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Effect of *Origanum vulgare* Hydroalcoholic Extract on Liver Enzymes, Cholesterol, Triglycerides, Cholesterol-HDL, Cholesterol-LDL, Total Bilirubin, Creatinine, Albumin, Total Protein in Rat.

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ABSTRACT

The *Origanum Vulgare* herbal plant is grown in different areas of the Mediterranean and some areas of Asia, and nowadays is also grown in Iran. This study investigates the effect of Origanum Vulgare hydroalcoholic extract on liver enzymes, cholesterol, Triglycerides, cholesterol-HDL, cholesterol-LDL, total bilirubin, creatinine, Albumin, total protein in rat. For this experiment, 50 adult male Wistar rats, weighing approximately 200 ± 5 g were divided into 5 groups. The control group (Group 1) was fed nothing except normal food and water, Group 2 , witness group, was given 2cc distilled water, three experimental groups (Groups 3 to 5) received *Origanum Vulgare* hydroalcholic extract at low dose (100 mg), middle dose (200 mg) and maximum dose (400 mg) for 28 days intraperitoneally. After 28 days, all rats were weighed and were bled out from cardiac vein. Biochemical parameters were measured by standard methods. Liver biopsy was also obtained for histopathy. Results revealed that Origanum Vulgare hydroalcoholic extract leads to significant reduction of liver enzymes, bilirubin, creatinin ($P \leq 0.05$). Furthermore, a significant increase was seen in triglycerides, cholesterol-HDL, and cholesterol-LDL levels respectively ($P \leq 0.05$ and $P \leq 0.01$). There were no effects on other parameters and there was no histopathological change.

Keywords: Origanum Vulgare, Liver test, liver enzyme, Lipid, biochemical parameter

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INTRODUCTION

Today, many researchers are interested in the application of herbal drugs. Most of these drugs have components with preventive effects and in some communities, because of their inhibitory features in special diseases, use of fruits and vegetables have increased as a result of their protective effects against diseases like cancer, cardiovascular and liver diseases. The protective properties are due to antioxidant compounds in plants that lead to the prevention of damages by free radicals [1]. *Origanum Vulgare* is from labiatae family and plantae kingdom [2]. This herb is seen in vast areas of Europe, especially in southern Europe, the north of Africa and vast areas of Asia. In Iran it is distributed in the north and northwest regions but has not been observed in the hot regions of the south [3].

Origanum Vulgare in traditional medicine is utilized as disinfectant, antispasmodic, anti-flatulence, anti-worm and is also used for liver and gallbladder discomforts [4-7]. Origanum Vulgare 1% essence consists of phenols, monoterpene main proportion of which consists of phenols, monoterpene hydrocarbons and alcohol. Generally Origanum Vulgare essence contains 25 compounds, such as 26.9% thymol, carvacrol 40.7% and 7.3% gamma Trypnyn [8-10]. Hyper lipidemia increases serum lipid levels like total cholesterol, LDL and TG, that can lead to the development of arthrosclerosis and finally myocarditis infarction [1]. Some chemical drugs are used in liver diseases; however, there is no herbal plant for use in liver diseases. Although Origanum Vulgare is used more traditionally, there is less study about its effect on liver. In this study the effect of Origanum Vulgare hydroalcoholic extract on some liver enzymes (AST, ALT and ALP), serum concentrations of cholesterol, triglyceride, HDL-cholesterol, LDL-cholesterol, Creatinine, albumin, Bilirubin and total protein in rat were investigated.

MATERIALS AND METHODS

Extract Preparation

The herb was purchased from a local herbal shop in Shiraz and identified in the Shiraz Agriculture Faculty according to standard protocol. The leaves were cleaned and powdered by electric blender and then extracted with 75% 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 extract (12.9% of leaf powder) was kept at 40°C until used. The extract was soluted in water before administration.

The rats were kept in the animal house of the Kazeroon Branch of Islamic Azad University and were fed with routine diet.

Blood Sampling

Blood samples were taken from the heart of fifty adult male Wistar rats weighing about 200 ±5 g. Animals were divided into five groups(ten rats in each group). 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 three experimental groups received intraperitoneally low (100 mg/kg BW), medium (200 mg/kg BW) and maximum (400 mg/kg BW) Origanum Vulgare

hydroalcoholic extract daily for 28 days respectively. After 28 days all animals 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.

Measurements

ALT, AST and ALP were measured using colorimetric standard methods. Serum total cholesterol was analyzed by BIOTRON BTR 820 Auto Analyzer using enzymatic method [11]. HDL cholesterol was measured by the above-mentioned analyzer using phosphotungstate [12]. LDL cholesterol was calculated based on Friedwald's equation [13]. Serum triglycerides were estimated by using Autopack Reagent Kit by enzymatic DHBC colorimetric method [14]. Serum creatinine and total bilirubin were determined according to Young and Pestaner, and Jendrassik methods respectively. Serum total protein was measured according to the Biuret method as modified by Hutson et al., using bovine serum albumin as standard [15-17]. Serum albumin were assayed by Bromocresol green method.

Histopathological examination

Rat liver slices were collected and immersed in 10% neutral formalin as fixative. The fixative liver samples were sent to the Cancer Institute for histopathological examination according to Bancroft et al., (1996)[18].

STATISTICAL ANALYSIS

The data were expressed in SI units and analyzed by repeated measurements ANOVA, Duncan, Spearman and T-test using SPSS/PC software [19]. All values were expressed as mean and standard error (SE) and $P<0.05$ was seen as statistically significant.

RESULTS

Table 1: The mean ±SE of ALT, AST, ALP, cholesterol, triglyceride, HDL-cholesterol, LDL-cholesterol, total protein and albumin.

Albumin (g/dl)	Total protein (g/dl)	ALP (U/L)	ALT (U/L)	AST (U/L)	LDL-Cholesterol (mg/dl)	HDL-Cholesterol (mg/dl)	Cholesterol (mg/dl)	Triglyceride	Parameters Groups
± 4.15 0.123	± 8.85 0.1201	520.875 ± 49.1133	± 55.5 3.0911	± 293 42.3893	± 36.25 1.501	± 26.00 1.331	± 56.97 2.69	± 38.99 3.12	Group1
± 4.45 0.19	± 8.61 0.11	± 499.25 83.99	± 53.25 3.99	± 290.00 11.30	± 37.87 0.81	± 25.50 0.72	± 47.45 2.11	± 48.12 3.91	Group2
± 3.66 0.09	± 8.58 0.14	± 461.50 56.75 *	± 52.00 4.10	± 277.12 30.41	± 39.12 0.9	± 29.12 0.61	± 55.15 2.50	± 40.9 2.12	Group3
± 4.00 0.17	± 8.30 0.19	± 412.87 45.58 *	± 45.62 3.23*	± 267.37 17.53 *	± 41.00 1.12*	± 31.00 0.89 **	± 56.12 2.61	± 53.00 2.61 **	Group4
± 3.60 0.12	± 7.60 0.11	± 369.37 23.11 *	± 38.52 3.50 *	191.00 ± 29.26 *	± 43.37 1.11**	± 34.00 1.19 **	± 55.00 3.01	± 55.1 2.19 *	Group5

Significant in $P<0.05^{*}$, Significant in $P<0.01^{**}$

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Table 2: The mean ±SE of creatinine and total bilirubin.

Total bilirubin (g/dl)	Creatinine (mg/dl)	Parameters Groups
± 7.67 0.57	± 0.67 0.101	Group1
± 7.68 0.45	± 0.69 0.14	Group2
± 7.61 0.42	± 0.59 0.14	Group3
± 7.22 0.91	± 0.57 0.23	Group4
± 6.45 0.32 *	± 0.50 0.11 *	Group5

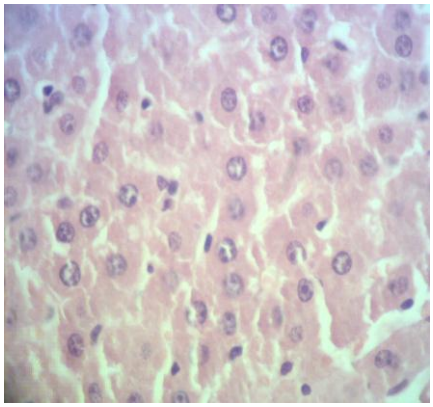
Significant in $P<0.05^{*}$

There were no significant changes in body weight between control and experimental groups($P>0.05$). The mean ±SE of ALT, AST, ALP, cholesterol, triglyceride, HDL-cholesterol, LDL-cholesterol, total protein, albumin and creatinine, and total protein are shown in Tables1 and 2 respectively.

Histopathological examination

Histological examination of liver sections from control and sham groups revealed normal structure (Figure 1, 2). Different concentrations of Origanum Vulgare hydroalcoholic extract indicated no changes Histopathologically Cellular disciplinary, keeping radial mode et cell and having normal liver cells (1or 2 nuclear, have abundant euchromatin and specified nucleolus) was obvious (Figure 3,4,5).

1



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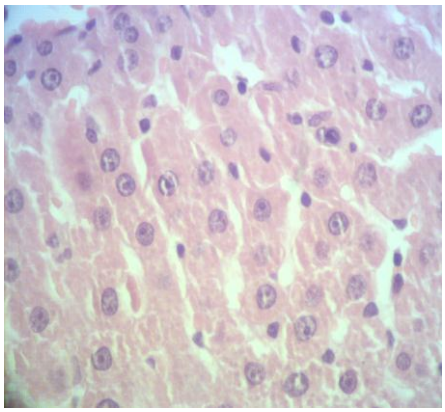

Figure 1: control group, (40×)

Figure 2: sham group, (40×)

Figure (1 and 2): control group: revealed the normal histological structure of hepatic lobule.

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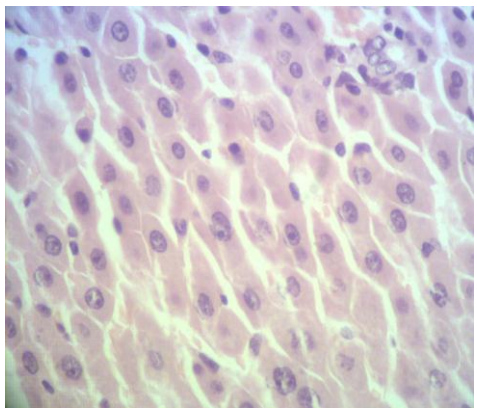

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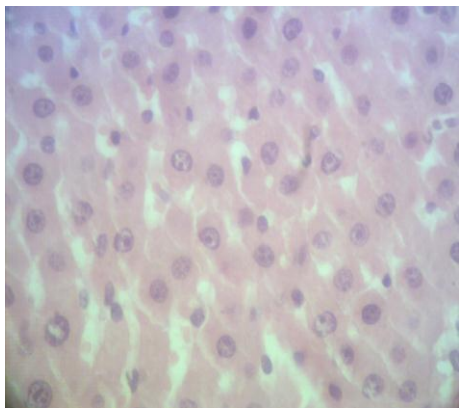

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Figure 3: low group (100 mg/kg BW), (40×) Figure 4: medium group (200 mg/kg BW), (40×)

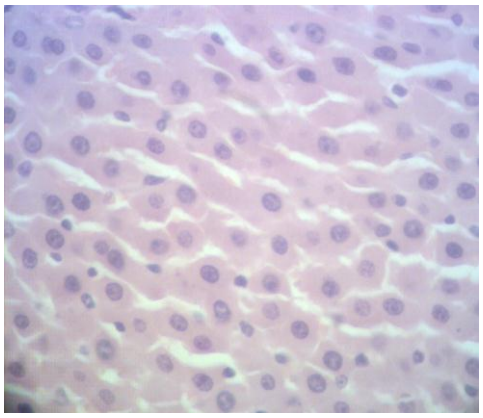

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Figure 5: maximum group (400 mg/kg BW),(40×)

Figure (3,4,5): Different concentrations of Origanum Vulgare hydroalcoholic extract make no changes Histopathologically Cellular disciplinary, keeping radial mode et cell and having normal liver cells.

DISCUSSION

On the basis of the results of enzyme ALT, the groups that received an extra 200 and 400 mg/kg show meanful reduction of $P<0.05$ in comparison with Control group. About enzyme AST, only the group receiving 400 mg/kg of extract shows meanful reduction of $P<0.05$ in contrast to control group. Regarding ALP enzyme, all 3 groups receiving 100, 200, 400 mg/kg show meaningful reduction at $P<0.05$ level, in contrast to control group. These results show the effect of Origanum Vulgare on liver cell and ALP, ALT, AST enzymes reduction [20-23]. Investigations proved that coffeic acid inhibits increase of serum enzymes against liver damages caused by tetrachloride methane which suppress liver protective activity by different mechanisms [20-22]. According to Shamavi's research (2005)the more live antioxidant feature, the less AST and ALT enzymes (24). Based on the research of Matsuura et al. and Bozin et al., it seems that Flavonoid components in Origanum Vulgare like Rosmarinic are able to neutralize (DPPH) 1,1-diphenyl1-2-picryl hydrazyl and inhibit its destructive effects through its antioxidant trait [25-27]. On the other hand, stimulation of DNA polymerase by flavonoid components cause rRNA synthesis to increase in liver repaired cells [25,29]. According to research done by Oktem et al., (2006), litospermic B, 12- Hydroxy

jasmonic, orsolic acid and other phenolic compounds reduce liver inflammation by inhibition of lipoxygenase cycle, leukotriene and free radical production in mouse liver kuppfer cell [28]. It can be concluded that when the antioxidant defence system of liver increases, the ALT, ALP, and AST enzymes rate probably reduces.

Regarding serum creatinine rate, the group that received Origanum Vulgare hydro alcoholic extract of 400 mg/kg Bw had meaningful reduction in $P<0.05$ level in contrast to control group. Investigation results of Sabten Ocak et al., (2007) show that caffeic acid, origanums antioxidant compound, inhibit high production of nitric oxide and reduce damages caused by nephrotoxic [29]. So, inhibition of oxidative damages leads to optimal nitric oxide system and improves kidney activity, causing an increase in creatinine excretion and a reduction of it in plasma. The statistical investigations showed Origanum Vulgare hydroalcoholic extract has no effect on serum concentrations of albumin and total proteins. Other results indicate that consumption of Origanum Vulgare has no significant effect on plasmas mean cholesterol concentration, so there was no statistical difference in mean cholesterol concentration between the various studied groups in comparison with control and witness group ($P<0.05$). Mean concentration of triglycerides and cholesterol-HDL are in contrast to control group so that mean rate of these 2 parameters in experimental groups 4,5 (which received 200 and 400 mg/kg Bw of Origanum Vulgare for 28 days) have have significant statistical statistical differences in contrast to control group ($P<0.05$), the greatest average of triglycerides and cholesterol concentration was observed in these groups.

The result of this study shows that use of Origanum Vulgare has more significant effects on cholesterol-LDL and most of it was shown in dosages of 100 and 200 mg/kg Bw, so that meaningful statistical differences were observed between mean rate of cholesterol-LDL in groups 3 and 4 in contrast to control group ($P<0.05$).

It seems that Origanum Vulgare effects lipid metabolism by dose-dependent mechanism and changes the concentration of plasmas triglycerides lipase which breaks down tissue triglycerides and increases their plasma rate. Triglycerides concentration of plasma in the group which consumed 200 mg/kg Bw of Origanum Vulgare daily was more than the group which consumed 400 mg/kg Bw of Origanum Vulgare, it seems that in dose of mg/kg Bw, elimination of plasmas triglycerides is higher than triglycerides lipolysis.

Normally, increasing concentration of plasmas triglycerides is associated with decreasing cholesterol-HDL concentration. But in this case, it is determined that consumption of Dittaws causes an increase in plasma triglycerides and cholesterol-HDL simultaneously.

The significant proportion of Dittany consists of phenols, monotropone hydrocarbons like P-Simine, Y-tripine and monotroponic alconols such as linalul, x-tronpine and Thujan 4-01 [30]. Also, in essence of Origanum vulgare there are other antioxidant compounds such as thymul, cavacrol, linalool, camphan, toylen [31].

Antioxidants are plant chemicals that have useful effects in reduction of Arthrosclerosis and other cardiovascular diseases Antioxidants inhibit the bound or adhesion of lipid to vessel walls and inhibit cellular degradation by reducing unsaturated

fatty acids and free radicals oxidation. In Sadeghi et al., in case of the effects of consumption of some plants on serum lipids rate, it is said that daily use of 500 mg/kg Dorema aucheri has anti hyperlipidemia and hypercholesterolemia effects and utilization of this plant affects ion at liver activity. These effects can be due to the antioxidant available in it . It must be noted that consumption of 500 mg/kg of this plant has toxic effects despite its anti lipidemic effects. So optimal consumption dosage must be determined [32, 33].

Extract of Artichoke affects serum lipids and total cholesterol and cholesterol-HDL rates reduce 18.5 % and 23 % respectively; these effects can be due to inhibition of cholesterol synthesis or cholesterol elimination by bile [34].

Beta Vulgaris extract as an antidiabetic compound significantly reduces triglycerides and cholesterol rates in healthy and patient group (35). Onoagbe et al. 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 Onoagbe et al [36].

Choi and Hwang., 2005 conducted a study in 2005 where the result of a number of plants (fruit of piper cubeba, flowering of physalis angulata and flower of Rosahybrida) caused an increase in activity of antioxidant enzymes and cholestrol-HDL and reduction in Malon di Aldehid. Moreover, the effects of these plants Vena reduces the potentiality of heart diseases [37].

CONCLUSION

Results have revealed Origanum Vulgare hydroalcoholic extract leads to reduction of liver enzymes, bilirubin, and creatinin to significant level of $P \leq 0.05$. Furthermore, there was a significant increase in triglycerides cholesterol-HDL, cholesterol-LDL levels to $P \leq 0.05$ and $P \leq 0.01$ respectively. No effects on other parameters were seen and there was no change histopathologically.

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