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Effect of *thymus vulgaris* essential oil on blood lipid and histological changes of kidneys and aorta in Experimentally Induced Hyperlipidemic Mice

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Abstract

The experiment was conducted to investigate the effect of induced hyperlipidemia in mice fed with high fat diet (HFD) for 4 weeks on blood lipid profile and possible pathological changes in aorta and kidney. There were significant differences in most mice which induced hyperlipidemia throughout the experimental period with the blood lipid levels and histopathology

Keywords: Anti-hyperlipidemic, High-fat diet, thymus vulgaris, essential oil, antioxidant effects, statin,

Introduction

The main risk factor for the development of atherosclerosis and heart disease, hyperlipidemia is brought on by an excess of lipids or fatty substances in the blood. Depending on the underlying reasons, hyperlipidemia can be classified as either primary or secondary. Changes in lipids, including those found in cholesterol, triglycerides, very low-density lipoproteins (VLDL), low-density lipoproteins (LDL), and intermediate-density lipoproteins (IDL), may lead to coronary heart disease, cerebral stroke, myocardial infarction, and renal failure are becoming a major health problem in the world recently (1).

Hypercholesterolemia can build up and develop into atherosclerosis, thus narrowing of blood vessels, especially in the heart, brain, kidneys, and eyes. Moreover, 22% of cardiac output passes through the kidneys, making them prone to endothelial capillaries damage (2).

Although drugs therapies available for the treatment of hyperlipidemia include the use of drugs like niacin, fibrates, HMG-CoA reductase inhibitors, bile acid binding resins, Omega-3 Polyunsaturated Fatty Acids (PUFA), and PCSK9 inhibitors but associated with lots of side effects. Therefore, herbal treatment for hyperlipidemia has been appreciated because of fewer side effects, less cost, and easy availability (3).

Thymus vulgaris, belonging to the Lamiaceae family, is a small scented perennial herb, predominantly found in the Mediterranean region, North Africa and Southern Europe (4). Intraspecific chemotype variations are seen in Thymus and are named geraniol, α-terpineol, thuyanol-4, linalool, carvacrol, and thymol after its dominant monoterpene (5).

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Thymus vulgaris essential oil (TEO) is a mixture of monoterpenes. The main compounds of this oil are the natural terpenoid thymol and its phenol isomer carvacrol (CVL) (6), which have antioxidative, antimicrobial, antitussive, expectorant, antispasmodic, and antibacterial effects (7-9). Terpenoids, flavonoid aglycones, flavonoids glycosides, and phenolic acids were also found in *Thymus spp*.(10).

Materials and methods

Fresh aerial parts (stems and leaves) of *T. vulgaris L*. were dried at room temperature in a shadow place for 3 days. Arial parts were ground in a mill passed through a sieve of 30 mesh separately and the powders obtained were stored in amber glass bottles at 4 °C (11)**.**

Isolation of essential oil

Air-dried aerial parts (stems and leaves) of T. vulgaris were hydro-distillated for 2.5h using a Clevenger type apparatus according to the standard procedure. The essential oil volume was measured directly in the extraction burette. The obtained essential oils were dried with anhydrous sodium sulphate (12, 13).

Experimental animals

The study was conducted from March 2022 through September 2022 at the department of pharmacology–College of Medicine /AL Nahrain University. The experiments were approved by the Ethical Committee at the College of Medicine /AL Nahrain University.

Thirty-two apparently healthy, albino male mice 2-3 months old, weight about 20-30g, were obtained from the National center for drug control and researches. The animals were acclimatized in standard environmental conditions and fed with food and water *ad libtum* for a week before commencement of the experiment.

Induction of Hyperlipidemia

Hyperlipidemia was induced in mice by addition of High Fat Diet (2% cholesterol and 1% peanut butter) along with the standard for 28 days (14).

Table1: standard and high fat diets composition

Standard diet	**High Fat Diet**
Seeds (sunflower, groundnut)	Seeds (sunflower, groundnut)
Cereals	Cereals
Fruits (grapes, apple)	Fruits (grapes, apple)
Vegetables	Vegetables
Vitamin A	Vitamin A
Vitamin D_3	Vitamin D_3
Vitamin E	Vitamin E

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	Cholesterol powder
	Peanut butter

Experimental design

The rats were feeding with standard diet for two weeks before starting the experiment as acclimatization period for adaptation, then for group A as blank group continue for four weeks as experimental period, for B group feeding with high fat diet for four weeks, while group C got treated by atorvastatin 10 mg/kg for further 4 weeks after induction. Group D got treated by thymus vulgaris essential oil 500 mg /kg for further 4 weeks after induction.

Blood collection

The animals were fasted for 12 hours prior blood collection. Blood was collected by piercing the facial vein with a lancet. The blood samples were collected in plain glass tubes and allowed to clot for 20 minutes at room temperature and centrifuged at 3000 RPM for 20 minutes .

The serum obtained was kept at 0°C until analyzed. Serum was used for the estimation of the serum lipid profile and liver function test.

Biochemical analysis

Serum lipid total cholesterol (TC), triglyceride (TG), low density lipoprotein (LDL), very low-density lipoprotein (vLDL), high density lipoprotein (HDL), aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP) levels of mice were detected with a biochemical auto-analyzer (Shimadzu , Japan) and respective commercial test kits (Abbott diagnostic, USA) according to the manual instructions.

Histopathological examination

The kidney and aorta obtained from each animal after sacrificed and fixed in 10% formalin solution, then processed by the paraffin technique. Sections of 5µm thickness were cut and stained by haematoxylin and eosin (H&E) for histological examination. The sections were analyzed using an Olympus light microscope with an attached photograph machine (15)**.**

Statistical analysis

Statistical analysis was performed using SPSS (Statistical Package for social Science) version (17), and Microsoft Excel Worksheet 2010. Crude data was analyzed to obtain mean and standard deviation (SD). Student t- test was used to compare between two groups. ANOVA test was used to compare between different groups. P-value of $\leq$ 0.05 considered being significant and P-value of $\leq$ 0.001 considered as highly significant.

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Results

Serum lipid profile

From the data presented in table 2 it is observed that the administration of high fat diet induced hyperlipidemia in mice (Group B). Concurrent administration of *Thymus vulgaris* essential oil at 500mg/kg body weight (Group IV) showed a highly significant reduction in the levels of serum total cholesterol, LDL, VLDL as well as triglycerides. In comparison with atorvastatin treated group, group treated with Thymus vulgaris essential oil showed significant increase in serum TG and statistically significant increase in serum TC, LDL and VLDL.

Table 2: Comparison between hyperlipidemic induced (non-treated) group and induced (hyperlipidemic) group Thymus vulgaris essential oil in relation to different parameters.

Group	Induced group Mean ±SD	Normal group Mean ±SD	*Thymus vulgaris* essential oil (500mg/kg) Mean ±SD
TC (mg/dl)	270.62 ±9.69	113.25 ±12.04[a**]	196.62 ±16.71[a**]
TG (mg/dl)	269.50 ±20.33	108.75 ±9.03 [a**]	136.62 ±15.26 [a**]
HDL (mg/dl)	47.25 ±1.39	54.87 ±2.54 [aNS]	73.50 ±5.80 [a**]
LDL (mg/dl)	246.37 ±12 .64	85.50 ±1.48 [a**]	89.00 ±0.78 [a**]
vLDL (mg/dl)	64.62 ±6.54	33.62 ±1.60 [a**]	39.62 ±0.70 [a**]
MDA (ng/ml)	101.56 ±4.40	20.34 ±2.19 [a**]	37.27 ±1.58 [a**]
GPx (ng/ml)	0.601 ±0.03	2.67 ±0.07 [a**]	1.691 ±0.21 [a**]

a: Comparison with induced group, **NS**: not statistically significant (p>0.05), ****:** Highly statistically significant (p≤0.001), **TC**: total cholesterol, **TG**: triglycerides, **HDL**: high density lipoprotein, **LDL**: low density lipoprotein, **vLDL**: very low-density lipoprotein, **MDA:** Malondialdehyde, **GPx**: glutathione peroxidase**.**

Table 3: Comparison of group treated with Thymus vulgaris essential oil with induced (non-treated) and Atorvastatin treated group in relation to different parameters.

Group	Induced (non-treated) group Mean ±SD	Atorvastatin treated group (10mg/kg) Mean ±SD	*Thymus vulgaris* essential oil (500mg/kg) Mean ±SD
TC (mg/dl)	270.62 ±9.69	178.62 ±27.98 [a**]	196.62 ±16.71[a**, bNS]
TG (mg/dl)	269.50 ±20.33	115.37 ±6.25 [a**]	136.62 ±15.26 [a**, bNS]
HDL (mg/dl)	47.25 ±1.39	47.37 ±1.52 [aNS]	73.50 ±5.80 [a**, b**]
LDL (mg/dl)	246.37 ±12 .64	82.50 ±2.07 [a**]	89.00 ±0.78 [a**, bNS]
vLDL (mg/dl)	64.62 ±6.54	22.75 ±1.27 [a**]	39.62 ±0.70 [a**, b**]
MDA (ng/ml)	101.56 ±4.40	25.43 ±1.93 [a**]	37.27 ±1.58 [a**, b**]
GPx (ng/ml)	0.601 ±0.03	1.672 ±0.18 [a**]	1.691 ±0.21 [a**, bNS]

a: Comparison with induced group, b: comparison with atorvastatin group, **NS**: not statistically significant (p>0.05), ****:** Highly statistically significant (p≤0.001), **TC**: total cholesterol, **TG**: triglycerides, **HDL**: high density lipoprotein, **LDL**: low density lipoprotein, **vLDL**: very low-density lipoprotein, **MDA:** Malondialdehyde, **GPx**: glutathione peroxidase**.**

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Histopathological examination of the aorta:

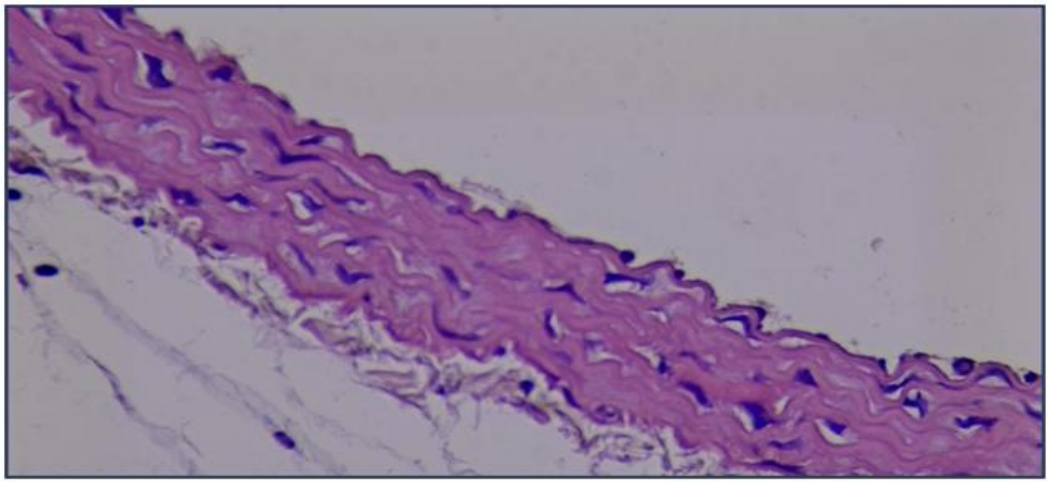

Figure1 Histopathology of mice aorta for normal group showing normal structure of aorta with no infiltration of the intima by foamy histiocytes (containing lipid). (H&E stain, 40X)

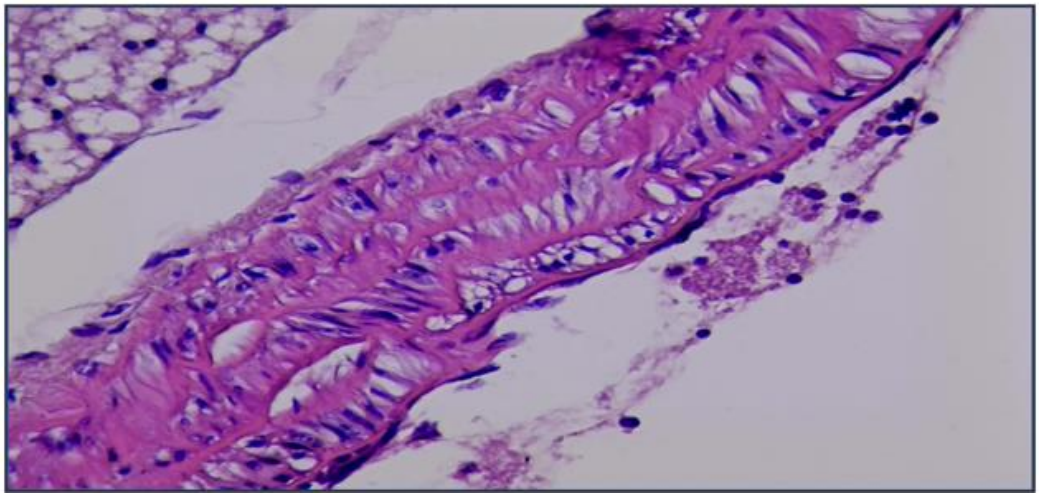

Figure 2 Histopathology of mice aorta for hyperlipidemic group showing moderate accumulation of abundant foamy histiocytes (containing lipid) inside the wall of aorta in the media layer near the intima. (H&E stain, 40X)

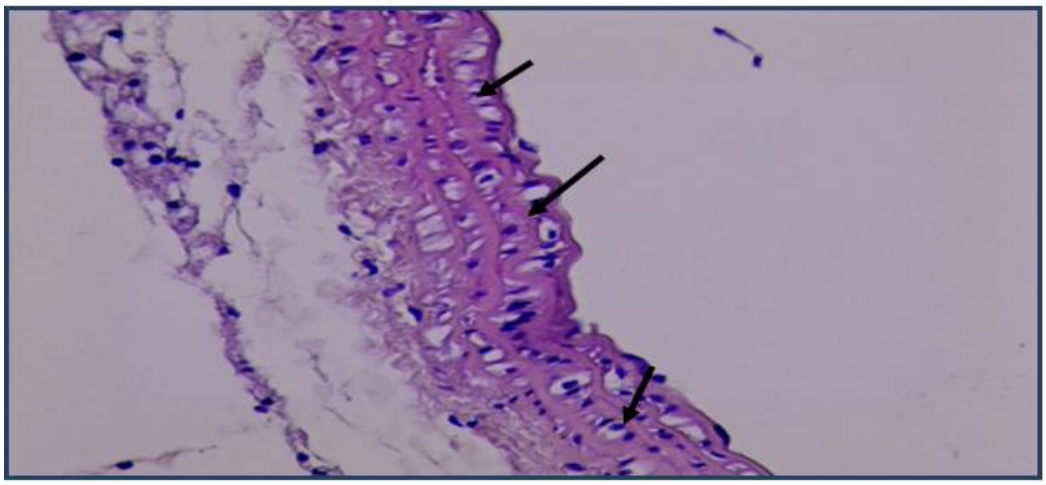

Figure 3 Histopathology of mice aorta for hyperlipidemic group treated with atorvastatin showing moderate accumulation of foamy histiocytes in the aortic intima. (H&E stain, 40X)

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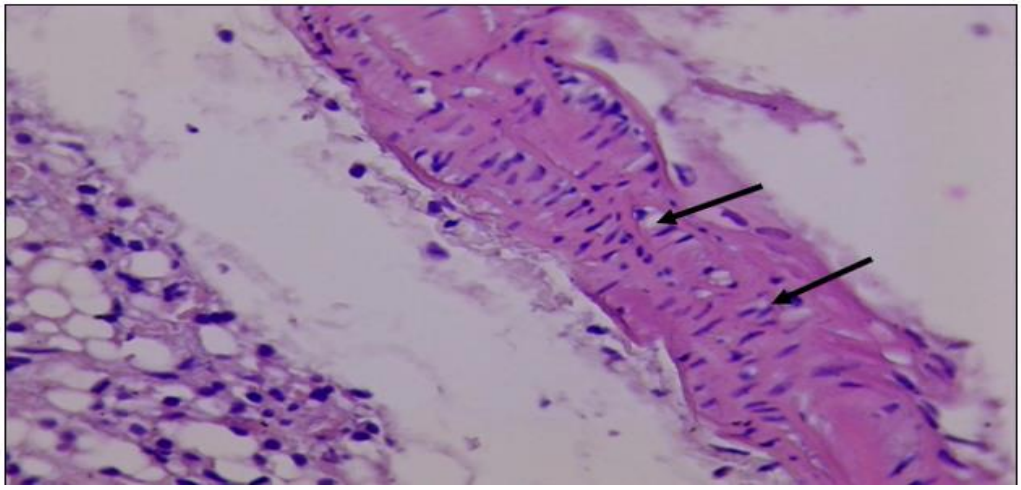

Figure 4 Histopathology of mice aorta for hyperlipidemic group treated with Thymus vulgaris essential oil group showing mild (few numbers of foamy histiocytes in intimal infiltration. (H&E stain, 40X)

Histopathological examination of the kidney:

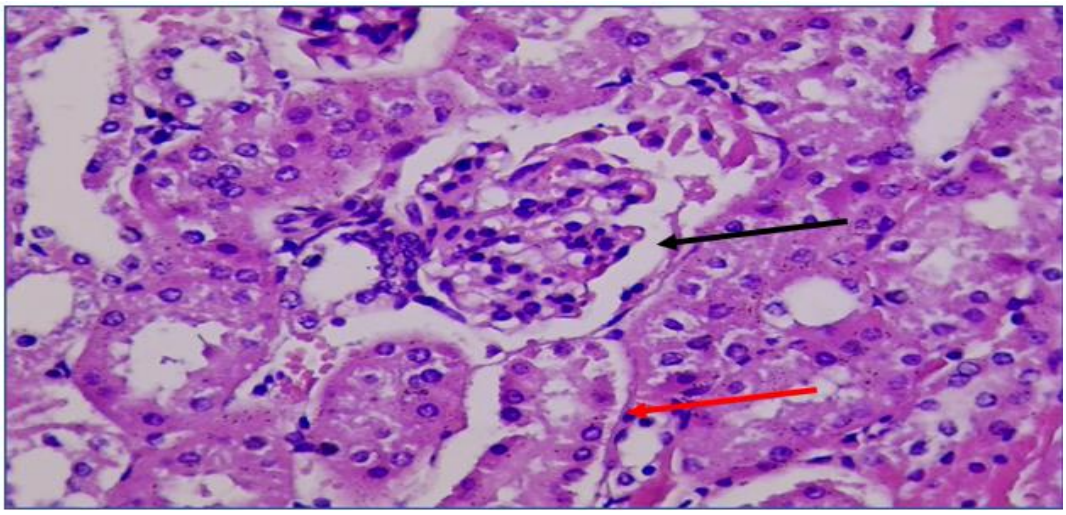

Figure 5 Histopathology of mice kidney tissue for normal group showing normal histological structure of the glomeruli (black arrow) with renal tubules (red arrow). (H&E stain, 40X)

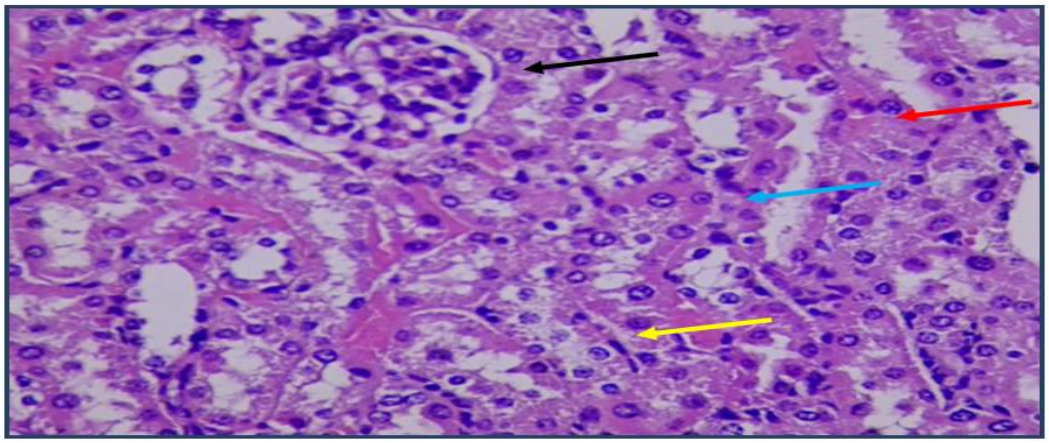

Figure 6 Histopathology of mice kidney tissue for hyperlipidemic group showing normal glomeruli (black arrow) with degeneration changes of tubules (vesiculation (yellow arrow), desquamation of cell (red arrow) and nuclear enlargement (blue arrow)). (H&E stain, 40)

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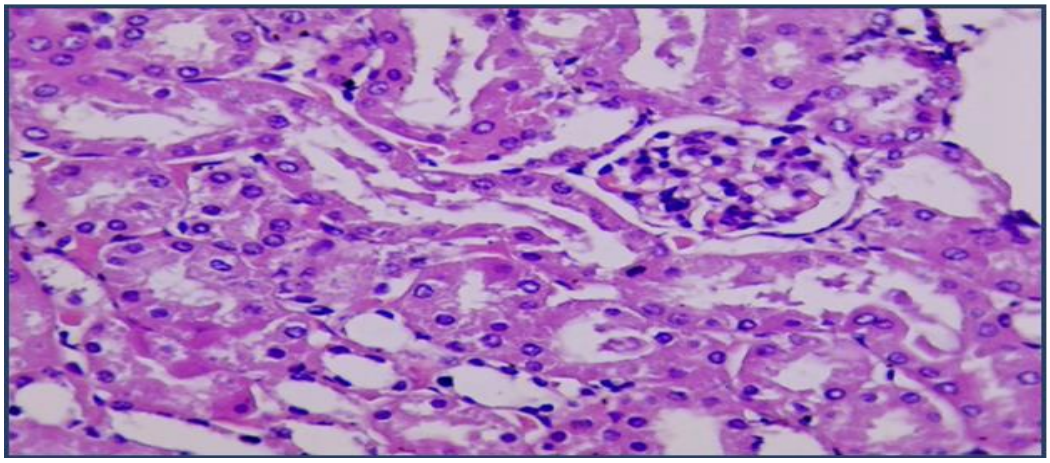

Figure 7 Histopathology of mice kidney tissue for hyperlipidemic group treated with atorvastatin showing normal histological structure of the glomeruli with renal tubule. (H&E stain, 40X)

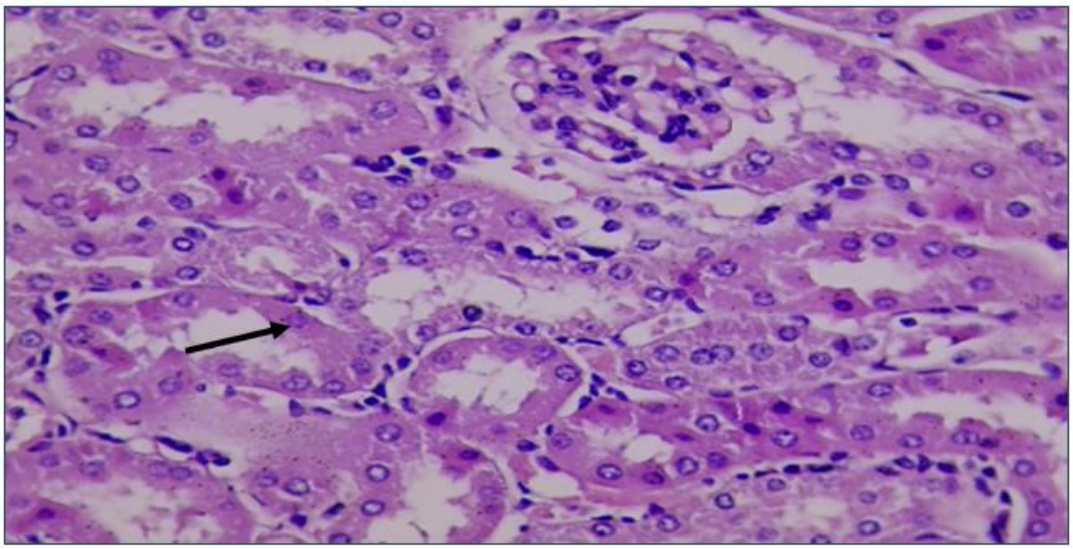

Figure 8 Histopathology of mice kidney tissue for hyperlipidemic group treated with Thymus vulgaris essential oil group showing focal tubular injury. (H&E stain, 40X)

Discussion

Hyperlipidemia, a group of metabolic disorders characterized by the elevated levels of lipids, is a major modifiable risk factor for atherosclerosis and cardiovascular disease (16). These lipids include cholesterol, cholesterol esters, phospholipids, and triglycerides. Increased levels of LDL are related to the development of atherosclerosis (17, 18). The result showed that there was significant increase in hyperlipidemia biomarker which includes TC, TG, LDL and HDL. There were histological changes in organs which confirm the result of blood lipid. That mean high fat diet successfully induces hyperlipidemia in rats, to make disease model of hyperlipidemia.

Malondialdehyde (MDA), known as a product of lipid peroxidation or reaction of oxygen with unsaturated lipids (19), was highly significant increase in induced (hyperlipidemic) mice. The elevated levels of MDA in induced (hyperlipidemic) mice suggest increased lipid peroxidation in fat deposits that could be released and have detrimental effects on hepatocytes. Besides, the results were supported by histological

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examination which showed degenerative changes in the aorta and kidney (Fig. 2 and 6). The serum lipid profile and MDA was found to be declined with *thymus vulgaris* essential oil in comparison with induced (non-treated) group. Glutathione peroxidase (GPx), which is enzyme involved in the termination reaction of ROS pathway whose function is to reduce the cumulative load of ROS within the cell, or intracellular space (20), was signifyingly increased in *thymus vulgaris* essential oil group in comparison to induced group.

The effect of *thymus vulgaris* essential oil and atorvastatin on serum TC, TG, LDL and VLDL was comparable although atorvastatin seems to be more effective in certain lipid profile parameters. The reason behind the reduction in lipid profile and liver enzymes activity mostly by *thymus vulgaris* essential oil may due to the diversity of phytochemical compounds of *thymus vulgaris* essential oil such as geraniol, α-terpineol, thuyanol-4, linalool, carvacrol, and thymol after its dominant monoterpene which possess a radical scavenging activity and hepatoprotective properties (21). They protect cells from damage induced by oxidative stress which is generally considered to be a cause of degenerative diseases. Flavonoids like carvacrol and thymol may have an additive effect to the endogenous scavenging compounds as they can increase the function of the endogenous antioxidants (22). Moreover, carvacrol and thymol may diminish TC, TG, LDL and, VLDL through inhibition of pancreatic lipase which responsible of cleavage of triglyceride into fatty acids and glycerol (23) since the activity of lipase greatly affects the metabolism of fat and the concentration of triacylglycerols in blood.

References

1. Abu-Raghif AR, Sahib HB, Abbas SNJIJPSRR. Anti-hyperlipidemic effect of Vitex agnus castus Extracts in Mice. 2015;35:120-5.
2. Shareef SM, Kathem SHJJoPNR. Gentiopicroside ameliorates lipopolysaccharide-induced acute kidney injury by inhibiting TLR4/NF-κB signaling in mice model. 2022;13(4):135-45.
3. Trevor AJ, Katzung BG, Masters SB, Kruidering-Hall M. Pharmacology examination & board review: McGraw-Hill Medical New York; 2010.
4. Boukhatem MN, Darwish NH, Sudha T, Bahlouli S, Kellou D, Benelmouffok AB, et al. In vitro antifungal and topical anti-inflammatory properties of essential oil from wild-growing Thymus vulgaris (Lamiaceae) used for medicinal purposes in Algeria: a new source of carvacrol. 2020;88(3):33.
5. Mandal S, DebMandal M. Thyme (Thymus vulgaris L.) oils. Essential oils in food preservation, flavor and safety: Elsevier; 2016. p. 825-34.
6. Absalan G, Barzegar S. Development and Validation of a Gas Chromatographic Method for Identification and Quantification of Thymol and Carvacrol in Pharmaceutical Samples. 2016.
7. Höferl M, Buchbauer G, Jirovetz L, Schmidt E, Stoyanova A, Denkova Z, et al. Correlation of antimicrobial activities of various essential oils and their main aromatic volatile constituents. 2009;21(5):459-63.
8. Youdim KA, Damien Dorman H, Deans SGJJoEOR. The antioxidant effectiveness of thyme oil, α-tocopherol and ascorbyl palmitate on evening primrose oil oxidation. 1999;11(5):643-8.
9. Dorman HD, Deans SGJJoam. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. 2000;88(2):308-16.

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10. Vila R. Flavonoids and further polyphenols in the genus Thymus. Thyme: CRC Press; 2002. p. 158-90.
11. Jannati N, Gharachorloo M, Honarvar MJJoMp, By-product. Extraction of thymol compound from Thymus vulgaris L. oil. 2021;10(1):81-4.
12. Borgarello AV, Mezza GN, Pramparo MC, Gayol MFJS, Technology P. Thymol enrichment from oregano essential oil by molecular distillation. 2015;153:60-6.
13. Salehi B, Mishra AP, Shukla I, Sharifi-Rad M, Contreras MdM, Segura-Carretero A, et al. Thymol, thyme, and other plant sources: Health and potential uses. 2018;32(9):1688-706.
14. Wang L-X, Liu K, Gao D-W, Hao J-KJWJoGW. Protective effects of two Lactobacillus plantarum strains in hyperlipidemic mice. 2013;19(20):3150.
15. Bancroft J, Stevens AJE, London Melbourne New York. Theory and practice of histological technique 3rd ED Churchill Livingston. 1990.
16. Bauer JJJotAVMA. Evaluation and dietary considerations in idiopathic hyperlipidemia in dogs. 1995;206(11):1684-8.
17. Smith Jr SC, Jackson R, Pearson TA, Fuster V, Yusuf S, Faergeman O, et al. Principles for national and regional guidelines on cardiovascular disease prevention: a scientific statement from the World Heart and Stroke Forum. 2004;109(25):3112-21.
18. Jacobson MSJTJop. Heart healthy diets for all children: no longer controversial. 1998;133(1):1-2.
19. Ayala A, Muñoz MF, Argüelles SJOm, longevity c. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. 2014;2014.
20. Melov SJTijob, biology c. Animal models of oxidative stress, aging, and therapeutic antioxidant interventions. 2002;34(11):1395-400.
21. Thompson JD, Chalchat J-C, Michet A, Linhart YB, Ehlers BJJoce. Qualitative and quantitative variation in monoterpene co-occurrence and composition in the essential oil of Thymus vulgaris chemotypes. 2003;29(4):859-80.
22. Gholami-Ahangaran M, Ahmadi-Dastgerdi A, Azizi S, Basiratpour A, Zokaei M, Derakhshan MJVM, et al. Thymol and carvacrol supplementation in poultry health and performance. 2022;8(1):267-88.
23. Ali-Shtayeh MS, Jamous RM, Abu-Zaitoun SY, Akkawi RJ, Kalbouneh SR, Dudai N, et al. Secondary treated effluent irrigation did not impact chemical composition, and enzyme inhibition activities of essential oils from Origanum syriacum var. syriacum. 2018;111:775-86.