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Effect of Thymus Vulgaris Essential Oil in Experimentally Induced Hyperlipidemic Mice

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Abstract

Aim of study is to investigate the possible effect of thymus vulgaris essential oil as anti-hyperlipidemic agent in mice. The. Thirty-two male albino mice were fed a high cholesterol diet for 28 days to construct hyperlipidemic models. The anti-hyperlipidemic activity thymus vulgaris essential oil against hyperlipidemia induced was evaluated in mice. Atorvastatin was used as a standard. Total cholesterol, triglycerides, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol levels were measured. Compared with normal mice, hyperlipidemic mice possessed significantly higher lipid and liver enzymes profile outcomes. After treatment thymus vulgaris essential oil, lipid levels and liver enzymatic activities in hyperlipidemic mice significantly decreased. Besides that, thymus vulgaris essential oil treated group showed significant improvement in levels of tissue MDA and GPx in hyperlipidemic mice.

Keywords

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

The major 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 consequences in humans include acute pancreatitis, blood vessel blockage, and cholesterol gallstones (1).

Although drugs therapies available for the treatment of hyperlipidemia includes 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 (2).

The genus Thymus comprises 300−400 species, several of which are utilized in folk medicine. Thymus vulgaris, belonging to the Lamiaceae family, is a small scented perennial herb, predominantly found in the Mediterranean region, North Africa and Southern Europe (3). In alternative medicine, flowering parts and leaves of Thymus species have been widely used as herbal tea, tonic, antitussive, carminative and antimicrobial, as well as for treating colds. It has been suggested that a fraction of these properties is related to the EO (4, 5). Thymus vulgaris L., exhibits polymorphic variation in monoterpene production (6). Intraspecific chemotype variations are seen in Thymus and are named geraniol, α-terpineol, thuyanol-4, linalool, carvacrol, and thymol after its dominant monoterpene (7).

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) (8), which have antioxidative, antimicrobial, antitussive, expectorant, antispasmodic, and antibacterial effects (9-11). Terpenoids, flavonoid aglycones, flavonoids glycosides, and phenolic acids were also found in Thymus spp.(12).

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 (13).

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 (14, 15).

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 (16).

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
	Cholesterol powder
	Peanut butter

Experimental design

The mice were divided into 4 groups, 8 mice each group:
Group 1 (normal): standard diet for 28 days .
Group 2 (induced): High Fat Diet (HFD) for 28 days.
Group 3 (treated): HFD for 28 days then atorvastatin 10 mg/kg for further 28 days.
Group 4: HFD for 28 days then thymus vulgaris essential oil 500 mg/kg for further 28 days.

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.

Measurement of oxidative stress

The liver was homogenized for malondialdehyde (MDA) and glutathione peroxidase (GPx) investigation. The liver was rinsed in ice-cold PBS (0.02mol/L, pH 7.2-7.4). Remove excess blood thoroughly and weighed before homogenization. The tissues were sliced into small pieces and homogenized them in a certain amount of Phosphate-buffered saline (PBS) (Usually 10mg tissue to 100µl PBS) with a glass homogenizer on ice. The resulting suspension was subjected to two freeze-thaw cycles to further break the cell membranes. After that, centrifugate homogenates for 15 minutes 5000 rpm.

Histopathological examination

The liver 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 (17).

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 ≤ 0.05 considered being significant and P-value of ≤ 0.001 considered as highly significant.

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 2). 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.

Liver enzymes activity

In this study, serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) and alkaline phosphatase (ALP) activities were significantly high in high-cholesterol fed diet than in normal mice. On the other hands, the Thymus vulgaris essential oil revealed a highly significant reduction in AST, ALT and ALP levels. In comparison with atorvastatin treated group, group treated with Thymus vulgaris essential oil showed a highly significant reduction in serum ALP, whereas, the serum ALT and AST showed statistically insignificant in comparison with atorvastatin treated group; table 3.

Antioxidant activities

The MDA were significantly increased in induced (non-treated) group and thymus vulgaris essential oil in comparison with healthy group. Meanwhile, the glutathione peroxidase level in induced (non-treated) group in comparison with healthy group decreased highly significant while it increased highly significant in Thymus vulgaris essential oi treated group; table 2.

In comparison with atorvastatin treated group, group treated with Thymus vulgaris essential oil showed a highly significant increment in MDA and statistically insignificant in glutathione peroxidase levels; table 3.

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**}
AST (U/l)	216.62 ±29.77	20.37 ±2.54 ^{a**}	145.87 ±11.98 ^{a**}
ALT (U/l)	161.25 ±16.06	26.62 ±3.64 ^{a**}	82.37 ±8.28 ^{a**}
ALP (U/I)	222.50 ±17.12	83.75 ±7.52 ^{a**}	154.62 ±4.12 ^{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, AST: aspartate aminotransferase, ALT: alanine aminotransferase, ALP: alkaline phosphatase, 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 $^{a**,\ b**}$
AST (U/l)	216.62 ±29.77	110.00 ±8.10 ^{a**}	145.87 ±11.98 $^{a**,\ bNS}$
ALT (U/l)	161.25 ±16.06	84.75 ±7.98 ^{a**}	82.37 ±8.28 $^{a**,\ bNS}$
ALP (U/I)	222.50 ±17.12	183.12 ±10.30 ^{a**}	154.62 ±4.12 $^{a**,\ b**}$
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, AST: aspartate aminotransferase, ALT: alanine aminotransferase, ALP: alkaline phosphatase, MDA: Malondialdehyde, GPx: glutathione peroxidase.

Histopathological examination of the liver:

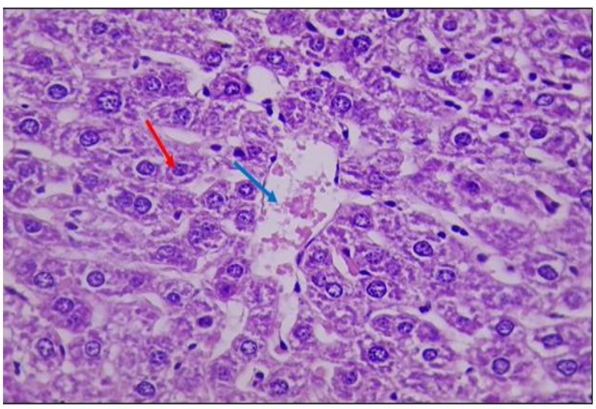

Figure 1: Histological section of liver tissue for normal group showing normal structure of hepatocytes (red arrow) and central vein (blue arrow). (H&E stain, 40X)

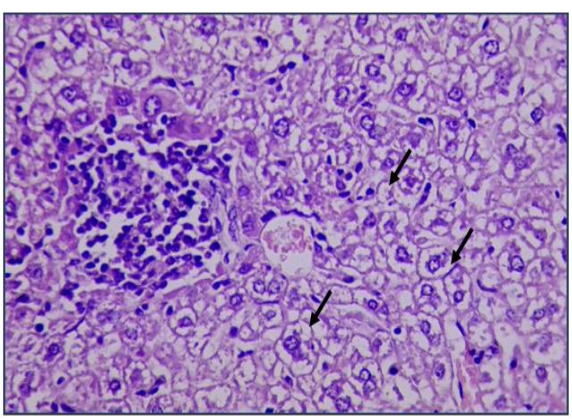

Figure 2: Histological section of liver tissue for hyperlipidemic group showing sever and diffuse cytoplasmic fatty infiltration (microvesicular steatosis). (H&E stain, 40X)

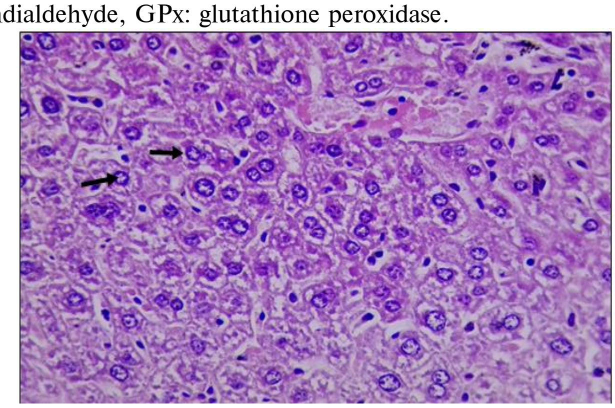

Figure 3: Histological section of liver for hyperlipidemic group treated with atorvastatin showing mild & focal microvesicular steatosis. (H&E stain, 40X)

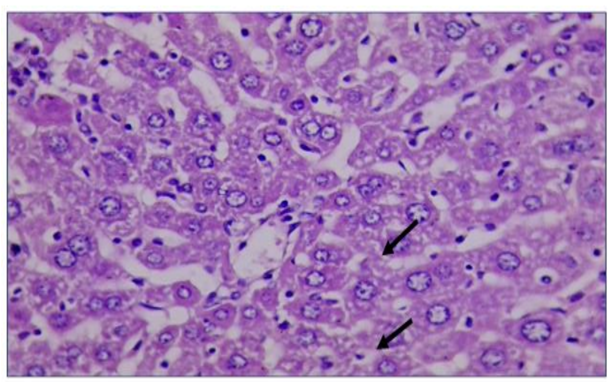

Figure 4: Histological section of liver for hyperlipidemic group treated with thymus vulgaris essential oil showing mild diffused and minor microvesicular steatosis. (H&E stain, 40X).

Discussion

Hyperlipidemia is characterized by increase in serum lipid profile namely, triglycerides (TG), total cholesterol (TC) and low-density lipoprotein-cholesterol (LDL-C) and thus is considered one of the primary risk factors leading to CVDs and myocardial infarction. Hyperlipidemia is directly linked with a prominent metabolic dysregulation in the affected patients (18). The above-mentioned changes in association with declining (HDL-C) serum level eventually result in hyperlipidemia, causing advanced cardiac pathological conditions (19). Moreover, the interfering harmful effect of HFD with the process of lipid metabolism in the liver is the primary factor responsible for the development of nonalcoholic fatty liver disease (20). Hyperlipidemia, in particular raised low-density lipoproteins (LDL hypercholesterolemia), is one of the primary risk factors contributing to the evolution of atherosclerotic cardiovascular disease (18). Practical strategies to treat hyperlipidemia, include the decrease lipids synthesis and their gut absorption using synthetic therapeutic agents as fibrates, statins and bile acid sequestrants. The use of these agents might be associated with series of side effects most notably myopathy, rhabdomyolysis and increase risk of gallstone formation. Hence, developing a novel and effective anti-hyperlipidemic therapeutic agents with minimal side effects is urgently required (21).

Different parts of thymus vulgaris show different pharmacological activity. Thymus vulgaris has been used since ancient times to achieve healing, cure chest congestion, and induce saliva; the fresh leaves are taken to relieve sore throats. The plant is also used as an effective remedy for chest infections (bronchitis, pharyngitis, whooping cough) as well as to treat worms in children. The plant has been used for its flavor in cooking (22). Thymus vulgaris essential oil (TEO) is used as an antiseptic, antiviral, and antimicrobial agent in folk medicine. Thyme also possesses carminative and antioxidative effects (7).

In the present study, feeding the mice high fat diet (HFD) for 4 weeks led to highly significant increase in serum total TC, TG, LDL and VLDL in induced (hyperlipidemic) mice as compared to normolipidemic group fed normal standard diet. These changes observed in hyperlipidemic group may be due to that HFD induced hyperlipidemia by demodulating lipid metabolism, mainly by decreasing β-oxidation and increasing cholesterol synthesis and oxidative stress by decreasing free radical scavenger enzyme gene expression (23). Also, Rui-Li (2008) reported that HFD induced abnormal increases in lipid peroxidation, serum concentrations of total cholesterol, triacylglycerol, and low-density lipoprotein cholesterol in addition to decreased lipoprotein lipase activity, accompanied by a depressed antioxidant defense system. Oxidative stress has been documented to play a pivotal role in the patho-physiology and progression of diverse human diseases including CVD, CVI and DM (24).

The serum ALT, AST and ALP levels were extensively elevated in high-cholesterol fed diet than in normal mice. This may due to the disturbance of lipid metabolism because of high fat intake, resulting in accumulation of TG in liver and an increased increment of the liver index, and hepatic steatosis occurred (25) since the liver has a crucial role in regulating plasma lipid level all the way through LDL clearance and HDL cholesterol recruitment (26). Moreover, the elevation in liver enzymes may also due to excess reactive oxygen species (ROS) production in the mitochondria as a result of lipid overload. The surplus ROS generation exhausted the endogenous antioxidants (27).

Malondialdehyde (MDA), which is a product of lipid peroxidation or reaction of oxygen with unsaturated lipids (28), 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 examination which showed degenerative changes in the liver (Fig. 2). 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 (29), 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 (6). 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 (30). In addition, carvacrol and thymol may reduce TC, TG, LDL and, VLDL through inhibition of pancreatic lipase which responsible of liberation of triglyceride into fatty acids and glycerol (31). The activity of lipase greatly affects the metabolism of fat and the concentration of triacylglycerols in blood.

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