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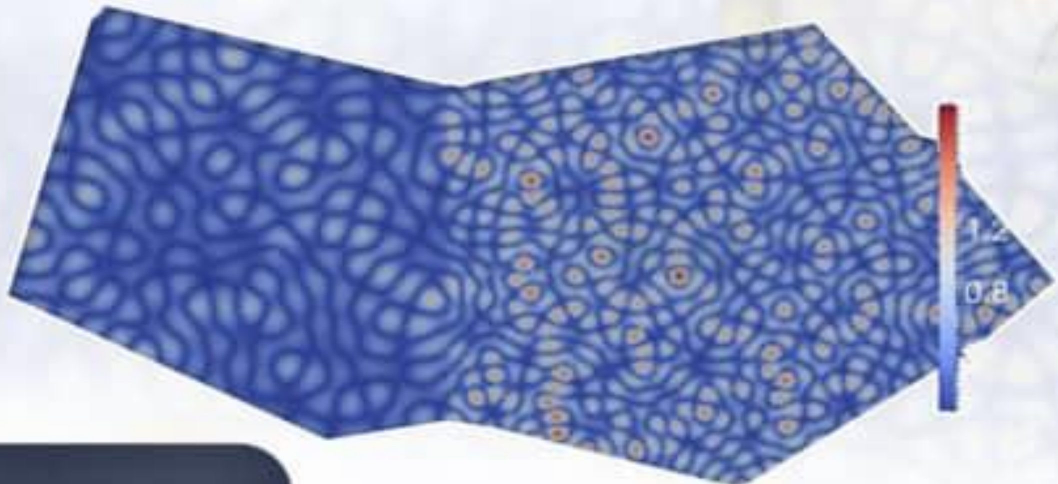

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Effect of Active Compounds for *Quercus* Fruit on Some Biochemical Parameters and Tissue Aorta in Induced Atherosclerosis Rats

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A B S T R A C T

This research included extractions of the active compounds from *Quercus* fruit, the identification of active compounds extract by using the capillary gas chromatography technique CGC and with high-performance liquid chromatography technology HPLC. The active dose of the aqueous extract (250 mg/kg) was studied in animals after induced arteriosclerosis with cholesterol (500mg/kg) dissolved in coconut oil for two weeks. The effect of the extracts oil, poly phenols, and at 7, 37.5, and 10 mg/kg respectively were studied also. The results showed a significant ($P \leq 0.05$) increase in catalase activity and the level of high density lipoprotein-cholesterol (HDL-C), However, there was a significant ($P \leq 0.05$) decrease in the high plasma kallikrein, caspase-3, cholesterol, triglycerides, and low-density lipoprotein cholesterol LDL-C in induced atherosclerosis rats treated with all extracts compared with affected control with the active extracts (oil, poly phenols) during the first and second week. The tissue aorta examination in the group of animals treated with the active extracts (aqueous, oily, flavonoid) after two weeks of the treatment showed that large parts of the tissues of the aorta were healed close to the normal state compared to the group of animals induced with atherosclerosis untreated whose tissues contained on thickenings and foam cells.

1 Introduction

The idea of a great healing power exists in medicinal plants for serious diseases that affect living organisms (humans and animals) (Tungmunnithum *et al*., 2018).

The *Quercus is acorn oak* Dicotyle donae from type Quercus aegilops tree was used to treat cough and hoarseness, *Quercus* leaves were also used to treat stomach pain and heart disease (Bahmani *et al*., 2015). Use to dilate blood vessels, and dermatitis and as an anti-fungal and anti-inflammatory). *Quercus* leaves and fruits were used to treat stomach diseases and hemorrhoids (Taib., 2020). They are used to treat ulcers that affect diabetes, treat high blood fats, and as an anti-cancer by inhibiting cancer-causing chromosomes, especially in the treatment of colon cancer (Chokpaisarn., 2020; Amedi., *et al* 2020).

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Atherosclerosis, which is one of the cardiovascular diseases (CVD), Atherosclerosis leads to the formation of active macrophages capable of producing enzymes that break down protein and break down collagen, which add strength. It has an adequate fibrous cover, making the cover brittle, weak, and more prone to rupture (Soehnlein *et al.*, 2021). Recent studies indicate a relationship between arteriosclerosis and lipids on the one hand, and inflammation on the other, according to the hypothesis of lipid oxidation, LDL-C cholesterol found in the lining of blood vessels increases absorption by macrophages (Libby, 2021 & Que *et al*., 2018). The research aims to study the positive effects of *Quercus* in preventing atherosclerosis by reducing some enzymes and lipids and improving damage to the aortic tissue.

2 Materials and Methods

Quercus was the local oak found in the city of Dohuk / Iraq was used taken and dry and grain in a Blender machine, distilled water was added, mixed for the electric motor in an ice bath, then filtered. The fatty acids and volatile oils were isolated from the Quercus plant by using (100) g of the plant's powder in petroleum ether (60-80 °C) for one day, then placed in the Soxhlet extractor until the color of the solvent became clear (Sayyar *et al.,2013*) The fatty acids of the oil isolated from the plant were diagnosed using capillary gas chromatography. Flavonoids were obtained from the remaining tissues (bagasse) were taken and dried to get rid of petroleum ether and then placed in the Soxhlet with absolute ethanol 90% until it became clear. The solvent became transparent (Kato *et al.*, 2010). Flavonoids were identified using HPLC.

3 Animal experiments

3.1. Experimental design:

Forty-five (45) adult male Albino rats (4) months old, with weights ranging about (250)g were used. Atherosclerosis was induced using cholesterol dissolved in coconut oil at a concentration of (500 mg/kg) (Ram *et al*..2014) were dosed orally for 15 days, then animals were divided into(9) groups each group including (5)rats.

Three groups were used to determine the active dose of aqueous extract. Then six groups were used to study the extracts 1st was healthy +ve control 2nd unhealthy -ve 3-6 groups were orally treated with active extracts table 1.

Table (1): effect of doses from Quercus on rate induced atherosclerosis.

Dose (mg/ kg)	Quercus extracts
250	**aqueous extract**
7	**Oil**
37.5	**Flavonoids**

Then the tissue samples (aorta) were taken in the experimental end and placed in a neutral physiological saline solution at a concentration of (0.9%) (Kassim., 2012).

3.2. Biochemical analysis in plasma

- **Determination activity of Plasma Kallikrein PK in blood plasma**.

The activity of the enzyme in blood plasma was estimated using chromogen by monitoring the change in absorbance at wavelength in (405) nm (Ito& Statland.,1981).

- **Estimation of the activity of enzyme caspase-3 in blood plasma (CasP-3)**

The activity of enzyme caspase-3 in was estimated (ELISA) kit from Nanjing Duly Biotech of China (Rica &Molly., 2012).

- **Determination of Plasma Catalase Activity (CAT)**

The activity of the catalase enzyme in plasma was estimated by the followed method (Hadwan &Abed., 2016) using hydrogen peroxide as a substrate.

- **Determination of lipid profile in plasma**

Lipid profile in blood was estimated using a ready-made analysis kit.

T-Cho., T.G French company (Biolabo), by the enzymatic method (Burtis *et al*.,2012). HDL-C was measured by the enzymatic method using a ready-made assay kit (Kostner, 1976). LDL-C in the blood plasma was calculated according to the equation (Friedewald *et al*., 2000):

LDL-C = Total Cholesterol – (HDL-C + T.G/5)

- **Statistical analysis**

The values of biochemical variables were analyzed using the statistical program SPSS 25, to find the standard statistical methods for determining the mean and standard deviation of the mean (SD). Duncun test was used to compare the results between more than two groups.

3.3 Results and Discussion

Active compounds were diagnosed using different techniques. The fatty acids were diagnosed using CGC technology, depending on the time of their purity in the separation rod, which in turn depends on the length of the hydrocarbon chain of fatty acid and the degree of saturation of fatty acid as shown in the figure1 and the table 2 The oil extract of the Quercus plant contains saturated and unsaturated fatty acids, which are essential acids for building eicosanoids and have important effects on anti-inflammatory and maintaining blood pressure. The composition of fatty acids and their physical and chemical properties of Quercus oil are very similar to the fatty acids found in Olive oil in terms of flavor, as Quercus oil is enhanced with unsaturated fatty acids by (70 _90%), so the unsaturated fatty acids play an important role to protect against heart disease and reduce blood fat as well as modify the metabolism and reduce sugar and urea in blood (AL-Rousan *et al*., 2013; Schulze., 2021).

Table (2): Fatty acids and their percentage in *Quercus* oil.

Fatty acid	Ratio
Palmatic	**7.8**
Oleic	**41.8**
Linoleic	**42.4**
Linolenic	**7.6**
Stearic	**2.7**
Elaidic	**1.4**
Heptadeanaoic	**0.9**
Eicosenoic	**2.4**
Behenic	**3.2**

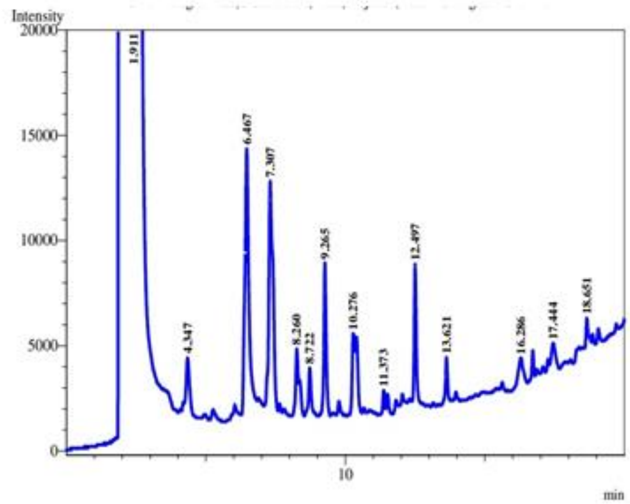

Figure (1). CGC chromatogram of fatty acids extracted from Quercus fruit oil

The phenols and flavonoids were also identified by HPLC technique by comparing the retention time of the standard compounds as shown in Table 3 and Figure 3 .

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Table (3): Retention time for standard substances and extract polyphenol

Peaks	Diagnosis of peaks	The retention time for standard substances	The retention time for polyphenol extract
1	**Gallic**	**13.7**	**13.7**
2	**Rutin**	**15.1**	**15**
3	**Kaemferol**	**18.6**	**20.2**
4	**Quercetin**	**20.20**	**22**
5	**Epigenen**	**23.4**	**23.4**
6	**Catechin**	**24.3**	**24.13**

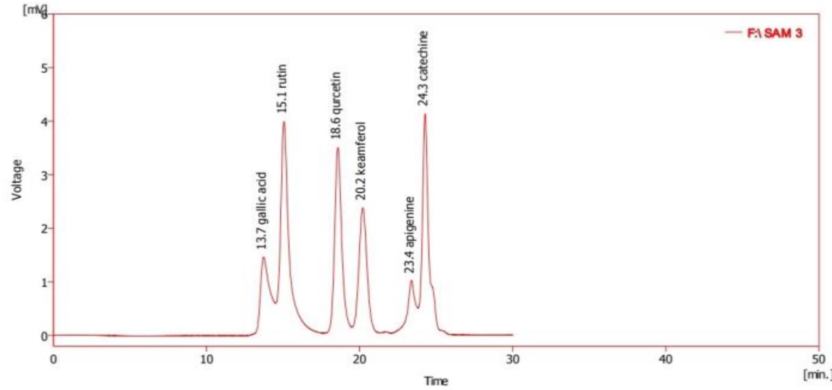

Figure. (2). HPLC chromatogram of flavonoids separated from Quercus

To study the effect of extracts of Quercus on the activity of the PK enzyme, The aqueous extract at a concentration of 250 mg/kg of body weight, which reduced the activity of the PK and casp-3 enzyme in induced atherosclerosis rats, the active doses of the extracts (oil, flavonoid) were calculated based on the active dose of the aqueous extract and the relationship between the effective dose of the extract and weight of extracts practically obtained. The doses for three extracts were (7, 37.5) mg/kg body weight for 2 weeks. Negative for infection through biochemical examinations and histological sections. The results are shown in Table (4) indicate an increase ($P \leq 0.05$) in the activity of PK enzyme in animals with induced atherosclerosis compared with the control group during treatment periods and for two weeks. High blood fats are also associated with thrombosis and inflammation and apoptosis (Larsen *et al*., 2000). Dosing animals with extracts (aqueous, oily, poly phenols) led to a significant increase ($P \leq 0.05$) for all extracts after 15 days of treatment. The decrease occurred from the first week with flavonoid and aqueous extract. The reason is attributed to the effective role of flavonoid compounds, as antioxidants in the night from oxidative stress to the active oxygen classes ROS that attack biomolecules, so the decrease of free radicals is related to a decrease in PK enzyme, as its chemical structure helps to endow hydrogen atom in hydroxyl group $OH^{\cdot}$ (Zhang *et al*., 2020) Also, unsaturated oils reduce the activity of the PK and caspa-3 enzyme by decreasing apoptosis (Elrasoul *et al*.., 2021)

Table (4): Effect of Quercus extracts on parameters plasma of male rats

Groups		PK(U/L)	Casp-(U/ml)3	CAT(KU/L)	Chol. mmol/L	T.g mmol/L	HDL-c mmol/L	LDL-c mmol/L
		Parameters						
Control		30.84± 0.597	42.7 ±1.494	9.915± 0.89	2.438 ±0.246	1.288± 0.203	2.131± 0.06	2.04 ±0.26
induced atherosclerosis	Time 0	35.38±1.305 a	47.6±1.1 a	7.256±0.75 a	4.22 ±0.66 a	1.96±0.25 a	1.91±0.3077 a	4.7 ±0.0.7 a
	1 week	35.3±1.83 a	48.99±2.0 b	7.64±1.187 b	7.256±0.819 b	2.4166±0.66 b	0.9±0.11 b	6.03±0.809 b
	2 week	37.83±1.753 b	49.311±2.19 b	6.4±0.345 c	9.232±1.127 c	3.833±1.169 c	0.69±0.164 c	7.93±1.1 c
induced atherosclerosis+Aqueous crude extract (250 mg/kg body weight	Time 0	38.59±1.35 a	48.68 ±2.5 a	6.56±0.352 a	9.015 ±0.737 a	4.04±0.811 a	0.953±0.065 c,a	7.49 ±0.72 a
	1 week	36.3±0.87 b	47.66 ±0.64 a	7.17±0.602 a	6.895 ±1.162 b	2.65±0.207 b	1.22±0.032 b	5.13 ±1.19 b
	2 week	36.65±1.42 b	42.34 ±1.56 b	8.27±0.65 b	4.95 ±0.781 c	1.966±0.25 c	1.24±0.05 c	3.61 ±0.86 c
induced atherosclerosis+ oil extract (7mg/kg body weigh)	Time 0	38.53±1.35 a	48.86 ±1.9 a	6.49±0.427 a	8.65 ±0.62 a	3.27±1.01 a	0.72±0.164 a	7.27 ±0.62 a
	1 week	36.3±0.876 b	47.08 ±0.832 ab	6.83±0.526 a	5.958 ±0.76 b	1.74±0.438 b	1.24±0.05 b	4.71 ±0.76 b

	2 week	30.63±1.42 b	43.8±1.6 b	8.61±0.65 b	6.31 ±0.748 c	1.401±0.13 b	1.28±0.2 c	4.39 ±0.78 c
induced atherosclerosis+ poly phenol extract (37.5mg/kg body weight)	Time 0	37.68±1.54 a	48.73 ±2.07 a	6.66±0.387 a	8.546 ±0.733 a	3.76±1.16 a	0.69±0.213 a	7.08 ±0.68 a
	1 week	37.26±1.89 b	47.6±1.1 a	7.25±0.75 ab	6.31 ±0.76 b	2.221±1.22 b	1.105±0.273 b	4.76±0.8 b
	2 week	34.166±0.983 c	45.86±1.21 b	7.95±1.29 b	4.788 ±0.66 c	1.266±0.0708 b	1.818±0.602 c	3.36 ±0.61 c

** The values in the above table refer to the average Mean Standard ± Deviation*

Different horizontal lowercase letters indicate a significant difference at the level of probability

The results in Table (4) show a significant decrease P≤ 0.05 in the activity of catalase enzyme in the blood plasma of animals used in atherosclerosis, especially during the last week, compared with the healthy control group. The reason for this is due to the antioxidant properties of the enzyme catalase. The formation of peroxy nitrite radical as a result of increased oxidative stress leads to inhibition of the activity of the CAT enzyme in atherosclerosis activity (Nandi *et al*., 2019). Whereas, the treatment of animals with plant extracts led to a significant increase in the activity of catalase enzyme in blood plasma, especially in the last week of treatment for animals used with arteriosclerosis, due to the properties of these extracts' anti-oxidation and resistance to free radicals by inhibiting the enzymes producing them such as Xanthine oxide enzyme, Reductase and NADPH - oxidase (Pedro *et al*., 2019).

The treatment of animals with cholesterol at a concentration of 500 mg/kg of body weight led to their infection with atherosclerosis with a significant P ≤ 0.05 increase in chol, T.G LDL-C in the blood plasma during the treatment period (15) days (Ram *et al*.,2014; Kang *et al*., 2019) as shown in Table (4). In the treatment of animals with extracts of Quercus (water, flavonoids, oil) there was a significant decrease (P≤0.05) for cholesterol in animals treated with extracts for 15 days compared with animals used with arteriosclerosis and the decrease occurred from the first week the reason for this decrease is that the extracts act as inhibitors of LDL receptors as they work On the inhibition of the enzyme HMG-COA responsible for the formation of cholesterol and thus reduce the level of excess cholesterol to bile acids and then to the intestinal tract and thus excreted by the seed and activation lipoprotein lipase enzyme (Yokoyama., *et al.,* 2022 ;Wen *etal*.,2022), Also, results shown in table (4) indicate a significant decrease in the concentration of HDL-C P≤ 0.05 in animals used with arteriosclerosis compared with healthy animals from the beginning of the treatment until after two weeks of treatment. The transfer of cholesterol ester and cholesterol ester transfer protein from HDL particles to VLDL particles and thus HDL bodies become rich in small triglycerides and thus lead to their destruction inside the body and the loss of Apo A protein with urine, which is an important component for building HDL particles with a low number of HDL particles (Pikto – Pictkiewicz *et al*., 2005; Casula *et al*., 2021), when animals were treated with Quercus extracts, a significant P≤ 0.05 rise occurred in the level of HDL in animals induced with arteriosclerosis during two weeks of treatment if the increase was observed from the first week as shown in the table (4) as fatty acids work in extract Oily, oils have a high level of HDL-C (Mazidi., 2022). Also, flavonoids work in the extract. Vegetarians raise the level of HDL-C by increasing the activity of the lecithin cholesterol acyl transferase enzyme LCAT (Rograni & Baluchnejadomojard., 2010; Ahn *et al.,* 2020)

Histological examination in animals used for arteriosclerosis with cholesterol at a concentration of (500 mg/kg) dissolved in oil for 15 days, compared with normal tissues, shows in figure (3) the presence of histological changes in the aorta compared with the control group through the presence of thickenings in three layers of the aorta with the existence of many Foam cells in cholesterol-containing macrophages with coagulation of smooth muscle fibers and cholesterol deposition in the artery. -1 (IL-1) in foam cells with artery stenosis due to inflammation and cholesterol accumulation. The reason for these histological changes is due to a metabolic disorder in lipids, oxidative stress, secretion of LDL-C cholesterol, and accumulation in the form of fat droplets with cell stimulation. foamy in the cytoplasm as shown in the figure (Poznyak *et al*., 2021).

The treatment of animals suffering from cholesterol-induced atherosclerosis with extracts of natural products of Quercus (aqueous, polyphenol, oily) during two weeks of treatment daily led to the healing of large parts of the aortic tissues, which are close to the normal state compared to animals affected by atherosclerosis, and the reason for these changes Histological in the ability of extracts to repair and restore damaged tissues by improving the mechanical properties of tissues of aorta while strengthening the cross-links of collagen by increasing activity of the endogenous lysyl oxidase-1 enzyme and lysyl oxidase-2, which work to enhance the tensile balance and elasticity of internal tissues (through catabolic processes). And building by enhancing bonds between the collagen (responsible for tissue tension) and elastin (responsible for elasticity) with the formation of covalent cross-links and their natural remodeling in fibroblasts, endothelial cells, and vascular smooth muscle cells (Sawada *et al*, 2022). Free radical formation on tissues (Martínez-González *et al*., 2019) as shown in the figure (3).

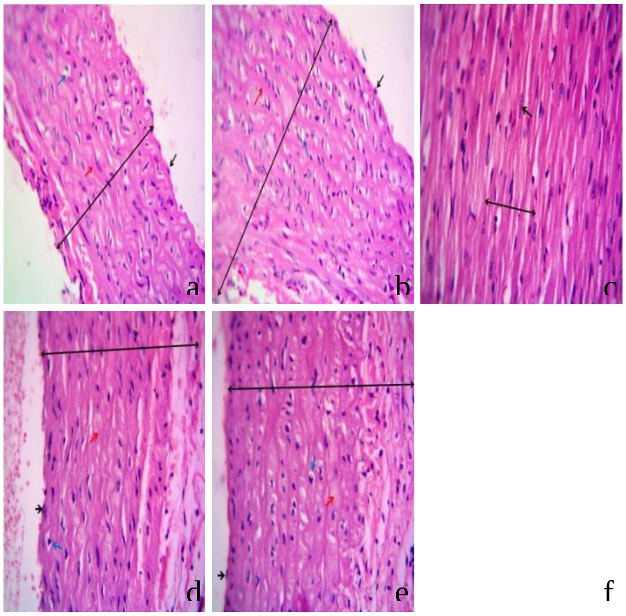

Figure (3). photomicrographs of aorta sections (a) Histological section of the aorta of a control rat showing the normal histological features of aortic layers (↔), endothelial cells (⟵), cells, and smooth muscle fibers (⟵), with the presence of some foamy cells (⟵). Hematoxylin (b) **-** Histological section of the aorta of a cholesterol-treated rat shows thickening of the aortic layers (↔), spherification of endothelial cells (⟵), thickening of smooth muscle fibers (⟵), and the presence of many foamy cells (cholesterol-containing macrophages) (⟵). Hematoxylin(c) Histological section of the aorta of a rat treated with Q aqueous extract showing the normal histological features of aortic layers (↔), endothelial cells (⟵), smooth muscle cells and fibers (⟵), with a slight presence of foamy cells (⟵).(d) **-** Histological section of the aorta of a rat treated with alcoholic extract A showing the normal histological features of aortic layers (↔), endothelial cells (⟵), cells and smooth muscle fibers (⟵), with a very slight presence of foamy cells (⟵).(e) **-** Histological section of the aorta of a rat treated with fatty extract F showing the normal histological features of aortic layers (↔), endothelial cells (⟵), cells and smooth muscle fibers (⟵), with a slight presence of foamy cells (⟵).all tssiue4Hematoxylin and Eosin Tincture, 400X

Conclusions

The extractions of the active compounds from *Quercus* fruit decrease in the high plasma kallikrein, caspase-3, cholesterol, triglycerides, and low-density lipoprotein cholesterol LDL-C in induced atherosclerosis rats treated with all extracts compared with affected control with the active extracts (oil, poly phenols) during the first and second week. The tissue aorta examination in the group of animals treated with the active extracts (aqueous, oily, flavonoid) after two weeks of the treatment showed that large in rats induced atherosclerosis

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