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Research Paper

The protective role of aqueous extract of rosemary leaves and vitamin E in protection against risk of induced hyperlipidemia in male white rats

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ABSTRACT

The study aimed to determine the protective effect of the aqueous extract of rosemary leaves and vitamin E against induced hyperlipidemia on some biochemical parameters in serum and aortic tissue in male white rats. Twenty rats weighing 250-300 g and aged 10-12 weeks were used and placed in plastic cages under laboratory conditions for 30 days. The animals were divided into four groups: the first was the control group fed a standard diet, the second was fed a standard diet with cholesterol (1% g/kg), the third was fed cholesterol with vitamin E (50 mg/kg), and the fourth was fed cholesterol with the aqueous extract (50 mg/kg).

The results showed that the cholesterol diet caused a significant increase ($P < 0.01$) in TG, LDL, VLDL, and HDL levels, while treatment with the aqueous extract and the extract with vitamin E resulted in a significant decrease in TC, TG, LDL, VLDL, and HDL levels. The reduction was more pronounced in the group treated with the extract and vitamin E. Additionally, the treatment improved aortic tissue compared to the cholesterol group.

The study concludes the preventive role of rosemary aqueous extract and vitamin E against the harmful effects of elevated lipid levels.

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1. INTRODUCTION

Hyperlipidemia is defined as high plasma lipid concentrations such as total cholesterol, triglyceride, low density lipoprotein (LDL-C), very low density lipoprotein (VLDL-c), and high density lipoprotein (HDL-c) [1]. It is a health problem that affects people's lives through the association with diseases such as ath erosclerosis and coronary heart disease, one of the most common causes of death in the world [2]. The use of common chemotherapy drugs in people with hyperlipidemia, atherosclerosis and disease cardiovascular disease that has a pathway inhibition of hepatic enzyme 3-Hydroxy3-Methylglutaryl CoA Reductase often carries with it side effects and most important carcinogenic factors [3]. In modern times, medicinal plants have raised many concerns through their use as drugs to treat many chronic diseases. They have become preventive and health tools in many developed countries. Moreover, the mechanism of their work is unknown. Rosmarinus officinalis is a medicinal plant that belongs to the family. Lamiaceae has been used as a medicinal herb for centuries because of its important activities against many diseases. It contains many effective biological properties. It is used as a hepatoprotective [4], anti-inflammatory [5], Anti-hyperlipidemia and anti-hyperglycemic [6].

These properties are attributed to the contents of the rosemary plant, which contains phenolic compounds such as rosemarinic acid, and turbines such as karnosic acid, rosemaryquin, rosemannol, carnosol [7]. Vitamin E is a fat-soluble antioxidant that stops the production of ROS, which is formed when fat levels increase. Vitamin E is used as an anti-inflammatory and anti-atherogenic and helps to prevent or delay chronic diseases associated with free radicals [8]. Vitamin E is one of the most important natural antioxidants that works against oxidative stress [9, 10]. The study aimed to know the protective effect of the aqueous extract of leaves of rosemary and vitamin E in rats with induced hyperlipidemia.

2. RELATED WORK

Previous literature related to this medicinal herb included a large number of studies from the [4,6]studies conducted on laboratory animals and showed valuable results related to the positive role of its alcoholic extract on lipid profile as it led to a significant increase in HDL-C and a significant decrease in Cholesterol, Tryglyceride, LDL-C, v LDL-C.

Recent research shows positive effects of rosemary extracts (Rosmarinus officinalis L.), below are some studies related to the impact of rosemary on lipid profiles:

Study on Mice: A 2023 study showed that rosemary extract helped reduce liver fat content and levels of total cholesterol and triglycerides in mice fed a high-fat diet. The results indicated an increase in HDL cholesterol (good cholesterol) levels and a decrease in plasma triglycerides, suggesting an improvement in the lipid profile [11].

Study on HepG2 Cells: Another study found that rosemary extract can regulate glucose and lipid metabolism by activating AMPK and PPAR pathways. It was revealed that rosemary increases glucose uptake and lowers cholesterol levels, indicating its potential use as a natural treatment for metabolic disorders [12].

Previous Study on Mice: In an earlier study, rosemary extract rich in carnosic acid was used, and the results showed it contributed to weight loss and increased fat levels in feces, leading to improved plasma cholesterol levels. These findings support the idea that rosemary could have beneficial effects on the lipid profile [13].

Blood Lipids and Antioxidant Effects: A study found that rosemary enhanced antioxidant activities and reduced total cholesterol and triglyceride levels in hyperlipidemic rats, suggesting its potential in preventing hyperlipidemia [14].

Diabetic Rats: Research indicated that rosemary administration decreased cholesterol by 22%, triglycerides by 24%, and LDL by 27%, while increasing HDL by 18% in both normal and diabetic rats [15].

Rosemary Oil in Diabetic Rats: A study demonstrated that rosemary oil significantly reduced triglycerides, total cholesterol, and LDL levels while increasing HDL levels in streptozotocin-induced diabetic rats [16].

High-Fat Diet: An investigation revealed that aqueous extract from rosemary improved lipid profiles and reduced oxidative stress in mice fed a high-fat diet, decreasing triglycerides and total cholesterol levels [17].

Mechanistic Insights: A study explored how rosemary activated AMPK and PPAR pathways, leading to improved lipid metabolism in liver cells, further supporting its beneficial effects on lipid levels.

These studies collectively underscore the potential of rosemary as a natural agent for improving lipid profiles and managing conditions related to dyslipidemia.

3. METHOD

3.1. Collection and Preparation of Plants

Rosmarinus officinalis L. was collected and diagnosed from the gardens of the University of Tikrit. The plant were washed with distilled water before the drying phase to obtain a pure sample free of impurities. Plant dried at room temperature then leaves were taken and placed in a non-transparent containers, at room temperature until extraction.

3.2. Extraction Methods

The leaves of the rosemary plant were extracted in cold water in accordance with the study of [18]. Leaves crushed with the electric mixer, the powder was mixed 40 g with 160 ml of distilled water, placed in a glass flask. After mixing, the mixture was kept in the refrigerator at 4 ° C for at least 24 hours to ensure the solubility was well completed. Filtering the mixture using filtration papers to remove the plant fiber and obtain a raw extract, transfer the filter mixture to the rotary evaporator at 40 C degree for no change in the chemical composition of the mixture. This process is performed for 30 minutes until it reaches a semi-solid strength and then transferred to a device Vibrator incubator at 35C degree until drying, and kept in plastic packaging until use.

3.3. Design Experience

Twenty animals of white male rats were weighed between 250 - 300 g and their ages between 10-12 weeks. Animals were placed in plastic cages in the animal house at the Faculty of Veterinary Medicine / University of Tikrit and underwent the necessary laboratory conditions during the 30 - day study period. The animals were randomly assigned to four groups and each group had 5 animals. Group 1 was treated as a control group and was given a regular diet with drinking water during the trial period. Second group treated with 1% g / kg cholesterol. Third group treated with aqueous extract of rosemary leaves 50mg / kg body weight) with a cholesterol-rich diet (1% g / kg); forth group treated with vitamin E (50 mg / kg) and fed with cholesterol-rich diets (1% g / kg).

3.4. Preparation of Blood Sample

After the end of the trial period (30 days), the animals were starved for 24 hours, then blood samples were taken by cutting the jugular vein, placing the blood in the test tubes and placed in the incubator at 37 ° C for 30 minutes, Using the centrifuge at 3000 cycles per minute for 15 minutes, separate the serum from the other ingredients using the micropipettes serum placed at -80 ° C until the biochemical tests are done. The concentration of Cholesterol, triglycerides, Density HDL-C using the analysis kit for each test which processed by the Spanish BioSystems and as instructed by the manufacturer [18]. LDL-C concentration in the serum was estimated as follows: [19] LDL-c (mg / dl) = Total cholesterol (HDL-C + VLDL-C). VLDL-C concentration in the serum was estimated according to the following equation [13]: VLDL-c (mg / dl) = (Triglycerides / 5).

3.5. Preparation of Histological Sections

The alveolar artery was pulled using special forceps and removed from the attached parts. It was kept in formalin at a concentration of 10% for 24 hours and then washed using water before it kept in alcohol at a concentration of 70% for tissue study. Tissue cutting was performed as reported [20]. The samples were placed with different concentrations of ethylene alcohol (50%, 75%, 95%, 100%) to withdraw the water molecules, then placed in xylenefor 30 min. after that placed in plastic molds containing paraffin wax and left in the refrigerator to be hardened. The samples were then cut by a rotary microtome (6 - 5) micrometer, and these pieces were passed on to concentrations Different from ethyl alcohol (100%, 95%, 75%, 50%) to remove the xylene for 5-10 min., a drop of hematoxylin and Eosin dye were used, then passed ethylene alcohol (70-90%), histological slides washed by xylene then left to dry.

3.6. Statistical Analysis

Significance differences were extracted using the ANOVA-one way test. These differences were confirmed by the standard error. The differences were determined by Duncan's multiple ranges and at a significant level (P< 0.01) [21].

4. RESULTS AND DISCUSSION

4.1. Results

The study showed that rats fed high cholesterol (1% g / kg) significantly increased TC, TG, LDL, VLDL, HDL, in the serum, Compare with control group. This is consistent with previous studies [22-23]. Treatment using (50mg / Kg) of aqueous extract of rosemary leaves showed a decrease in TC, TG, LDL-c, VLDL-c, HDL-c, while no significant differences in HDL concentration -c in serum compare to cholesterol group. The results of the vitamin E group (50 mg / kg) showed a significant decrease in the TC, TG, VLDL-c, while no significant differences were observed in the concentration of LDL-c, HDL-c in serum, compare to cholesterol group. (50 mg / kg) of vitamin E with(50 mg / kg) of rosemary extract showed a decrease in the levels of TC, TG, LDL-c, VLDL-c, and no significant differences in HDL -c in serum, in compare to cholesterol group.

Table 1. Shows Effect of Treatments in Blood Parameters

Parameters / Groups	Control	Chol	R+Chol	E+Chol	R+E+Chol
Triglyceride (mg/dL)	27.283±1.666 b	55.446±1.439 a	38.504±1.492 b	31.573±0.708 b	29.043±0.859 b
Cholesterol (mg/dL)	119.181±0.372 b,c	159.757±0.114 a	98.239±0.386 b	130.877±0.727 b	121.532±0.115 b,c
HDL (mg/dL)	70.271±0.133 b,c	89.722±0.054 a	52.144±0.218 d	60.504±0.319 c,d	74.409±0.166 b
LDL (mg/dL)	43.453±0.103 b	58.946±0.157 a	38.393±0.614 b	64.058±0.594 a	41.315±0.103 b
VLDL (mg/dL)	5.457±0.333 b	11.089±0.288 a	7.701±0.298 b	6.315±0.142 b	5.809±0.172 b

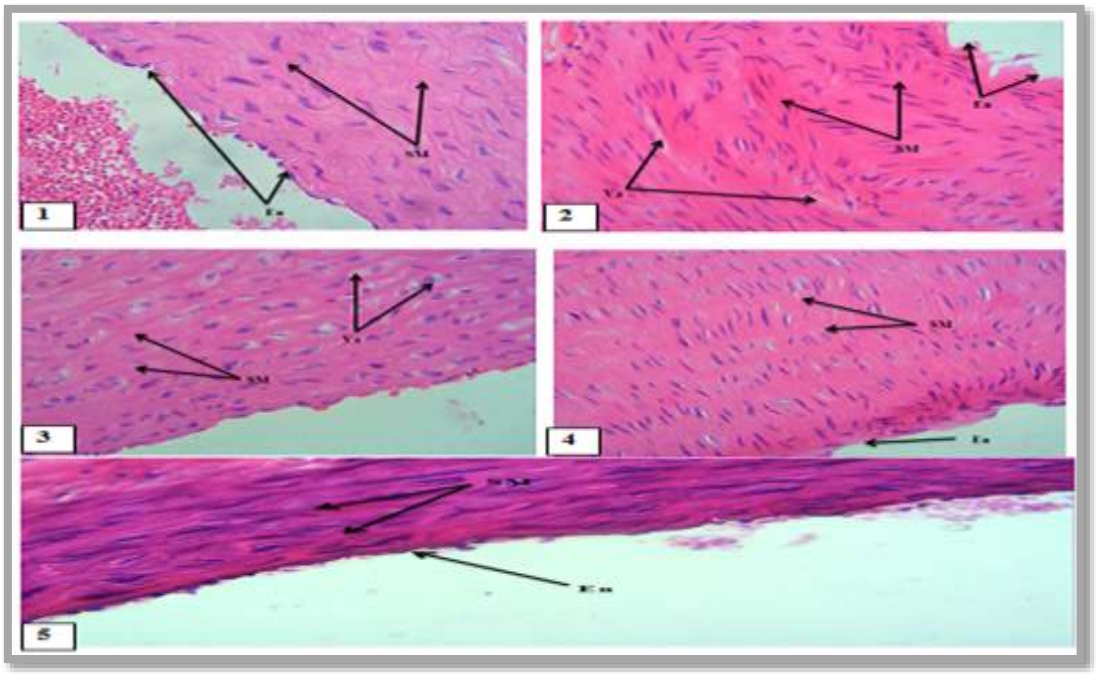

Figure 1. Shows effect of treatments in Tissue

Figure 1 IMG 1 control group showing the smooth muscle fiber (SM) and possibility of distinguishing the endothelial layer(En) of the artery naturally, Figure 1 IMG 2 the cholesterol group shows the irregularity of smooth muscle fiber (SM) with the presence of vapors between muscle fiber (Va), noting the non-discrimination of the layer of epithelium of the artery. Figure 1 IMG 3 the cholesterol with vitamin E group shows the smooth muscle fiber (SM) with presence of vapors between muscle fiber (Va). Figure 1 IMG 4 cholesterol with rosemary group shows the regularity of smooth muscle fiber (SM) in the wall of the artery also show the internal lining (En) of the artery almost naturally. Figure 1 IMG 5 cholesterol with rosemary and vitamin E group shows smooth muscle fiber (SM) as well as internal lining of the artery (En) naturally. (H & E 400X).

4.2. Discussion

Increasing the dietary content of cholesterol and its absorption by the intestines stimulates the liver to produce large amounts of VLDL and LDL molecules, cholesterol-rich foods increase the level of apoB mRNA in the liver, which is involved in the formation of an LDL molecule [24], Lipid reduces LDL receptor activity, inhibits lipoprotein lipase and hepatic lipase, and increases the level of VLDL and LDL in blood plasma [25]. The liver stimulates the production of HDL molecules to transport cholesterol from blood plasma to the liver through reverse cholesterol transport. Increased levels of LDL, VLDL and cholesterol, despite elevated HDL levels, may indicate inhibition of the enzyme Lecithin cholesterol acyltransferase (LCAT) activity, which transfers cholesterol from the surface of the HDL molecule to its core to continue taking another cholesterol molecule, as well as a decrease in the production of Cholestryl ester transfer protein (CETP) which transfer triglyceride to HDL molecule [26]. The increase of fat content in the liver cause oxidative stress observed through the high level of MDA in plasma blood, low level of antioxidants and increase LDL oxidation that produce free radicals and the occurrence of oxidative stress, which it is one of the main factors of atherosclerosis and cardiovascular diseases [22]. Figure 1 IMG 2 observed that the smooth muscle fibers were irregular with the presence of muscle fiber vapors and the non-discrimination of the epithelial layer of the artery, which leads to a change in membrane permeability and characteristics.

Vitamin E soluble in fat is an antioxidant that has the ability to inhibit the production of free radicals resulting from fat oxidation as well as its immune role and regulation of gene expression and other metabolic processes. Vitamin E contributes to the activation of LPL in the body tissues and Hepatic lipase, which stimulate cells uptake of cholesterol and triglycerides from blood [26], and thus reduce the level of fat and harmful lipoproteins in the blood and contribute to maintain the lining of the internal surface of the blood vessel Figure 1 IMG 3 and helps to resist the adhesion of blood content , also inhibits the effectiveness of protein kinase C, which contributes of abnormal proliferation and differentiation of smooth muscle, platelet and Monocytes [27]. As well as increase the secretion of Prostacyclin from the lining of the epithelium of the blood vessel, which in turn works to expand the blood vessel and prevent the platelet aggregation, Noted through the study increase in the level of antioxidants as well as reducing the peroxidation of fat and inhibition of LDL-c oxidation within the blood vessel, which is the basic step in the Atherosclerosis, so the vitamin plays a vital role in the prevention of coronary heart disease [26]. While the study confirmed the ability of vitamin E to improve the system of antioxidants in the body by raising the concentration of GSH and SOD and reduce concentrations of MDA and thus protect cells from the risk of peroxidation of fat

[30] Noted the vital importance of plant-derived active substances such as Rosmarinic acid and Carnosic acid, through their ability to scavenging the free radicals and prevent oxidative stress [31]. It maintains the smooth musculature of the artery wall and the inner lining of the artery (Endothelium) naturally Figure 1 IMG 4. The level of the Atherogenic index plasma decreased further than in other studied treatments. These compounds also increase the flow of bile and the release it with stool and thus decrease the concentration of cholesterol in the intestines [32]. As well as the ability of these chemical compounds in the conversion of saturated fatty acids to unsaturated monounsaturated fatty acids by activating the effectiveness of Cholesterol Acyltransferase, which converts the free cholesterol in the liver to cholesterol esters, and reduce the release of cholesterol and triglyceride from liver into the bloodstream and thus lower the level of LDL -C and VLDL [33-34].

The plant contains manganese and zinc [35]. Which enter the chemical composition of SOD [36] and glutathione as zinc contributes to the maintenance of the sulfhydral group in the synthesis of glutathione from oxidation [37]. The plant extract has an effective role in protecting the liver from the toxicity of oxidative stress which observed in this study by lowering the level of LDL-c and MDA in the serum more than in the rest of the studied treatments and upgrading Antioxidants which maintains the tissue of the liver from the condition of stress [38].

5. CONCLUSION

We conclude from the above results in our study the preventive role of rosemary alone or with vitamin E in reducing the increase in harmful fat levels in the body and preventing their accumulation in various tissues of the body, especially blood vessels and the heart, and this is what leads to reduce the lesions of atherosclerosis and various heart diseases..

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Author Contributions Statement

All authors acknowledge their individual contributions to this research according to the Contributor Roles Taxonomy (CRediT) to ensure transparency and clarify each researcher's role in the study. The table below presents the distribution of roles and contributions for each author.

Name of Author	C	M	So	Va	Fo	I	R	D	O	E	Vi	Su	P	Fu
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C : **C**onceptualization
M : **M**ethodology
So : **S**oftware
Va : **V**alidation
Fo : **Fo**rmal analysis
I : **I**nvestigation
R : **R**esources
D : **D**ata Curation
O : Writing - **O**riginal Draft
E : Writing - Review & **E**diting
Vi : **V**isualization
Su : **S**upervision
P : **P**roject administration
Fu : **Fu**nding acquisition

Conflict of Interest Statement

The authors declare that there are no conflicts of interest related to this research, its findings, or its publication.

Informed Consent

Informed consent was obtained from the relevant authority before conducting the study, which involved using male rats to evaluate the protective effect of aqueous rosemary leaf extract and vitamin E against induced hyperlipidemia. All ethical and experimental standards were strictly followed, ensuring animal care in accordance with international guidelines for the use of animals in scientific research. The rats were closely monitored throughout the study, with all necessary measures taken to minimize any potential distress.

Ethical Approval

Ethical approval was obtained from the Ethics Committee at the University of Kirkuk for conducting this study using laboratory animals (rats). All ethical guidelines were followed to ensure the welfare and protection of the animals throughout the experiment.

Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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