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Gallic Acid and Olive oil Ameliorate Atherosclerosis in Experimentally Induced in Male Rats

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Abstract | Common cardiovascular illnesses include atherosclerosis, where small aggregations of cholesterol and other components, similar to tiny tumors, develop in the artery wall and subsequently affect blood flow. The current study aimed to investigate the antiatherogenic effects of a combination of olive oil and gallic acid. Forty male atherogenic rats were arbitrarily divided into five groups (n=8). One group was given 1 mL of distilled water as a control (G1), while groups G2 and G3 were treated with 1 mL of Olive oil and 100 mg/kg body weight of Gallic acid, respectively. In addition to G4, which received 40mg/kg body weight of Atorvastatin, and G5, which served as a combination group, the animals were treated with the same dose of G2 and G3 together for 30 consecutive days. Gas chromatography-mass spectrometry (GC-MS) is used to evaluate the phytoconstituents of Olive oil. The results revealed reasonable quantities of unsaturated fatty acids (oleic, palmitic, linoleic, and linolenic acids, among others), which possess cardioprotective and antioxidant properties. The lipid profile indicated that the groups supplemented with Olive oil, Gallic acid, and the combination were significantly improved. Similarly, Atherosclerosis was revealed by increased TC, LDL-C, TG, and VLDL-C, but decreased HDL levels in the G1 group. The results of the G4 and G5 groups showed the best improvement. Similarly, the levels of TNF- α in rats with atherosclerosis were significantly elevated ($P < 0.05$) compared to those after treatment. The best improvement in TNF- α was observed in G4, which underwent intubation with 40 mg/kg body weight of Atorvastatin. In conclusion, the results suggest that Olive oil and Gallic acid exhibit potential antiatherogenic effects; however, further investigation may be necessary to optimize their therapeutic benefits.

Keywords | Gallic acid, Olive oil, Atherosclerosis, Rat, and Atorvastatin

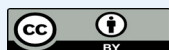
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INTRODUCTION

Still, cardiovascular disease (CVD) is the primary cause of death worldwide (Kaminsky *et al.*, 2022). Atherosclerosis and atherosclerotic disorders continue to be the most serious issues in modern medicine and health care, including myocardial infarction, stroke, sudden death, and other

prevalent causes of mortality and disability. Atherosclerotic lesion formation has a long asymptomatic period (Jebari-Benslaiman *et al.*, 2022). Thus, in many situations, the initial clinical signs of atherosclerosis emerge when the lesion is already well-formed, causing significant narrowing of the arterial lumen. Atherosclerosis is the predominant pathological mechanism involved in the majority of cases

(Fan and Watanabe, 2022). Pathological changes to the artery wall layers, known as atherosclerosis, begin to develop in childhood and often remain unnoticed for many years until they progress to a more advanced stage (Bonafiglia *et al.*, 2022). Multiple atherosclerosis animal models demonstrate that the onset of inflammation coincides with the initiation of lipid accumulation in the arterial wall. In both animals and humans, the first lesions of atherosclerosis are populated by blood leukocytes, which play a crucial role in host defense and inflammation (Soehnlein and Libby, 2021; Ali *et al.*, 2024; Al-Sailawi *et al.*, 2024). New insights into the processes underlying leukocyte recruitment have been gained by applying the fundamental principles of inflammatory biology to atherosclerosis (Ajoolabady *et al.*, 2024). Polyphenols, such as those found in Olive oil (OO), have antioxidant effects by reducing the levels of free radicals; they can also help improve patients' inflammatory and lipidemic profiles. The antioxidative polyphenols in Olive oil effectively neutralize free radicals that contribute to the oxidation of low-density lipoprotein cholesterol. Consumption of OO is linked to the primary or secondary prevention of cardiovascular illnesses, according to recent studies. Still unclear, however, is whether a lower dosage of OO with a higher concentration of polyphenols or a higher dosage of OO with a lower concentration would be preferable (Kourek *et al.*, 2024). Trihydroxybenzoic acid, also known as Gallic acid, is present in certain plant materials and tea leaves. In both animal and laboratory studies, Gallic acid has been shown to mitigate the effects of transverse aortic constriction (TAC) on heart hypertrophy, dysfunction, and fibrosis. The end-diastolic and end-systolic diameters of the left ventricle are decreased, and the diminished fractional shortening in TAC is recovered (Ayad *et al.*, 2021). Furthermore, it reduces the production of skeletal α -actin, β -myosin heavy chain, brain natriuretic peptide, and atrial natriuretic peptide. Gallic acid administration diminishes perivascular fibrosis (as measured by Trichrome II Blue staining) and connective tissue growth factor and collagen type I expression (Okafor *et al.*, 2024). In this study, we report the effect of Olive oil and Gallic acid on cardiac dysfunction in a rat model of atherosclerosis and compare these effects with those of Atorvastatin.

MATERIALS AND METHODS

CHEMICALS

Olive oil purchased from the herbal store (Al-Hikma) at Babylon city, as well as Gallic acid obtained from Sigma Aldrich (USA), cholesterol, Hydrogen peroxide, and Tritone (Biokemica-India).

GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS) ANALYSIS

The GC-MS analysis of Olive oil was conducted using

an Agilent 7820A GC system connected to a mass spectrometer (Agilent, USA), as shown in Figure 1. The analytical column employed was an Agilent HP-5 MS Ultra Inert (30 mm $\times$ 250 μ m $\times$ 0.25 μ m). A one μ L injection volume was used with a pressure of 11.933 psi. The GC inlet line, auxiliary heaters, and injector (splitless) temperatures were set at 250 $^{\circ}$ C, 300 $^{\circ}$ C, and 250 $^{\circ}$ C, respectively. The carrier gas used was helium with a purity of 99.99%. The oven program temperature included four ramps: the first ramp was held at 60 $^{\circ}$ C for 3 minutes, the second ramp increased from 60 $^{\circ}$ C to 180 $^{\circ}$ C at a rate of 7 $^{\circ}$ C/min, the third ramp increased from 180 $^{\circ}$ C to 280 $^{\circ}$ C at a rate of 8 $^{\circ}$ C/min, and the fourth ramp was held at 280 $^{\circ}$ C for 3 min.

EXPERIMENTAL ANIMALS AND MANAGEMENT

The male albino rats utilized in this investigation (n=40) were acquired from the animal house of the College of Veterinary Medicine/University of Al-Qasim. The rats were at least 2 months old and weighed 180-200 g. The rats were provided with tap water and a standard pellet diet. For the first two weeks, the animals were housed in the College of Veterinary Medicine's animal house at the University of Al-Qasim. They were kept in a controlled environment with a 12-hour light-dark cycle, temperatures ranging from 20 $^{\circ}$ C to 25 $^{\circ}$ C, and air conditioning. Mulch was added to the bed twice weekly.

INDUCTION OF ATHEROSCLEROSIS

Forty rats were used to induce atherosclerosis by administering 0.5% hydrogen peroxide in their drinking water and a high-cholesterol diet (1.50% cholesterol) daily for three weeks. Additionally, a single dose of Triton (10 mg/kg) was administered intraperitoneally at the end of the last three weeks of the experiment. After the end of the periods, the levels of cholesterol were estimated to ensure the disease was induced (Pashaie *et al.*, 2017).

EXPERIMENTAL DESIGN

After the induction of atherosclerosis, the animals were divided into five groups (n=8), and the rats were divided equally into five treatment groups according to the following:

- Group 1: 8 rats drenched with (1ml/rat/day) of distilled water as a control positive.
- Group 2: 8 rats drenched with (1ml/rat/day) of Olive Oil daily (Vazquez *et al.*, 2019).
- Group 3: 8 rats drenched with (1ml/rat/day) of distilled water containing (100mg/kg.bw) of Gallic acid (Jin *et al.*, 2017).
- Group 4: 8 rats drenched with (1ml/rat/day) of distilled water containing (40mg/kg.bw) of Atorvastatin (Oh *et al.*, 2021).
- Group 5: 8 rats drenched with (1ml/rat/day) of Virgin

Olive Oil and (100mg/kg.bw) of Gallic Acid, for 30 days.

LIPID PROFILE ASSESSMENTS

Tests for total cholesterol, triglycerides, and HDL-cholesterol were conducted using Randox kits, manufactured by Randox Laboratories Ltd. in Antrim, UK. Lipoprotein cholesterol and extremely low-density lipoprotein cholesterol levels were determined using the Friedewald *et al.* (1972) method.

TUMOR NECROSIS FACTOR

In the present study, an ELISA kit was used to measure the serum levels of tumor necrosis factor, following the manufacturer’s instructions. The absorbance at 450 nm was tested using a microplate ELISA (Aboktifa *et al.*, 2025).

STATISTICAL ANALYSIS

The data from the second experiment were analyzed statistically using one-way and two-way ANOVA, and the least significant differences (LSD) test was employed to determine whether there were substantial differences in the group means. SAS (Statistical Analysis System, version 9.1) was used for this purpose. Statistical significance was determined when the p-value was less than 0.05, and the findings were presented as the mean plus or minus the standard error.

RESULTS AND DISCUSSION

The fatty acid compositions of olive oil and other vegetable oils can be studied, and any adulteration detected using GC-MS. Table 2 displays the contents and composition of Olive oil as determined by GC-MS analysis (Figure

1). Oleic acid, a monounsaturated fatty acid, was found to compose 59.51% of the thirteen fatty acid methyl esters that were discovered. According to Table 1 and Figure 1, the area percentages of palmitic acid, linalic acid, linolenic acid, cis-11-docosanoic acid, and eicosanoic acid were 14.10%, 8.61%, 3.37%, 3.17%, and 2.95%, respectively.

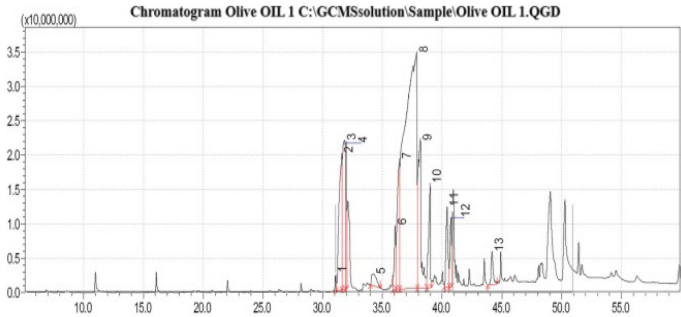


Figure 1: GC-MS analysis of olive oil.

LIPID PROFILE

The results, as illustrated in Table 2, indicated that the groups supplemented with Olive oil, Gallic acid, and the combination (Olive+Gallic) in the present study showed significant improvements. Similarly, Atherosclerosis, revealed by increased TC (76.92 ± 1.35 mg/dL), LDL-C (58.38 ± 1.31 mg/dL), TG (43.75 ± 1.57 mg/dL), and VLDL-C (10.34 ± 0.11 mg/dL) but decreased HDL (11.54 ± 2.48 mg/dL) levels in the control group. These alterations were markedly ameliorated when the rats were treated with Gallic acid, Olive oil, Atorvastatin, and a combination of these (Table 1). The results of the G4 and G5 groups showed the best improvement, characterized by a decrease in TC, LDL, TG, and VLDL, and an increase in HDL.

Table 1: Phytoconstituents of olive oil.

Biological activity	Name	Area %	Retention time (min)
Anti-inflammatory	Palmitic acid, methyl ester	14.10	31.829
Antioxidant	cis-10-Heptadecenoic acid, methyl ester	1.61	32.291
Antibacterial	Oleic acid methyl ester	59.51	33.428
Cardioprotective	Linoleic acid, methyl ester	8.61	34.175
Cardioprotective	Linolenic acid, methyl ester	3.37	35.982
Anti-inflammatory	Eicosanoic acid, methyl ester	2.95	36.421
Cardioprotective	cis-13-Eicosenoic acid, methyl ester	2.19	36.837
Anti-cancer	Docosanoic acid, methyl ester	1.28	38.182
Anti-inflammatory	cis-11-Eicosenoic acid, methyl ester	0.82	38.704
Antioxidant	Heneicosanoic acid, methyl ester	0.56	39.144
Anti-cancer	cis-11-Docosanoic acid, methyl ester	3.17	40.342
Antioxidant	Tricosanoic acid, methyl ester	1.10	41.541
Antioxidant	Dodecanoic acid, methyl ester	0.73	43.964

Table 2: Serum concentrations of lipid profiles.

TC Mean± S.E (mg\dl)	TG Mean± S.E (mg\dl)	HDL Mean± S.E (mg\dl)	LDL Mean± S.E (mg\dl)	VLDL Mean± S.E (mg\dl)	Parameters Groups
129.06±3.26A	43.75±1.57A	11.54±2.48D	79.87±6.42A	10.34±0.11A	G1(control)
89.51±9.63B	30.40±5.63B	17.28±5.06C	31.83±4.53 B	6.45±1.12B	G2 (Olive oil)
82.27±2.85B	33.69±7.16B	21.75±4.65B	26.45±3.44B	6.57±1.43B	G3 (Gallic acid)
53.74±1.78C	21.43±4.98C	34.06±3.27A	13.27±1.57D	1.48±0.99D	G4 (Atorvastatin)
66.22±10.10C	28.26±6.18 C	25.93±1.85 B	19.07±6.73 C	4.01±2.24 C	G5 (Olive Gallic acid)
18.05	9.61	6.51	7.76	2.32	LSD

Different capital letters denote significant differences ($P \leq 0.05$) among groups.

TNF- α

The results in Table 3 demonstrate that levels of TNF- α (mean $\pm$ SE) for rats with induced atherosclerosis were significantly higher (P -value < 0.05) compared to the TNF levels after treatment. After 30 days of treatment, the results for TNF- α in all treated groups (G2, G3, G4, and G5) showed a significant reduction compared to the control group. In addition, there were no significant differences among (G2, G3, and G5), which involved intubation with Olive oil, Gallic acid, and a combination, respectively. In contrast, the best improvement in TNF- α was observed in G4, which underwent intubation with 40 mg/kg body weight of Atorvastatin.

Table 3: Proinflammatory cytokine TNF levels.

Groups	TNF At induction Ng/L	TNF after treatment Ng/L
G1(control positive)	241.31±11.34Aa	145.01±8.65Ab
G2 (Olive oil)	219.04±13.00Ba	122.77±10.06Bb
G3 (Gallic acid)	193.80±9.74Ca	117.70±13.77Bb
G4 (Atorvastatin)	211.44±10.58Ba	89.43±10.06Cb
G5 (Olive+ Gallic acid)	185.92±13.65Ca	104.08±7.34Bb
LSD	21.3	

Among groups, different capital letters indicate a statistically significant difference ($P \leq 0.05$). Variegated small letters indicate statistical significance ($P \leq 0.05$) over time intervals.

DISCUSSION

An epidemic of atherosclerotic disease, a risk factor for cardiovascular disease, has been expanding over the past several years. Therefore, the purpose of this research was to determine whether two herbal components, Gallic acid and Olive oil, could help restore the cardiac and lipid profiles to normal following atherosclerosis induction. The high concentration of this monounsaturated v-9 fatty acid in Olive oil is one reason for its health advantages. Numerous studies have found that Olive oil has a higher concentration of unsaturated fatty acids (UFAs), and the present study's GC-MS analysis results corroborate these

findings (Poulli *et al.*, 2006; Abdelrahman *et al.*, 2019). Additionally, numerous studies have shown that Olive oil's high relative levels of unsaturated fatty acids may make it an even more effective tool in the fight against coronary heart disease (Grundy, 1986; Diraman and Dibeklioglu, 2009; Asik and Özkan, 2011). One class of plant polyphenols called gallotannins includes Gallic acid. Some foods that contain it include blackberries, tea leaves, grapes, fruits, and vegetables (Kawada *et al.*, 2001; Choubey *et al.*, 2015). One example is gallnuts. Antioxidant properties are just one of its many biological effects (Soong and Barlow, 2006). However, there is considerable evidence that GA protects the heart (Appeldoorn *et al.*, 2005; Kee *et al.*, 2014; Jin *et al.*, 2017; Al-Mansury *et al.*, 2021). When calculating risk for coronary heart disease, the total cholesterol/HDL ratio outperforms both total cholesterol and LDL cholesterol values. Treatments combining Gallic acid, Olive oil, and atorvastatin considerably improved HDL levels while lowering total, triglyceride, LDL, and VLDL levels. Table 2 shows that the best lipid profile was achieved by combining Olive oil with Gallic acid (G5). This suggests that there may be a synergistic effect between the two that could be useful in clinical settings for controlling lipid levels and risk of cardiovascular disease the significant change in the lipid profile during experimental atherosclerosis development points to Triton-induced disruptions in lipid metabolism. Approximately 30–40% of dyslipidemia cases are secondary, meaning they are triggered by other medical conditions or medications (Yanai and Yoshida, 2021). Hyperlipidemia, caused by a combination of triton and hydrogen peroxide, occurs when peripheral tissues are unable to absorb plasma lipoproteins high in triacylglycerol. It alters the VLDL-C structure, which slows or prevents its clearance from circulation by blood and tissue lipases, and it exacerbates hyperlipidemia by stimulating the liver to produce more cholesterol (Ayad *et al.*, 2022; Okafor *et al.*, 2024; Abdul-Ameer *et al.*, 2024). The results showed that Gallic acid and Olive oil significantly reduced Triton-induced atherosclerosis. The high concentration of bioactive phytochemicals in the G5 group may explain its notable hypolipidemic effects, which have been associated with anti-atherosclerotic and other pharmacological actions (Vazquez *et al.*, 2019;

Akbari, 2020; Okafor *et al.*, 2024; Serreli *et al.*, 2024). Atherosclerosis contributes to the development of tumor necrosis factor- α , a multifunctional proinflammatory cytokine (Bruunsgaard *et al.*, 2000). Therefore, a low ankle-brachial arterial pressure index, indicative of peripheral atherosclerosis, is associated with high TNF- α level in the current investigation following atherosclerosis induction, as shown in Table 3. On the other hand, animals given Gallic acid, Olive oil, or atorvastatin (groups G2, G3, G4, and G5) showed a notable decrease in TNF- α serum level.

CONCLUSIONS

The results indicate that Olive oil and Gallic acid showed potential antiatherogenic effects, but may require further exploration to maximize their therapeutic benefits. Further studies could investigate the mechanisms underlying these observations and assess their long-term impact on cardiovascular health.

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NOVELTY STATEMENT

The novelty of this research lies in investigating the antiatherogenic effects of a combination of olive oil and gallic acid. by analyzing the lipid profile and tumor necrotic factor.

AUTHOR'S CONTRIBUTION

Ghadeer D. Danan conceptualized and designed the study Ali I. Al-ameedi and Hassan K. Al-Awadi participated in the investigation and the drafting of the paper as well as, analyzed and interpreted the data. Ali I Al-ameedi revised the paper critically for intellectual content and approved the final version of the paper to be published. All authors agree to be accountable for all aspects of the work.

GENERATIVE AI OR AI-ASSISTED TECHNOLOGY STATEMENT

The authors declare that no Genrative AI was used in the creation of this manuscript.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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