2% reduction in the prevalence of coronary artery diseases [11].

Some chemical drugs are used in liver diseases; however there is no medical plant for use in liver diseases.

Although *Zataria multiflora* is used more traditionally, there is less study about its effect on liver. In this study the effect of *Zataria multiflora* hydroalcoholic extract on some liver enzymes (AST, ALT and ALP), the serum concentrations of cholesterol,

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ISSN: 1814-8085 / E-ISSN: 1927-5129/12

triglyceride, HDL-cholesterol, LDL-cholesterol, albumin and total protein in rat were investigated.

MATERIALS AND METHODS

Extract Preparation

The herb was purchased from a local herbal shop in Shiraz city and identified in the Shiraz Agriculture Faculty. The leaves were then cleaned and powdered by electric blender and the powder was extracted with 70% alcohol for 72 hours using macerated method. The mixer was filtered with Whatman No 1 filter paper. The solvent of the filtrate was evaporated at ambient temperature and the extracted powder (13.1% of leaf powder) was kept at $4o^0$C until used and was solvent in water before administration.

The rats were kept in animal house of Kazerun Branch of Islamic Azad University and were feed with routine diet.

Blood Sampling

Blood samples were taken from the heart of sixty adult male Wistar rats weighing about 200 to 220 *g*, divided into six groups of ten. The control group (group 1) did not receive any drug. In the sham group (group 2) 2 cc of distilled water was used. The other four experimental groups (groups 3 to 6) including very low (100 mg/kg BW), low (200 mg/kg BW), medium (300 mg/kg BW) and maximum (400 mg/kg BW) received *Zataria multiflora* hydroalcoholic extract daily for 28 days intraperitoneal. After 28 days all animals in the different groups were weighed and their blood was collected from heart vein. Blood samples were collected into vacutainers and serum was separated by centrifugation at 750 *g* for 15 min and stored at -20°C until use. The samples with hemolysis were thrown away.

Measurements

Serum activities of ALT, AST and ALP were measured using colorimetric standard methods. Serum total cholesterol was analyzed by BIOTRON BTR 820 Auto Blood Analyzer using enzymatic method ([12]. Serum high density lipoprotein cholesterol (HDL-cholesterol) was measured by using BIOTRON, BTR 820 using phosphotungstate method [13]. The value of serum low density lipoprotein cholesterol (LDL-cholesterol) was calculated based on Friedwald's equation [14]. Serum triglycerides were estimated by using Autopack Reagent Kit by method of enzymatic DHBC colorimetric method [15]. The serum total protein concentration was measured according to the Biuret method as modified by Donninger *et al*. (1972) using bovine serum albumin as standard [16]. Serum albumin levels were assayed by Bromocresol green method.

Statistical Analysis

The data were expressed in SI units and analyzed by repeated measurements ANOVA, Duncan, Spearman and T-test using SPSS/PC software [17]. All values were expressed as mean and standard error (SE), and $P<0.05$ was seen as statistically significant.

Table1: The Mean ± SD of Serum Concentration of Biochemical Parameters of 60 Rats in Six Groups

Parameters Groups	Triglyceride (mg/dl)	Cholesterol (mg/dl)	HDL-cholesterol (mg/dl)	LDL-cholesterol (mg/dl)	AST (U/L)	ALT (U/L)	ALP (U/L)	Total protein (g/dl)	Albumin (g/dl)
Control (group 1)	39.12± 2.16	47.37± 2.93	26.625± 1.2527	35.25± 1.6008	313± 41.3893	55.5± 3.9097	529.875± 49.2303	8.85± 0.1150	4.5625± 0.1133
Sham (group 2)	50.12± 4.34	48.25± 2.44	29.50± 0.62	38.87± 0.61	243.00± 8.20	56.25± 3.94	584.25± 84.07	8.61± 0.13	4.45± 0.09
Group3	41.25± 1.88	56.75± 2.81	30.12± 0.81	41.12± 0.95	307.12± 35.41	62.00± 4.19	478.50± 57.75	8.68± 0.15	4.66± 0.07
Group4	45.00± 3.64	57.00± 2.86	31.00± 0.92	40.00± 1.37	367.37± 28.53	65.62± 4.23	451.87± 46.58	8.70± 0.21	4.70± 0.18
Group5	57.12± 2.74	56.37± 3.93	35.00± 1.06	44.37± 1.03	354.00± 27.26	58.25± 3.50	408.37± 23.78	8.60± 0.10	4.60± 0.10
Group6	54.62± 4.52	56.12± 4.73	32.87± 1.25	40.25± 1.09	277.25± 16.84	46.25± 3.35	401.50± 67.42	8.66± 0.27	4.50± 0.14

RESULTS

The mean ±SE of ALT, AST, ALP, cholesterol, triglyceride, HDL-cholesterol, LDL-cholesterol, total protein and albumin in 60 rats in six groups is shown in Table **1**. The significant differences between each parameter in the different groups are shown in Table **2**. Table **2** show *Zattaria multiflora* increases the mean concentration of triglyceride, HDL-cholesterol and LDL-cholesterol, and there is a significant difference between the mean concentration of triglyceride and HDL-cholesterol in groups 5 and 6 in comparison with

Table 2: The Significant Differences Between Serum Concentrations of Biochemical Parameters in Different Groups in Comparison with Control Group

Parameter	Groups	Sig.
Triglyceride	Sham(2)	0.394
	3	1
	4	1
	5	0.008**
	6	0.034*
Cholesterol	Sham(2)	1
	3	0.85
	4	0.76
	5	1
	6	1
HDL-Cholesterol	Sham(2)	0.782
	3	0.29
	4	0.061
	5	0.000**
	6	0.001**
LDL-Cholesterol	Sham(2)	0.480
	3	0.013*
	4	0.087
	5	0.000**
	6	0.058
AST	Sham(2)	1
	3	1
	4	1
	5	1
	6	1
ALT	Sham(2)	1
	3	1
	4	1
	5	1
	6	1
ALP	Sham(2)	1
	3	1
	4	1
	5	1
	6	1
Total protein	Sham(2)	1
	3	1
	4	1
	5	1
	6	1
Albumin	Sham(2)	1
	3	1
	4	1
	5	1
	6	1

*Significant in P<0.05.
**Significant in P<0.01.

control group; also there is a significant difference between the mean concentration of LDL-cholesterol in groups 3 and 5 in comparison to control group ($P<0.05$).

DISCUSSION

In this study there were no significant differences between the serum concentrations of cholesterol in the sham and experimental groups in comparison with control group, ($P>0.05$) (Table **2**), but the mean concentrations of triglyceride and HDL-cholesterol in the different experimental groups have gradually increased in comparison with the control group, and there are significant differences between the mean serum concentration of triglyceride and HDL-cholesterol in groups 5 and 6 (received 300 and 400mg/kg of *Zattaria multiflora* respectively) in comparison with the control ($P<0.05$)) (Table **2**), with the highest serum concentration of triglyceride and HDL-cholesterol observed in group 5 (Table **2**). The results of this study showed *Zattaria multiflora* has increasing effects on LDL-cholesterol levels, with the greatest effects of this plant in 100 and 300 mg/kg doses. Significant differences between groups 3 and 5 in comparison to control group were also observed ($P<0.05$).

It seems *Zattaria multiflora* affects lipid metabolism with a mechanism related to dose and changes the serum concentration of triglyceride and HDL-cholesterol, LDL-cholesterol; this effect may be a result of triglyceride lipase activation which analyzes lipid tissue triglycerides and increases their plasma levels. The serum triglyceride concentration in a 300 mg/kg dose is higher than serum triglyceride concentration in 400mg/kg. It seems in this dose the serum lipid elimination is more than lipolysis.

In routine cases increase in triglyceride concentration decreases the HDL-cholesterol concentration, but in this study the effect of *Zattaria multiflora* resulted in a simultaneous increase in triglyceride and HDL-cholesterol concentration. This finding shows that after *Zattaria multiflora* hydroalcoholic extract administration, lipoproteins change proportional to lipid tissue analysis to help the lipid transfer of tissue to blood and its elimination.

On the other hand, *Zattaria multiflora* hydroalcoholic extract decreases lipid metabolism, lipid accumulation and increases endogenous lipolysis, consequently serum free triglycerides increase relative to the dose mechanism.

It is interesting that after *Zattaraia multiflora* hydroalcoholic extract administration, HDL-cholesterol changes in the same way as triglyceride, thus helping lipids elimination.

Anita (2004) reported that *Zattaria multiflora* has an antioxidant characteristic (this property can be due flavonoids) and, using this property, controls serum lipids. Antioxidants are plant chemical materials and have many beneficial effects in decreasing arthrosclerosis and other cardiovascular diseases. Antioxidant prevents cholesterol adhesion to the artery walls, and by decreasing unsaturated lipid acids oxidation and free radicals, prevent cell degradation [18].

Sadeghi *et al*. (2007a,b) reported Dorema aucheri in a 500mg/kg dose has antihyperlipidemic, hypercholesterolemic and hepatic effects, and these characteristics can be due to its antioxidant agents, but they also stoke that this extract at a dose of 500mg/kg is toxic for rats [19,20].

Artichoke extract affects serum lipids, decreasing total cholesterol 18.5% and LDL-cholesterol 23%; these effects may be due to cholesterol synthesis inhibition and/or cholesterol elimination from bile [11,21].

Vitex angus castus leaf extract with 20 and 40 mg doses not change the mean concentration of triglyceride and cholesterol in the test (experimental) groups in comparison with the control group, but there are significant differences between the triglyceride concentration in different experimental groups, and these changes are a result of the effects of this plant extract on lipid metabolism [22].

Beta vulgaris extract as an anti-diabetic component decreases triglyceride and cholesterol in both control and diabetes groups considerably [23].

Onoagbe *et al*. (2010) reported the ability of U. lobata to effectively reduce the elevated levels of blood glucose and cholesterol, as well as liver triglyceride in the diabetic rats, rendering valid the claimed anti-diabetic activities of the medicinal plant [24].

Choi *et al*. (2005) showed that the intake of medicinal plants (Piper cubeba (fruit), Physalis angulata (flower), Rosa hybrida (flower)) in rats results in an increase in antioxidant enzyme activity and HDL-cholesterol, and a decrease in malondialdehyde, which

may reduce the risk of inflammatory and heart disease [25].

There were no significant differences between mean serum activities of AST, ALT and ALP in the *Zattaria multiflora* received groups in comparison with the control group (P>0.05).

The fact that there was no change in the serum activities of liver enzymes after *Zattaria multiflora* extract administration shows this plant´s effects on lipid metabolism were done with no effects on liver enzymes synthesis and cell membrane infiltration.

CCl_4 toxicocity decrease serum ALP, ALT and ALP activities and *Berberis Vulgaris* leaf extract returns these parameters serum activities to normal values [26].

The statistical investigations showed Zattaria multiflora hydroalcoholic extract has no effect on serum concentrations of albumin and total proteins.

Mirzaee *et al*. (2005) stated that *Dorema aucheri* in a higher dose was more effects on serum proteins and BUN than in lower doses [27].

CONCLUSION

Our study concludes that *Zataria multiflora* has hepatoprotective potential as is being evident by non-significant changes in the liver function tests. This plant can be used as a beneficial drug in hyperlipidemic cases. *Zataria multiflora* was analyzed various lipids in lipid tissues and transfer to blood for elimination from body.

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Received on 21-02-2012 Accepted on 29-03-2012 Published on 11-04-2012

DOI: http://dx.doi.org/10.6000/1927-5129.2012.08.01.33