The investigation of the Anti-hypercholesterolemic Activities of Thymbra Spicata Labiata Oil (*Karabas* Thyme Oil) and Atorvastatin

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(Receıved on 2nd August 2010, accepted in revised form 14th January 2011)

Summary: The leaves of TSL (*Thymbra Spicata Labiatae*), which include plant sterols and stanols, are used in the treatment of hypercholesterolemia, which is a highly prevalent health problem. This study aimed to determine the histopathological effects of TSL oil and atorvastatin (ATS), also known as *Karabas* thyme, an herb in the Isparta region, on the biochemical parameters and the kidney tissues of the rats who received TSL oil and ATS in varying doses .

42 male four month-old Wistar albino rats with weights ranging from 200 to 250 g were used in this study. They were divided into 6 equal groups with 7 rats in each. The control group was fed standard pellet chow. The cholesterol (Chol) group and the other groups, except control groups, were fed on pellet chow with a cholesterol level of 1%. The 100 TSL, 200 TSL and 300 TSL groups were fed with a gavage tube a mixture of TSL oil, 0.5% Sodium Carboxy Methyl Cellulose (SCMC) of 100, 200 and 300 mg/kg, respectively. On the other hand, a 50 mg/kg Atorvastatin (ATS) 0.5% Sodium Carboxy Methyl Cellulose (SCMC) suspension was prepared and administered with a gavage tube to the 50 ATS group. Control group was given 0.5 ml with probed %0.5 SCMC.30 days later, the experiment was ended, and blood and tissue samples were collected from the rats. The following concentrations were assessed in the blood samples: serum glucose, Blood Urea Nitrogen (BUN), creatine, total protein, albumin, alanine aminotrasferase (ALT), aspartate aminotrasferase (AST), alkaline phosphatase (ALP), creatine kinase (CK), lactate dehydrogenase (LDH), Total Cholesterol (TC), Low Density Lipoprotein Cholesterol (LDL-C), High Density Lipoprotein Cholesterol (HDL-C), Triglyceride (TG), Oxidized LDL (ox-LDL), fibrinogen, homocysteine, and Vascular Endothelial Growth Factor (VEGF). The kidney tissues were examined histopathologically.

It was observed in this study that TSL and ATS did not affect the biochemical test results in general. It was also found that TSL could be influential in hypercholesterolemia and that a 300 mg/kg of TSL and ATS showed an antihypercholesterolemic effect of approximately the same degree. With TSL and ATS, minor kidney defects were observed. However, the effects of TSL increased with larger doses.

Introduction

Cholesterol is an important constituent of biological membranes and lipoproteins. Moreover, it plays a role in many structures such as steroid hormones, bile acids and vitamin D biosynthesis. The cholesterol concentration in blood is affected by both cholesterol-enriched diet and cholesterol synthesis in the liver [1].

While cholesterol has such a vital place in the human body, hypercholesterolemia is one of the most important health problems. The excessive consumption of animal fat and hydrogenated vegetable oils today increases lipid and cholesterol concentrations. As a result, many serious disorders such as atherosclerosis [2,3] cerebral vascular diseases [4], and fatty liver [5] can develop.

Even though pharmacological advancements in synthetic chemistry decreased the use of herbs for treatment of diseases at the beginning of the 20th century, towards the end of the same century, herbs regained their popularity under the name of "alternative medicine" as they were, natural, cheap, easily accessible, less toxic with lower side effects. In 1994, Europe and Japan spent 6 billion dollars and 2.1 billion dollars, respectively, on pharmaceutical preparations [6].

It was reported that plant sterol and stanols decreased cholesterol concentration by means of various mechanisms [7]. While plant sterols, campesterols and sitosterols are similar to cholesterol in structure, they are a lot more hydrophobic than cholesterol, and are, therefore, absorbed by replacing cholesterol in the mixed micelles. This replacement leads to a decrease in cholesterol concentrations and their absorption [4].

Thymbra Spicata Labiatae (TSL) is a herb, which has been grown and known for years in the Mediterranean Region. It includes volatile and scented compounds. The most important volatile oil compounds are carvacrol, p-cymen, γ-terpinen, borneol, thymol, linalool, β-pinene and camphen [8].

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Hypercholesterolemia, a highly prevalent health problem, can be treated by traditional means. TSL leaves have been used in the treatment of hypercholesterolemia for years [9]. The effect and side effects of this herb, which is frequently used among people, should be investigated.

Statins constitute the most powerful group of the lipid reducing drugs, which inhibit the reductive enzyme of 3-hydroxy-3-methilglutaryl Co-enzyme A (HMG-CoA) available today [10]. HMG CoA reductase inhibitors are frequently preferred owing to their lower side effects when compared to those of other drugs. Yet, because they are metabolized in the liver to a great extent, it is important to beware of liver toxicities [11,12]. An increase of 2-5% in ALT and AST in the liver as a side-effect of statins was previously reported [13].

In this study we used TSL, also known as *Karabas Thyme* (*Kara Thyme*) in the Isparta region. By administering TSL oil and atorvastatin (ATS) of various doses, we aimed to assess whether structural changes occur in the rats, kidney tissues, and evaluate their association with such biochemical parameters as Total cholesterol (TC), LDL-C, HDL-C, Triglyceride (TG), Oxidized LDL (ox-LDL), fibrinogen, homocysteine, and Vascular Endothelial Growth Factor (VEGF).

Results and Discussion

The average ± standard deviation values of glucose, BUN, creatine, total protein, albumin, ALT, AST, ALP, CK and LDH concentration of the 6 groups are presented in Table-1. No significant statistical difference was observed between the control group and the other groups in terms of these parameters ($p>0.05$).

The average ± standard deviation values of the TC, HDL-C, LDL-C and TG concentration of the control, Chol, 100 TSL, 200 TSL, 300 TSL and 50 ATS groups, and the paired comparison values calculated using the "Mann-Whitney U" test of the TC, HDL-C, LDL-C and TG concentrations of the same groups are presented in Table 2.

When the TC concentration of group Chol. and group 100 TSL were compared to those of the control group, a statistically significant increase was observed ($p<0.01$).

`No statistically significant difference was observed in the HDL-C concentration of Chol, 100 TSL and 200 TSL groups when compared to those of the control group ($p>0.05$), while a statistically significant increase was observed between the HDL-C concentration of group 300 TSL and group 50 ATS and those of the control group ($p<0.05$) (Table 2).

No statistically significant difference was found between the LDL-C concentration of group Chol. and group 100 TSL and those of the control group ($p>0.05$), while a statistically significant decrease was observed in the LDL-C concentration of group 200 TSL and group 300 TSL when compared to those of the control group ($p>0.05$). (Table 2).

The average ± standard deviation values of the homocysteine, fibrinogen, VEGF and ox-LDL concentration of the control, Chol., 100 TSL, 200 TSL, 300 TSL and 50 ATS groups, and the paired comparison results measured using the "Mann-Whitney U" test of the 6 groups are presented in Table-3.

Table-1: All group's concentration of serum glucose, BUN, creatinin, total protein, albumin and activations of ALT, AST, ALP, CK, LDH.

Groups *	**Glucose (mg/dl)**	**BUN (mg/dl)**	**KRE (mg/dl)**	**T.PROTEİN (g/dl)**	**ALBUMİN (g/dl)**	**AST (U/L)**	**ALT (U/L)**	**ALP (U/L)**	**CK (U/L)**	**LDH (U/L)**
50 Ator Groups (n=7)	**141.0±8.7**	**17.7±1.9**	**0.56±0.03**	**5.49±0.21**	**2.73±0.14**	**91.2±17.8**	**54.28±8.8**	**212.5±63.8**	**183.7±37.1**	**794.2±253.6**
300 TSL Groups (n=7)	**143.7±12.4**	**17.3±3.0**	**0.56±0.08**	**5.57±0.39**	**2.75±0.35**	**99.5±11.0**	**66.7±8.7**	**265.5±65.9**	**228.7±31.7**	**1001,5±270.9**
200 TSL Groups (n=7)	**150.8±18.7**	**17.9±1.3**	**0.52±0.02**	**5.54±0.30**	**2.75±0.10**	**109.5±29.1**	**56.8±7.8**	**295.0±73.1**	**239.8±107.3**	**1217.1±515.0**
100 TSL Groups (n=7)	**156.8±14.8**	**18.1±2.2**	**0.50±0.19**	**5.71±0.42**	**2.88±0.22**	**112.7±22.2**	**61.±8.6**	**240.4±46.2**	**187.7±90.8**	**1179.4±282.6**
Chol. Groups (n=7)	**149.1±12.7**	**18.5±0.5**	**0.56±0.03**	**5.6±0.21**	**2.89±0.6**	**107.92±16.8**	**61,8±11.3**	**272.7±48.1**	**178.4±70.9**	**1036.4±289.3**
Control Groups (n=7)	**144.5±17.7**	**20.2±2.2**	**0.55±0.03**	**5.2±0.14**	**2.60±0.21**	**127.8±27.7**	**65.0±5.0**	**281.0±40.2**	**209.4±79.1**	**1194.5±385**

*Concentrations of albumin, total protein, creatinin, BUN, serum glucose and activation of ALT, AST, ALP, CK, LDH which are belonging to six groups, no find meaning statistical difference in comparisions of groups ($p>0.05$).

Table2: TC, HDL-C, LDL-C and TG concentrations belonging to chol. control, 100 TSL, 200 TSL, 300 TSL and 50 ATS groups.

Groups	TC (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	TG (mg/dL)
Control (n=7)	53.1±4.5	33.7±3.0	16.5±1.8	56.5±17.5
Chol. (n=7)	71.7±10.65	32.7±2.2	21.3±7.9	83.7±12.9
100 TSL (n=7)	66.0±9.4	36.5±2.7	16.8±4.0	77.0±27.2
200 TSL (n=7)	62.0±11.2	35.8±5.9	10.3±3.	66.4±20.0
300 TSL (n=7)	57.0±6.7	38.4±2.3	10.8±2.5	52.1±20.0
50 ATS (n=7)	56.0±6.7	39.1±3.2	17.5±3.5	70.7±15.9
Control- Chol.	p < 0.01	p > 0.05	p > 0.05	p < 0.05
Control-100 TSL	p < 0.01	p > 0.05	p > 0.05	p > 0.05
Control-200 TSL	p > 0.05	p > 0.05	p < 0.01	p > 0.05
Control-300 TSL	p > 0.05	p < 0.05	p < 0.01	p > 0.05
Control-50 ATS	p > 0.05	p < 0.05	p > 0.05	p > 0.05
Chol. -100 TSL	p > 0.05	p < 0.05	p > 0.05	p > 0.05
Chol. -200 TSL	p > 0.05	p > 0.05	p < 0.01	p > 0.05
Chol. -300 TSL	p < 0.01	p < 0.01	p < 0.01	p < 0.01
Chol. -50 ATS	p < 0.05	p < 0.01	p > 0.05	p > 0.05

Doubly comparison of groups did with "Mann-Whitney U" test. Accepted meaningly p<0.05.

Table-3: Homocysteine, fibrinogen, VEGF and ox-LDL concentrations belonging to groups of Control, Chol., 100 TSL, 200 TSL, 300 TSL and 50 ATS.

Groups	Homosistein µmol/L	Fibrinojen mg/dL	VEGF pg/mL	Ox-LDL ng/mL
Control (n=7)	3.75±1.01	111.4±29.0	6.43±2.03	10.09±1.8
Chol. (n=7)	4.55±0.53	169.7±35.7	8.36±1.07	17.96±6.04
100 TSL (n=7)	3.29±0.4	119.2±41.7	7.68±1.98	14.94±4.39
200 TSL (n=7)	3.28±1.57	109.4±29.	5.8±1.41	13.18±2.11
300 TSL (n=7)	3.33±0.89	96.7±34.7	5.69±1.25	10.87±1.90
50 ATS (n=7)	3.38±0.55	100.5±9.5	6.5±1.11	10.77±2.97
Control-Chol.	p > 0.05	p < 0.01	p > 0.05	p < 0.05
Control-100 TSL	p > 0.05	p > 0.05	p > 0.05	p < 0.01
Control-200 TSL	p > 0.05	p > 0.05	p > 0.05	p < 0.05
Control-300 TSL	p > 0.05	p > 0.05	p > 0.05	p > 0.05
Control –50 ATS	p > 0.05	p > 0.05	p > 0.05	p > 0.05
Chol. -100 TSL	p < 0.01	p > 0.05	p > 0.05	p > 0.05
Chol.-200 TSL	p > 0.05	p < 0.05	p < 0.05	p > 0.05
Chol.-300 TSL	p < 0.05	p < 0.01	p < 0.01	p < 0.05
Chol.-50 ATS	p < 0.01	p < 0.01	p < 0.01	p < 0.05

Doubly comparison of groups did with "Mann-Whitney U" test. Accepted meaningly p<0.05.

A statistically significant difference was not observed between the homocysteine concentrations of the Chol, 100 TSL, 200 TSL, 300 TSL and 50 ATS groups and those of the control group (p>0.05) (Table 3).

While a statistically significant increase was observed in the fibrinogen concentrations of group Chol. when compared to those of the control group (p<0.01), there was no statistically significant difference in the fibrinogen concentrations between the control group and the 100 TSL, 200 TSL, 300 TSL and 50 ATS groups (p>0.05).

No statistically significant difference was observed between the VEGF concentrations of the control group and those of the Chol, 100 TSL, 200 TSL, 300 TSL and 50 ATS groups (p>0,05). While no statistically significant difference was observed between the VEGF concentrations of group 100 TSL and those of group Chol. (p>0.05), a statistically significant decrease was observed in the VEGF concentrations of groups 200 TSL, 300 TSL and 50 ATS when compared to those of group Chol. (p<0.05, p<0.01, p<0.01, respectively) (Table 3).

While a statistically significant increase was observed in the ox-LDL concentration of groups Chol, 100 TSL and 200 TSL when compared to those of the control group (p<0.05, p<0.01, p<0.05, respectively), no statistically significant difference was found between group 300 TSL and group 50 ATS (p>0.05) (Table 3).

A statistically significant, positive difference was observed between the TC concentrations and the LDL-C, TG, homocysteine, fibrinogen, VEGF and ox-LDL concentration of the control, 100 TSL, 200 TSL, 300 TSL and 50 ATS groups (p<0.01) .

A statistically significant, positive relationship was observed between the TG concentrations and LDL-C, fibrinogen, VEGF and ox-LDL concentration (p<0.01).

A statistically significant, positive relationship between homocysteine and TC, LDL-C, fibrinogen concentration was observed (p<0.01).

A statistically significant, positive relationship between the Ox-LDL concentration and TC, TG, fibrinogen, and VEGF concentration was observed (p<0.01).

When the kidney tissue cross-sections of the control, Chol, 100 TSL, 200 TSL, 300 TSL, and 50 ATS groups were evaluated, a very slight tubular defect was detected in one rat in group 100 TSL, in four rats in group 200 TSL, in six rats in group 300 TSL and in five rats in group 50 ATS (Fig. 1-6).

An antihypercholesterolemic effect of the Thymbra Spicata Labiatae (TSL) thyme was detected [9, 14]. It was also found that the thyme herb could have some side effects [14]. This study aimed to determine the antihypercholesterolemic effect and side-effects of TSL. In addition, the antihypercholesterolemic activities of TSL and ATS were compared.

The routine measurements of serum glucose, BUN, creatine, total protein, albumin, ALT, AST, ALP, CK, and LDH were made. In these measurements, no significant difference was

observed between the control the group and the groups which received TSL and ATS.

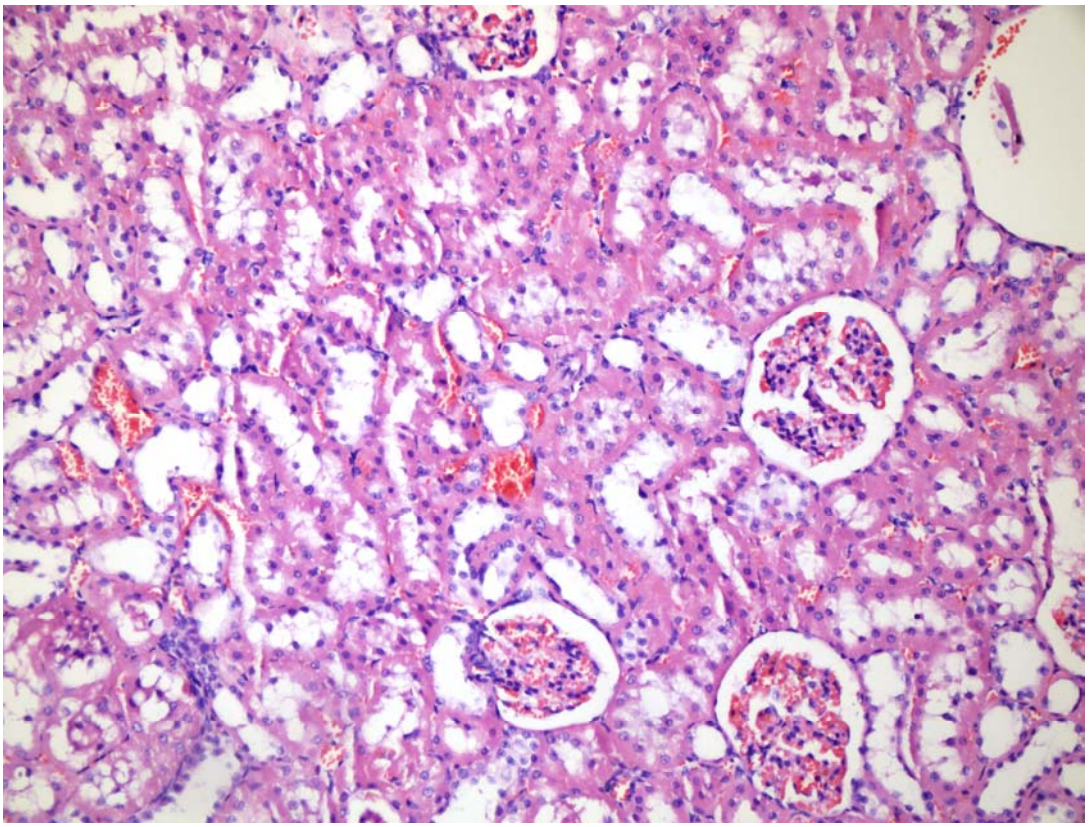

Fig. 1: Normal kidney tissue belonging to control group (Hematoxylin-Eosin, x100).

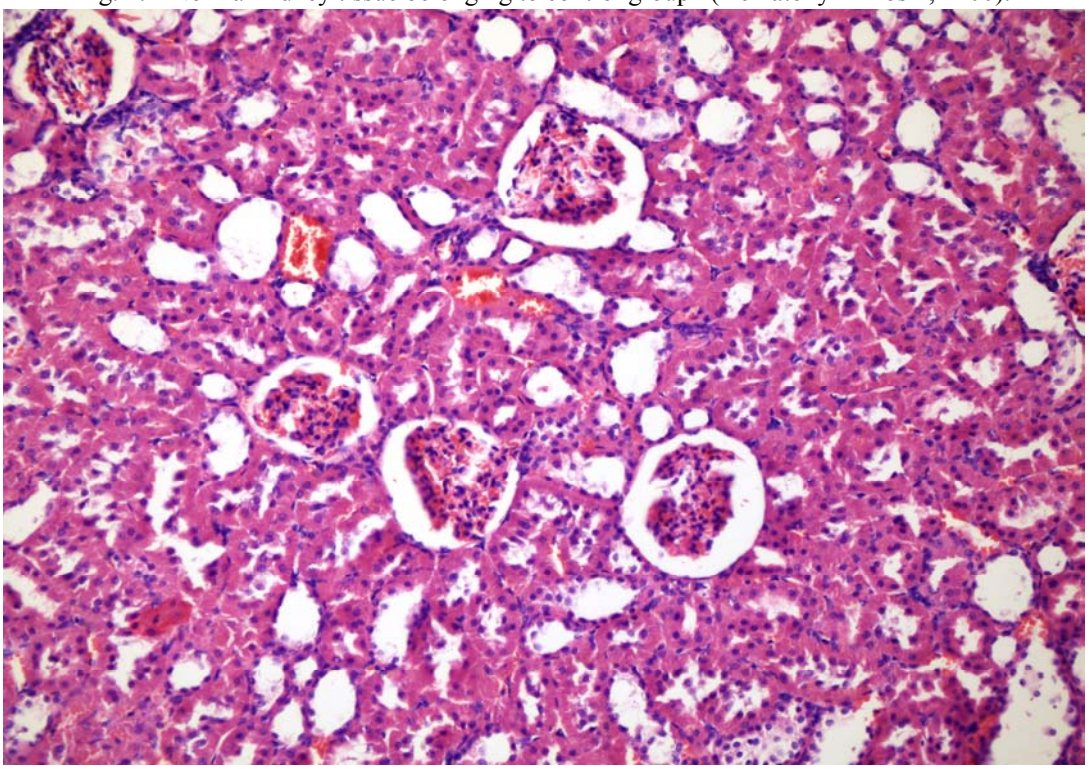

Fig. 2: Normal kidney tissue belonging to Chol. group (Hematoxylin-Eosin, x200).

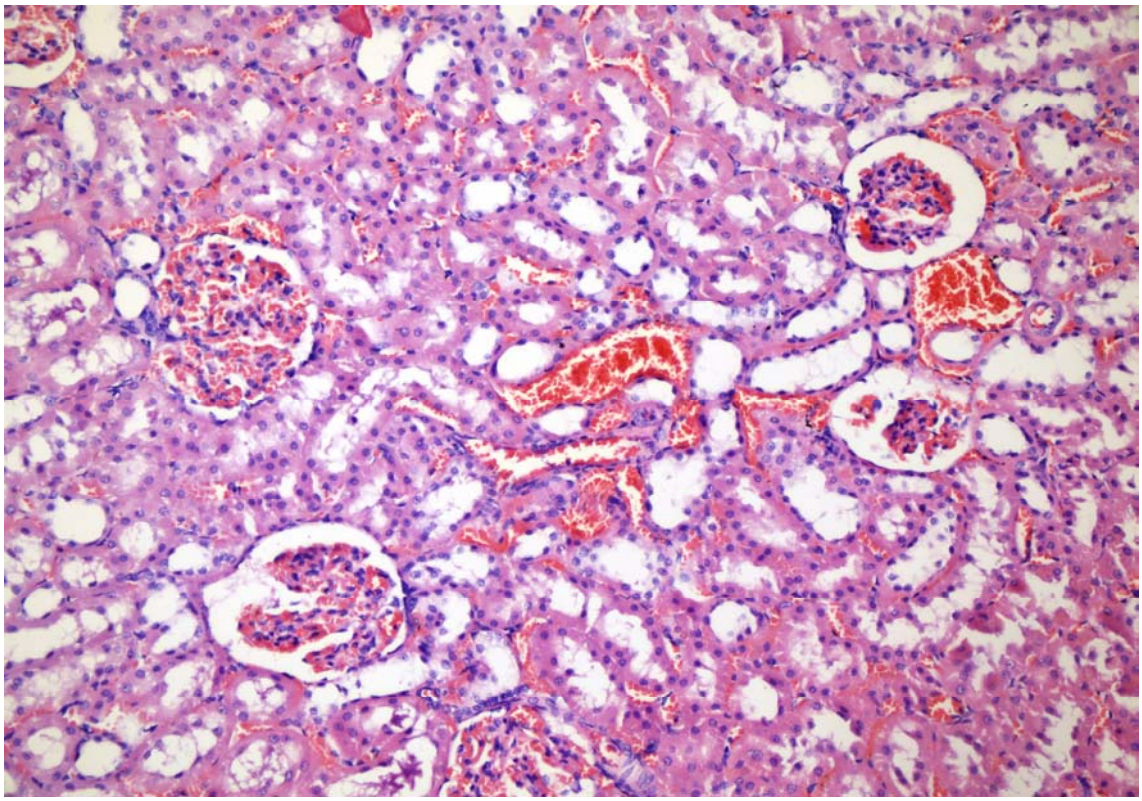

Fig. 3: Normal kidney tissue belonging to 100 TSL group (Hematoxylin-Eosin, x200).

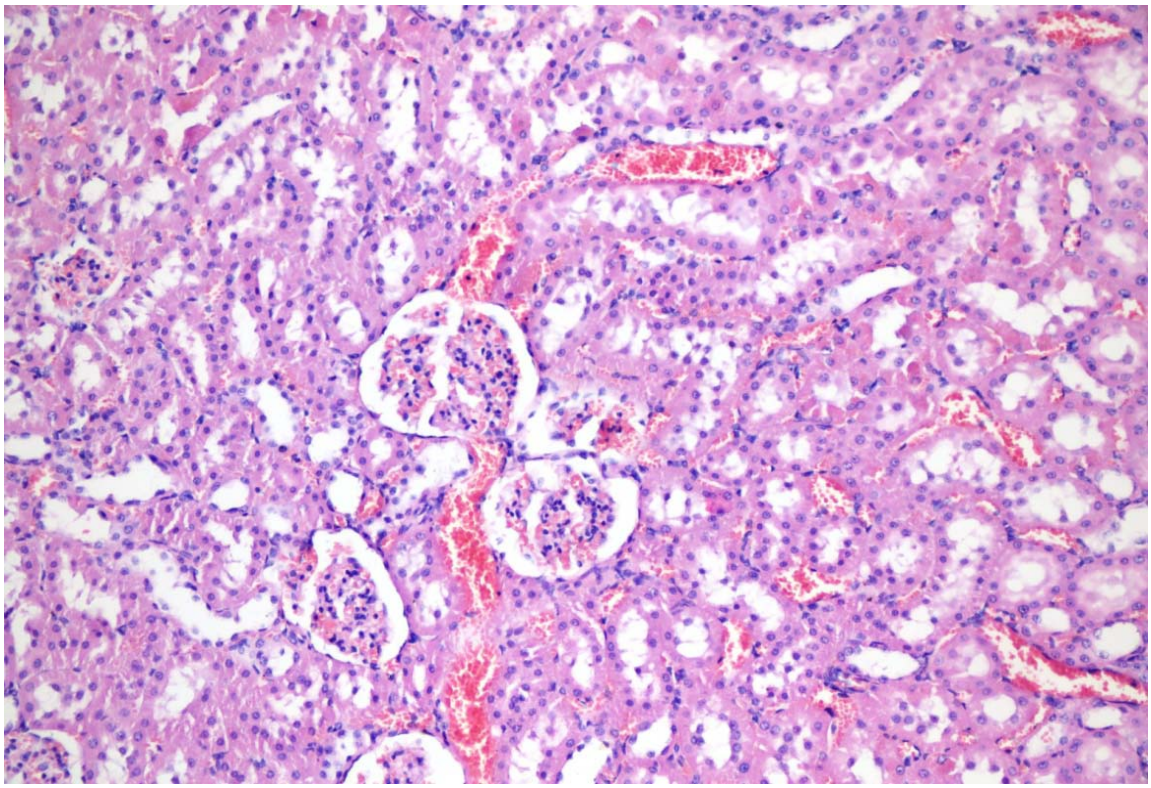

Fig. 4. Normal kidney tissue in 200 TSL group (Hematoxylin-Eosin, x400).

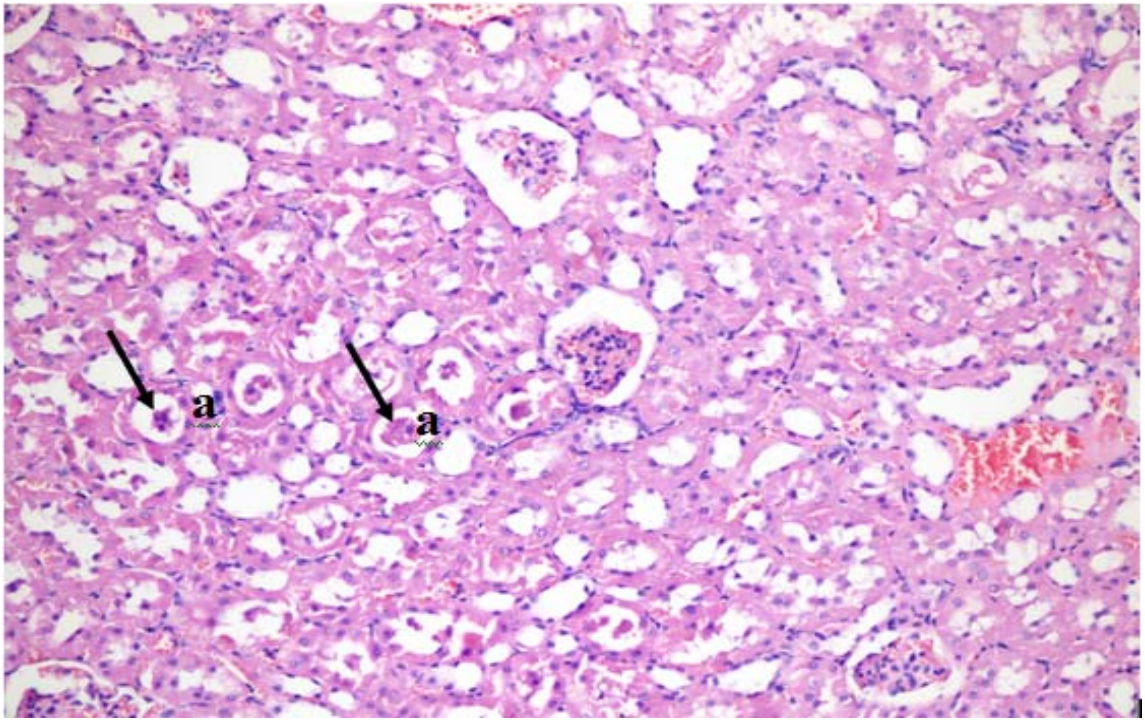

Fig. 5: Hydropic degeneration and cast (a) structures in tubular spaces of the kidney in 300 TSL group (Hematoxylin-Eosin, x200).

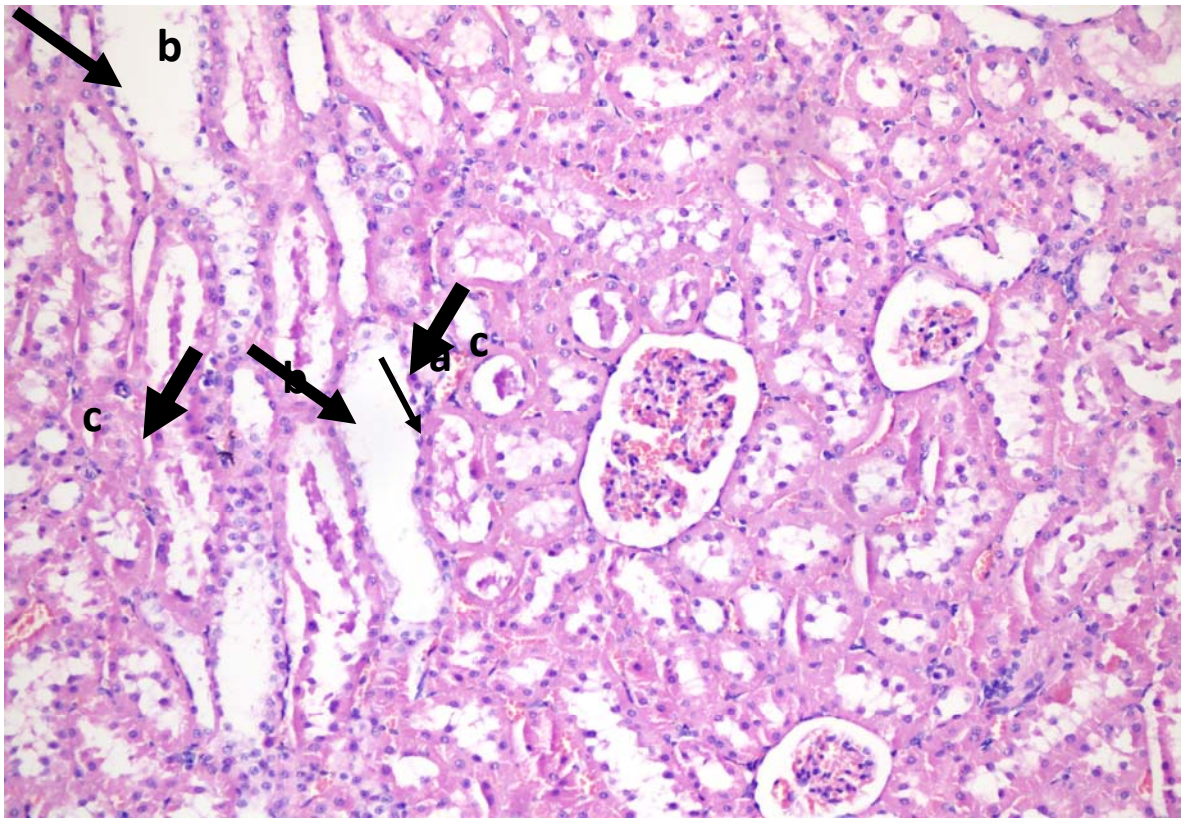

Fig. 6: Hydropic degeneration (c), dilatation (b) and cast (a) structures in tubular spaces of the kidney in 300 TSL group (Hematoxylin-Eosin, x200).

In one study, 100 mg/kg ethanolic extract of TSL, as a mixture of 0.5% Carboxy Methyl Cellulose, was given to the rats for 30 days. A control group, a group with a cholesterol added diet and a group to which TSL ethanolic extract was given were formed. In one other study, just as in our study, it was found that TSL did not show a significant difference in AST and ALT activities, but glucose concentrations, when compared to the group with a cholesterol added diet, significantly decreased AST and ALT [14].

In another similar study, different from our study, chow with 4% cholesterol and Tymus Fallax Fisch, a different type of thyme, were used. In the rats of this study, significant increases in the AST, ALP, BUN and creatine values of the rats in the group that were given 4% cholesterol and thyme. Fatty liver and the pathology related to prerenal uremia in the kidney were attributed to this thyme [15].

As a side-effect of statins, a 2.5% of slight increase in ALT and AST were reported [16]. However, in this study, ATS did not increase liver enzymes. In conclusion, with the doses used in this study, no side-effects reflected in the TSL oil and ATS routine measurements were observed.

In this study, the addition of cholesterol to the diet increased TC concentration, while it did not cause a significant change in LDL-C. High doses of TSL and ATS increased TC. A high dose of TSL increased LDL-C more than it increased ATS. The amount of increase that high doses of TSL and ATS caused were close to each other. The addition of cholesterol to the diet increased the TG concentration in group Chol. A high dose of TSL decreased TG to a significant degree when compared to that of group Chol.

In another related study, it was found that the ethanolic serum of TSL significantly decreased serum TG, LDL-C and TC concentration when compared to the goup with a cholesterol-added diet, but significantly decreased the HDL-C concentration [14]. Another study reported a different kind of thyme group significantly increasing HDL-C [15].

In a study in which high doses of statins were used, a higher decrease in LDL-C was reported when compared to that observed in low doses [17]. On the other hand, in this study, a significant decrease in LDL-C in group TSL, when compared to group Chol group, was observed in connection with increase in dose, but it did not cause a significant decrease in group ATS.

These findings are consistent with a study reporting that TSL decreased cholesterol concentration in rats fed with a high level of cholesterol [14] and the study stating that different types of thyme increased (by 3.5) serum HDL-C concentrations, thus showing an antiatherogenic activity [15]). A study indicating that thyme does not decrease cholesterol has not been encountered. Consequently, with further studies assessing the side-effects of thyme group herbs, we are of the opinion that they can be used in the treatment of hypercholesterolemia.

In this study, ox-LDL increased in the groups receiving a cholesterol added diet and in the groups that received a diet to which both cholesterol and a low dose of TSL were added. On the other hand, in groups receiving a diet to which cholesterol and a high dose of TSL and ATS were added, ox-LDL decreased. It was found that statins increased the number of LDL receptors and decreased LDL-C and ox-LDL in the plasma [18]. It was reported that TSL-related ethanolic extract TSL significantly decreased malondialdehit concentration [14]. A relationship between thyme group herbs and oil oxidation was detected [19]. However, no study indicating a relationship between thyme group drugs and ox-LDL was encountered. A high level of cholesterol increases the formation of free radicals, like superoxide radicals, by various means [20]. The effect of thyme on ox-LDL is derived from the thymol it entails and the antioxide feature of carvacrol. It was reported that the hypocholesterolemic effects of antioxidants emerged as a decrease in lipid peroxidation and antioxidant enzyme activity [15].

This shows that the antioxidant and antihypercholesterolemic effect of the thyme can decrease free radicals and ox-LDL, and thus, decrease the risk of coronary heart diseases. In conclusion, the antioxidant characteristic of thymol and carvacrol in thyme group herbs can be made use of.

In this study, while there was no significant difference in VEGF in the group to which cholesterol-added diet was given, a significant difference in the VEGF was observed in groups 200 TSL, 300 TSL and 50 ATS. This suggests that a decrease in cholesterol would decrease VEGF. In some studies where hypercholesterolemia was induced in different species of animals, changes in

the intima and an impairment in vascular vasodilator in the rabbit aorta was observed, yet there was no endothelial-related vascular response in pigs and dogs as a result of hypercholesterolemia. The increase in VEGF led to endothelial dysfunctions in the monkey's vein and the artery of the hypercholesterolemic dog [21].

It was indicated that hypercholesterolemia increased VEGF synthesis [22]. Hypercholesterolemia and other risk factors led to the impairment of endothelial vazodilator functions in humans peripheric blood veins. This pathological condition can be diagnosed in the first phases of life before the development of vascular lesion [23].

In hypercholesterolemia and atherosclerosis, the resistance of endothelial cells is lost, and their numbers increase especially in bifurcations with their physiological orientations impaired [24,25]. The morphological change in the endothelium causes an increase in serum cholesterol and EKGF synthesis. In hypercholesterolemia, as a result of the increase in the superoxide anions in the endothelial cells, the discretion of VEGF increases and this leads to impairment in endothelial-related diastole [26].

In hypercholesterolemia, VEGF is among the most important risk factors in arteriosclerosis [27,28].

The decrease in VEGF observed in the group that received a diet supplemented with cholesterol and high doses of TSL and ATS, suggests that VEGF has a positive relation with cholesterol.

In this study, there was an increase in the fibrinogen concentration of the group receiving a diet supplemented with cholesterol. A high dose of TSL and ATS, on the other hand, decreased fibrinogen. One study reported a positive relation between level of fibrinogen and LDL among 10.500 men whose ages ranged between 50 and 59, and a negative relation between HDL and fibrinogen [29]. These findings support our results. A similar association was reported in one more study [30]. In another study in which rats were given a diet deprived of cholesterol for one year, the correlations between fibrinogen and TG and HDL before and after the diet were found significant [31]. Different results were obtained in studies assessing the effects of statins on fibrinogen levels [32]. As a result of the administration of statin combined with fibratin in hypercholesterolemia treatment, significant decreases in fibrinogen levels were observed [33]. In studies where lovastatin was used, no change was reported in fibrinogen concentration [34,35]. Hypercholesterolemia impairs the procoagulant-anticoagulant balance. In coronary arteries, cholesterol and coagulant levels, which cause the formation of atherothrombosis, which in turn leads to the formation of MI, should be decreased [30]. It was found both in this and other studie that hypercholesterolemia treatment decreased fibrinogen levels. Thus, decreasing cholesterol, one of the risk factors of atherosclerosis, will lead to a decrease in fibrinogen, another risk factor.

While no change was observed in the homocyseine concentration in the rats receiving a cholesterol-added diet, a significant decrease was observed in the rats receiving a diet supplemented with both cholesterol and high doses of TSL and ATS.

In one study, 75 premature atherosclerotic patients were screened for hyperhomocysteinemia, and hyperhomocysteinemia was detected in approximately one third of the patients with cerebrovascular and perpheric vein disorder [36]. In other studies, a positive relation between homocystein and TC, and a negative relation between homocystein and HDL-C was reported [37,38]. Yet in another study, no significant relation between serum lipids and homocystein was observed [39]. Homocystein and hypercholesterolemia were presented as separate risk factors for cardiovascular patients [39].

The vascular defect mechanism in hyperhomocysteinia could not be completely understood. However, it was stated that hyperhomocysteinia could lead to this defect by increasing free radical production and lipid peroxidation, impairing endothelial functions, thrombosis functions or coagulation system functions, and by causing a decrease in adenosine production [40]. The findings of this study and some other studies show that there could be a positive relation between cholesterol and homocysteine.

No abnormal finding was observed in the liver and heart muscle cross-sections of all the groups. A slight tubular defect was observed in group ATS and TSL groups. The number of rats with tubular defects increased in direct proportion with the TSL dose. In a study in which Tymus Fallax Fisch thyme was used, a pathology which was associated with fatty liver and prerenal uremia in the kidneys

[15]. Apart from this, no histopathological study related to types of thyme was encountered. In this study, the slight tubular defect observed especially in group 300 TSL and group 50 ATS is an important point that needs to be taken into consideration when using these types of substances at these doses.

In conclusion, this study showed that TSL given in three different doses can be effective in hypercholesterolemia. It was found that 300 mg/kg of TSL and 50 mg/kg of ATS had the same degree of antihypercholesterolemic effect. TSL and ATS in these doses caused a minimum defect in the kidneys.

We believe that in the near future, medicinal herbs will be popular in the treatment of many disorders, primarily heart disease and the need for these herbs will gradually increase. We hope that this study will lead to further studies, and believe that more comprehensive studies will be conducted on the side effects caused especially in the kidneys in order to benefit from the antihypercholesterolemic and antioxidant effects of TSL.

Experimental

This study was carried out in Suleyman Demirel University, Faculty of Medicine, the Experimental Animal Laboratory, and the Deparment of Medical Biochemistry, Research Laboratory.

Animals

In this study, 42 adult male Wistar albino rats of weights ranging between 200-250g, obtained from Suleyman Demirel University, Experimental Animal Laboratory, were used. The animals were divided into 6 equal groups of 7: a control group, a group receiving a diet supplemented with 1% cholesterol (Chol group), groups receiving a diet supplemented with 1% cholesterol and 100-200-300 mg/kg of TSL (group 100 TSL, group 200 TSL, group 300 TSL), and a group receiving a diet supplemented with 1% cholesterol as well as atorvastatine (group 50 ATS). The rats were kept under standard light (12 hours of day light/12 hours of darkness) and at a temperature of 25 ^{o}C. They were fed 30 days in specially prepared cages.

On the first day of the study, the body weight of the rats were measured. 30 days after the beginning of the study, the experiment was ended with the collection of blood samples and kidney tissues under ketamine anesthesia. The kidneys for pathological study were fixed in a 10% neutral formaldehyde solution.

Materials

TSL Oil

In the study, the rats received 100, 200, 300 mg/kg doses of Thymbra Spicata Labiatae oil (produced via the distillation method by Semasen Baharat-Arsen, a Food, Drugs and Cosmetics Firm in Isparta). A mixture of TSL dissolved in 0.5% Sodium Carboxy Methyl Cellulose and given 0.5 ml

Atorvastatin (ATS)

In this study, the rats received a 50 mg/kg dose of Atorvastatin (Sanovel Inc. 10 mg Atorfilin film). Atorvastatin was prepared dissolved 0.5% Sodium Carboxy Methyl Cellulose and given 0.5 ml.

The Preparation of the Serum Samples

At the end of 30 days, all groups were decapitated under anesthesia (Ketamine 90 mg/kg + Xylazine 10 mg/kg). Approximately 6-7 ml of blood samples from the abdominal aorta were collected into standard tubes, citrate tubes and EDTA tubes placed in ice. While the tubes without anticoagulant and the citrate tubes were centrifuged at 4000 rpm for 10 minutes at room temperature, the EDTA tubes were centrifuged in a cooling centrifuge. The serum from the tube without anticoagulant was analysed for levels of glucose, BUN, creatine, total protein, albumin, ALP, ALT, AST, CK, LDH, TC, and HDL-C, LDL-C on the same day using an Olympus AU2700 autoanalyser (Japan) and the TG Olympus brand commercial kit.

The homocysteine concentration of the plasma in the EDTA tubes was measured using the USA commercial kit of Siemens Medical Solutions Diagnostics Los Angeles, CA 90045-6900 and the Immulite 2000 (USA) device.

The fibrinogen concentration of the plasma in the citrate tubes was measured using the Multifibren (DADE Behring Marburg GmbH, Germany) commercial kit and the DADE Behring BSC (USA) coagulation device.

The Vascular Endothelial Growth Factor from the plasma in the EDTA tubes was measured using the rat specific RayBiotech brand ELISA kit and the ELX808 Ultra Microplate Reader (USA) ELISA device.

Oxide-LDL Immune Diagnostic AG was measured by using the D64625 Bensheim brand Ox-

LDL ELISA kit and the ELX808 Ultra Microplate Reader (USA) brand ELISA device.

The Preparation of the Tissue Samples

The kidney tissues taken from the rats were fixed in a 10% formalin (pH 7.4 Phosphate buffer) solution. After routine follow-ups, 5-6 micron thick cross-sections were taken and stained with hematoxylin-eosin dye. Their microscopic pictures were taken and analyzed.

Thymbra Spicata Labiatae Oil Analysis

The Thymbra Spicata Labiatae oil was analyzed at the Suleyman Demirel University, Experimental and Observatory Student Research and Application Center Laboratory using the GC-17A Shimadzu (Japan) brand QP 5050 model mass spectrometer detector.

The Thympra Spicaya oil was found to compose of α-pirene (1.47%), Myrcene (0.57%), α-terpinene (0.93%), γ-terpinene (7.39%), p-cymene (18.73%), Linalool (4.28%), Caryolphyllene (1.94%), Borneol (0.96%), Thymol (0.79%), and Carvacrol (62.43%).

Histopathological Analysis

The kidney tissue specimens taken from the rats were fixed in neutral formalin. We worked in the S.D.U. Faculty of Medicine Pathology Laboratory. Cross sections in 5-6 micron of thickness were taken from kidney. After the slides were stained with Hematoxylin-Eosin and Masson's Trichrome, they were evaluated with Olympus BO 71 light microscope.

Statistical Analysis

Statistical analyses were performed using the SPSS for Windows Version 9.05 program. The "Kruskal-Wallis Variance analysis" test was used to determine whether there was a significant difference among the groups in general. The pairwise comparisons were conducted using the "Mann-Whitney U" test. The significance level was accepted as $p<0.05$. Whether there was a significant relationship between two variables was analyzed using the "Spearman's Rank Correlational Analysis". The results were reported as average ± SD values.

Conclusion

Herbal medicines are used in traditional treatment of the diseases for many years and we believe that it will be very popular in the near future. This study is designed in order to lead to the future studies in herbal medicine though side effects of these herbs must be taken into consideration so we hope that more comprehensive studies will be conducted on the side effects caused especially in the kidney in order to benefit from the antihypercholesterolemic and antioxidant effects of TSL.

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