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In Vitro study of the effect of Vanillin derivatives on aryl esterase, troponine and lipid profile in atherosclerosis patients as compared to normal subjects

Murooj Mousa Atallah[1], Fadhil Dawood Khalid[1], Yaser A.M Sulaiman[2]

[1]*Department of Chemistry / College of Science / Tikrit University*
[2]*Department of Clinical Laboratory Science/ College of pharmacy/ Tikrit University*

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***Corresponding author:**
Yaser A.M Sulaiman
neimiyaser@tu.edu.iq

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Abstract:

The study describes the effect of vanillin derivative on the activity of aryl esterase in vitro. After that, the estimated effectiveness of the enzyme aryl esterase, troponin T and lipids. The research included the collection of 120 blood samples distributed to 60 blood samples of healthy people as a control group and 60 blood samples of patients with atherosclerosis who were diagnosed under the supervision of specialized doctors at Salah Eddin General Hospital / Catheterization Unit.
Results showed that the vanillin derivative has an invigorating effect on the activity of aryl esterase, and that the degree of enzyme activation increases with the increase in the concentration of the derivative, while there was lower with a probability level ($P \leq 0.0006$) in the activity of aryl esterase from the serum of patients with atherosclerosis in addition, a significant increase at the probability level ($P \leq 0.001$) in the level of total cholesterol, triglycerides, LDL-C and VLDL-C , while there was decrease at the probability level ($P \leq 0.0004$) in the level of HDL-C.

دراسة مختبرية لدور مشتق الفانيلين في نشاط انزيم الأريل استيريز

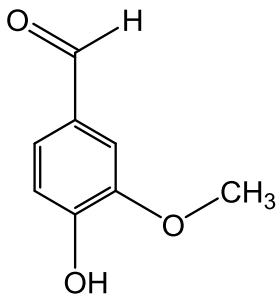
مروج موسى عطاالله فاضل داود خالد ياسر أحمد موفق

الخلاصة

تضمن البحث جمع 120 عينة دم موزعة على 60 عينة دم لأشخاص أصحاء كمجموعة سيطرة و 60 عينة دم لمرضى تصلب الشرايين ، تم تشخيصهم تحت إشراف أطباء مختصين بمستشفى صلاح الدين العام / وحدة القسطرة. اشتملت الدراسة على وصف تأثير مشتق الفانيلين على نشاط انزيم الأريل استيريز خارج الجسم. بعدها تم تقدير فعالية إنزيم أريل استيريز، تروبونين تي والدهون في مرضى تصلب الشرايين ومجموعة السيطرة. حيث أظهرت النتائج أن مشتق الفانيلين له تأثير تنشيطي على نشاط أريل استيريز، وأن درجة تنشيط الإنزيم تزداد مع زيادة تركيز المشتق، بينما كان هناك انخفاض معنوي مرتفع بمستوى احتمالية ($P\leq 0.0006$) في نشاط أريل استيريز لدى مرضى تصلب الشرايين وانخفاض عند مستوى الاحتمال ($P\leq 0.0004$) في مستوى HDL-C، بالإضافة إلى ذلك لوحظ زيادة كبيرة عند مستوى الاحتمالية ($P\leq 0.001$) في مستوى كل من (TCH ، TG، LDL-C، VLDL-C) لدى مرضى تصلب الشرايين.

Introduction

Atherosclerosis is one of the most important diseases of the Cardiovascular disease (CVD) and the most important basic causes of human death in the developed world, if the reports of the American Heart Association show that the disease kills one million people annually and is considered more than the death rate of cancer therefore, there is an urgent need to find effective preventive and therapeutic strategies to change this fact because of its health and economic consequences[1]. Atherosclerosis results from a primary injury to the lining of the arteries, resulting from several physiological and environmental factors, which leads to a response to inflammation and necrosis[2], Atherosclerosis leads to the formation of active macrophages capable of producing enzymes that degrade protein and destroy collagen, which adds strength to the ample fibrous covering, making the covering fragile, weak and more prone to rupture[3]. Recent studies indicate a relationship between atherosclerosis and fat on the one hand, and inflammation on the other hand, and according to the hypothesis of lipid oxidation, (LDL-C) (present in the lining of blood vessels) increases its uptake by macrophages [4] [5] so it works to promote the development of foam cells, which is a feature of the development of plaques in atherosclerosis, and maintains cytokines and their receptors in directing immune cells and works on the response of white blood cells stimulating the incidence of atherosclerosis, which increases the risk and complication of atherosclerosis disease with an increase in the imbalance in the function of mitochondria within the cell of the organism The neighborhood and increased levels of reactive oxygen[6]. The presence of foam cells and macrophages in the lining of blood vessels is the main feature of atherosclerotic lesions and their development, as they act on the abnormal absorption of low-density lipoprotein cholesterol while not sequestering cholesterol, which leads to its free accumulation in the form of fatty droplets, thus stimulating foam cells[7] and increased coagulation, inflammation, and programmed cell death[8]. Vanillin (4-hydroxy-3-methoxybenzaldehyde) is the major component of natural vanilla, which is one of the most widely used and important flavoring materials worldwide. This substance is also relevant for the synthesis of different agrochemicals, antifoaming and pharmaceutical products [9].



P-Vanillin

Figure (1): Vanillin structure

important phenol compounds include the dihydroxybenzene, and its derivatives [18]. Vanillin presents different functional groups, including ether and aldehyde moieties, besides the phenolic group. The presence of a methoxy group adjacent to the phenol hydroxyl gives origin to a group of compounds isolated from plants called vanillin, which includes substances such as vanillin, eugenol and capsaicin [19]. The presence of the aldehyde functional group turns possible the synthesis of vanillin derivatives by condensation reactions for the production of Schiff base derivative [20] (E)-N-(4-hydroxy-3-methoxybenylidene)-2-hydroxybenzohydrazide) this class of compounds is particularly interesting because the structural organization and the presence of moieties with different polarities: polar region due hydroxyl groups in the upper rim and nonpolar region originated by the benzene rings. The condensation of p-Vanillin and of Salicylic acid (Scheme 1) enables the preparation of the compound (E)-N-(4-hydroxy-3-methoxybenylidene)-2-hydroxybenzohydrazide), here identified as Vanillin derivative (Figure 2).

Besides its industrial and food application this compound has been the subject of several scientific investigations in the last years, such as the identification of antioxidant properties [10], antimicrobial activity [11] [12] [13], as well as antibiotic [14] [15] and anticancer actions [16]. Conversely, vanillin may also induce oxidative stress in yeast cells [17]. Part of these biological properties can be attributed to the fact that vanillin is a phenolic compound. The antioxidant activity of phenols is attributed to their ability to scavenge free radicals. Other

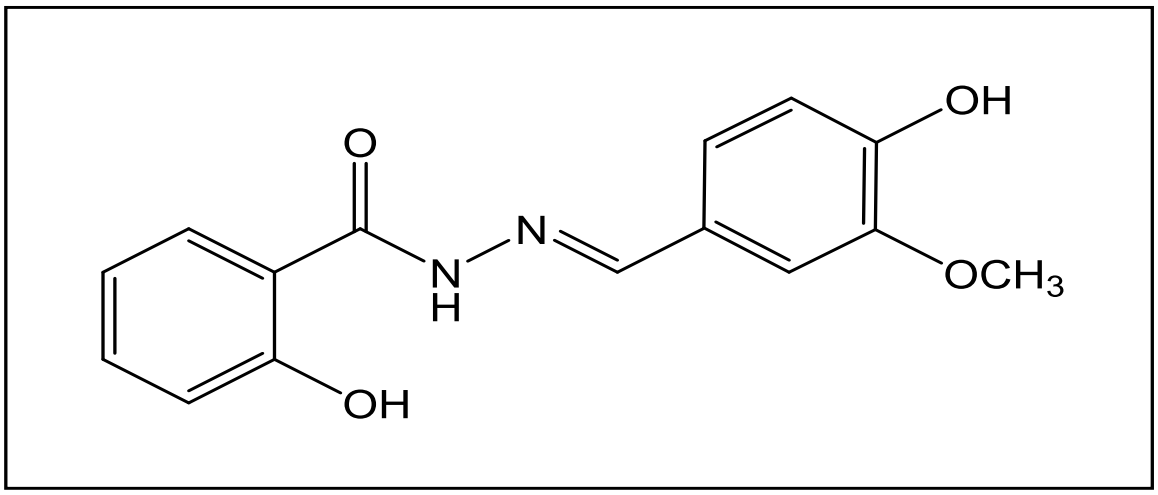


Figure (2): (E)-N-(4-hydroxy-3-methoxybenylidene)-2-hydroxybenzohydrazide).

This study aims to study the level of the enzyme aryl esterase (PON1) in patients with atherosclerosis and compare it with apparently healthy people as a control group. Also this study was aimed to study some biochemical variables in

atherosclerosis such as (troponin T, cholesterol, triglycerides, high-density lipoprotein, low-density lipoprotein, very low-density lipoprotein) and compare to healthy people

Materials and methods

The study was conducted in Tikrit City, from September to December 2022. Blood samples were taken from 60 atherosclerosis patients, and 60 from healthy subjects (control). The blood was drawn from the vein using a (5 ml medical syringe) and the blood was placed in new, sterile plain tubes free of any additives. The blood was left for (10) minutes, after which the blood serum was separated from the coagulated part with a centrifuge, the samples were kept at a temperature of 4ºC until biochemical variables were measured.

Study the effect of the prepared vanillin derivative on the activity of arylesterases:

1- we prepared Different concentrations of the compound (E)-N-(4-hydroxy-3-methoxybenzylidene)-2-hydroxybenzohydrazide) that was prepared solution of 500 mg/ml and dissolved in 10 ml of DMSO.
2- After that, the concentrated initial solution of 500 mg/ml that was prepared in step (1) is diluted into a series of dilute solutions, as the following concentrations are prepared:

   (500 mg/ml, 50 mg/ml, 5 mg/ml, 0.5 mg/ml, 0.05 mg/ml, 0.005 mg/ml, 0.0005 mg/ml)

3- The concentrations prepared from the compound were added to the reaction solution with the same quantity or volume of the enzyme concentrations that were prepared previously, after which the enzyme was added, and so the addition of the rest of the concentrations was completed according to the method of action described for the enzyme [21].

Estimation of aryl esterase activity in serum:

The activity of the aryl esterase enzyme in the serum was estimated using an ELISA kit manufactured by (Cloud-Clone Crop) company, where the method relies on the use of wells plate containing antibodies of the enzyme, as standard solutions are added to the wells to bind the antigen with the antibody of the enzyme present in those pits and incubated for a period of time, after completing the washing process, the Substrate solution is added, and only the samples containing PON1 show a change in color. The interaction of the enzyme with the base material stops when the sulfuric acid solution is added, and the color change is measured at a wavelength of 10-540 nm. The enzyme concentration in the sample is determined by comparing the absorbance of the sample with the standard solution by drawing a curve between them [21].

Estimation of troponin T level:

cTnT was estimated based on the ELISA method [22], as the microplate prepared in this kit was pre-coated with cTnT antibody, and cTnT-HRP conjugated. The assay sample and buffer solution with conjugated cTnT-HRP coated in the microplate pre-incubated for 1 hour. After the incubation period, the liquid is discarded and washed five times, then the associated standard solutions are added, then the base material for the Horseradish Peroxidase HRP enzyme is added. The product of the enzymatic substrate reaction is a blue compound. Then the stop solution is added as the blue color turns yellow. Optical effectiveness reads at a wavelength of 450nm.

Estimation of cholesterol in serum TC:

The level of total cholesterol in serum was estimated using the ready-made analysis kit from the French company and the enzymatic method (Biolabo) [23].

Estimation of triglycerides in serum TG:

The level of triglycerides in serum was estimated using the ready-made analysis kit from the French company and the enzymatic method (Biolabo) [23].

Estimation of high-density lipoprotein (HDL-C) cholesterol in serum:

The level of high-density lipoprotein cholesterol in serum was measured by the enzymatic method using a ready-made analysis kit [24]. **Estimation of the level of low-density lipoprotein LDL-C cholesterol in serum:**

LDL-cholesterol concentrations were calculated according to the formula [25]:

LDL-C (Conc.) = Conc. Total Cholesterol - (Conc. HDL-C +Conc. VLDL C) .

Estimation of very low-density lipoprotein VLDL-C cholesterol in serum:

After finding the concentration of triglycerides according to the amount of VLDL-C, depending on the method [26]:

VLDL-C = TG / 5.

Results and Discussion

The results included the statistical values of the clinical variables that were measured in the current study in the blood serum of patients with atherosclerosis and the control group (aryl esterase, troponin T, lipid profile) and according to the working methods described as shown in Table (1):

Table (1): The arithmetic mean of the measured variables

Parameter	Control	Patients	P value
	Mean ±SE N=60	Mean ±SE N=60	
PON1 (U/L)	135.36 ± 1.7	71.2 ± 3.7	≤ 0.0006
cTnT (ng/ml)	0.190± 0.020	2.010± 0.145	≤ 0.0003
TC (mg/dl)	165.1± 11.2	215.2 ± 8.9	≤ 0.001
TG (mg/dl)	100.9± 4.0	200.9± 3.6	≤ 0.001
HDL –C (mg/dl)	31.61± 1.0	20.69 ± 0.47	≤ 0.0004
LDL -C (mg/dl)	82.9 ± 2.9	169.72± 1.9	≤ 0.001
VLDL –C (mg/dl)	21.36 ± 1.1	40.28 ± 0.69	≤ 0.001

Measuring the level of the aryl esterase:

The results shown in Table (1) showed a high significant decrease with a probability level (P≤ 0.0006) in the activity of aryl esterase in the serums of patients with atherosclerosis (71.2 ± 3.7 U/L) compared to control group (135.36 ± 1.7 U/L) as well. Shown in Figure (3):

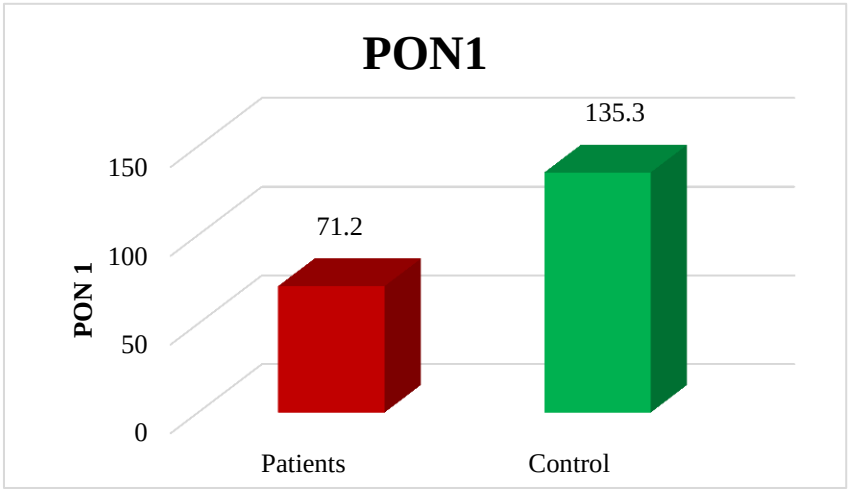


Figure (3): Concentration of aryl esterase (U/L) in the serums patients compared to control group.

The results of the current study agreed with the previous results [27] [28], which showed that aryl esterase activity was inversely related to the risk of developing cardiovascular diseases, including atherosclerosis, as the results showed a decrease in the concentration of aryl esterase in patients with atherosclerosis compared to healthy subjects, and this is due to the increase in oxidative stress processes in patients, causing an increase in lipid peroxides in the serum, which works to inhibit the enzyme, so its effectiveness decreases in patients [29]

Troponin T level:

The results shown in Table (1) showed a significant increase at the probability level (P ≤ 0.0003) in the level of cTnT in the sera of patients with atherosclerosis (2.010 ± 0.145) ng/ml compared to control group (0.190 ± 0.020), as shown in the figure (4).

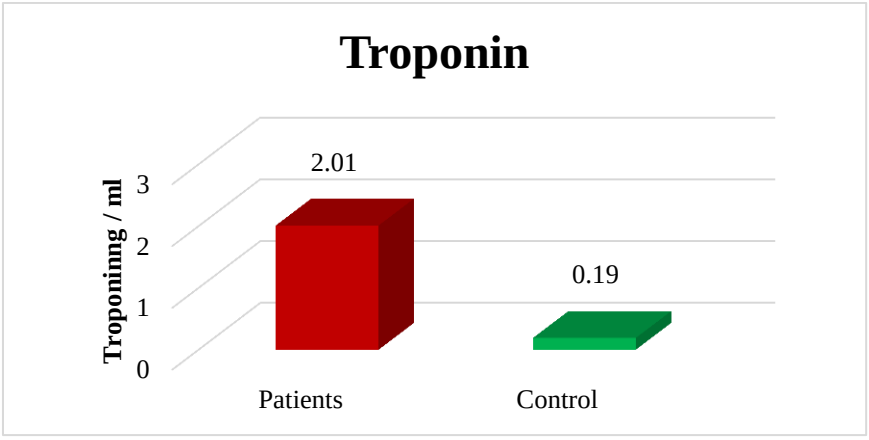


Figure (4): Troponin T level in the serums patients compared to control group.

The results of the current study agreed with the results of Yamini et al., which indicated that the level of cTnT was significantly increased in the serum of patients with atherosclerosis compared to healthy people, and that the predictive and diagnostic result of cTnT function may be an important marker of myocardial physiology [30] .The high level of cTnT in the serum of patients is attributed to the occurrence of necrosis in the heart muscle due to insufficient blood flow, and the lack of oxygen as a result of blockage of one of the coronary arteries feeding the heart muscle cells, so the part that is fed by this blocked artery dies in what is known as infarction [31] .After injury, cTnT, cTnI, is released into the circulation due to its tight binding to the thin, insoluble filaments of the myocardium [32], cTnT is slowly released into the circulation after injury [33] ,and often remains elevated for days to weeks [34]. However, it was observed that cTnT reaches peak concentrations lower than [35] cTnI. cTn is one of the most important diagnostic markers for heart muscle damage, but it can be elevated in other cases, including diabetes, because it affects the inner lining of blood vessels and their diameter, leading to calcifications within the walls, and as a result, atherosclerosis develops [36].

Lipid profile

The results shown in Table (1) showed a significant increase in the level of total cholesterol (TC) in the serum of patients with atherosclerosis (215.2 ± 8.9 mg/dl) compared to healthy people (165.1 ± 11.2 mg/dl) at the level of probability P ≤ 0.001 as shown in the figure (5).

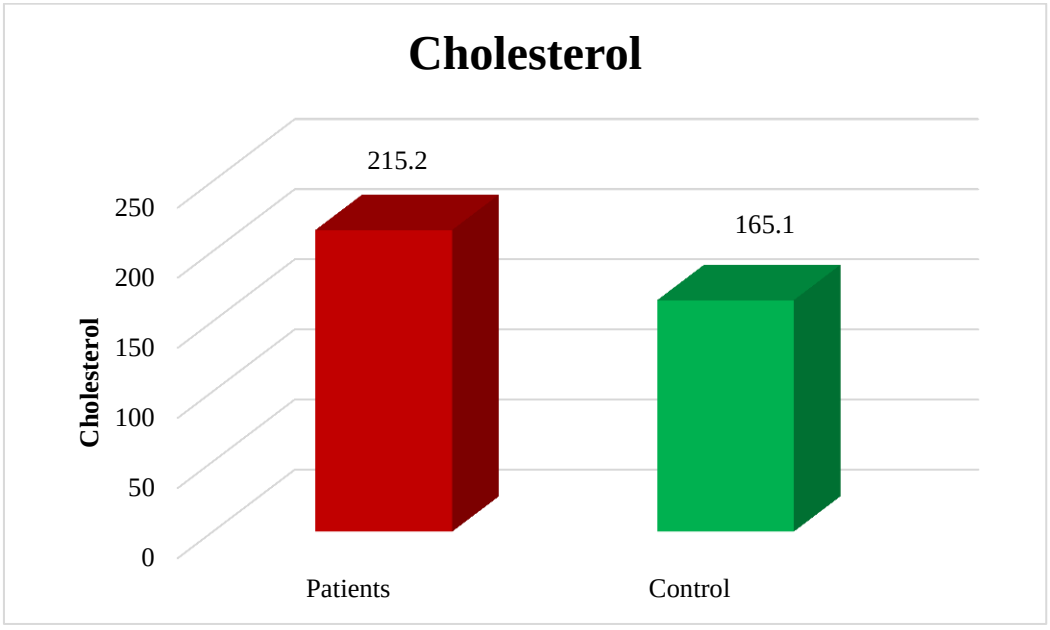


Figure (5): TC level (mg/dl) in the serums patients compared to control group.

The results shown in Table (1) showed a significant increase at the probability level ($P \leq 0.0007$)) in the level of triglycerides (TG) in the serum of patients with atherosclerosis (200.9 ± 3.6 mg/dl) compared to control group (100.9 ± 4.0 mg/dl) as shown. In Figure (6).

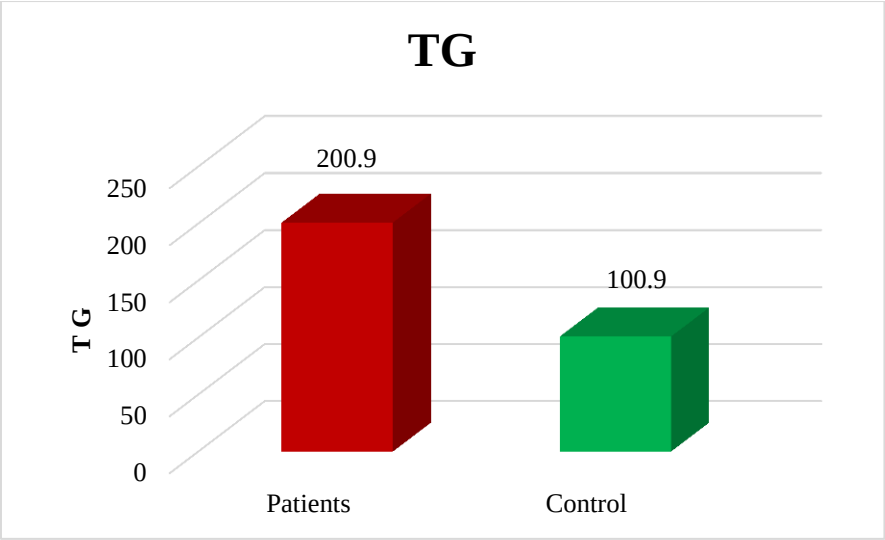


Figure (6): TG level (mg/dl) in the serums patients compared to control group.

Also, the results shown in Table (1) showed a significant decrease at the level of probability ($P \leq 0.0004$) in the level of (HDL-C) in the sera of patients with atherosclerosis (20.69 ± 0.47 mg/dl) compared to control group (31.61 ± 1.0 mg/dl). dl) as shown in Figure (7).

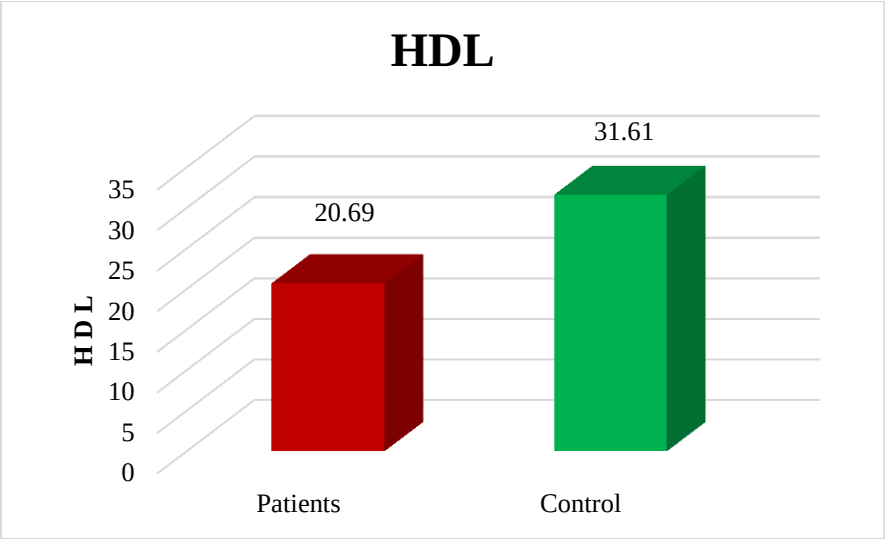


Figure (7): HDL-C level (mg/dl) in the serums patients compared to control group.

The results shown in Table (1) showed a significant increase at the level of probability ($P \leq 0.01$) in the level of (LDL-C) in the serum of patients with atherosclerosis (169.72 ± 1.9 mg/dl) compared to control group (82.9 ± 2.9 mg/dl) as well. Shown in Figure (8).

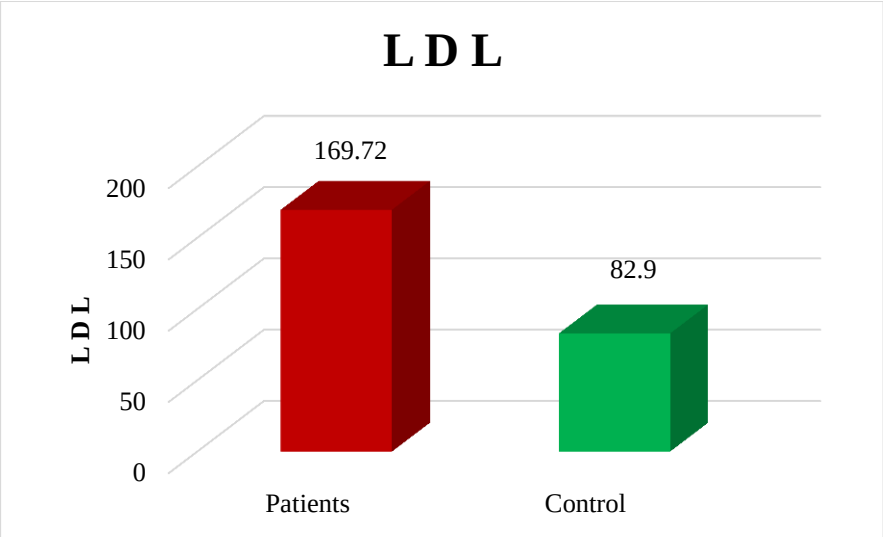


Figure (8): LDL-C level (mg/dl) in the serums patients compared to control group.

The results shown in Table (1) showed a significant increase at the level of probability ($P \leq 0.0006$) in the level of (VLDL-C) in the sera of patients with atherosclerosis (40.28 ± 0.69 mg/dl) compared to control group (21.36 ± 1.1 mg/dl).as shown in Figure (9).

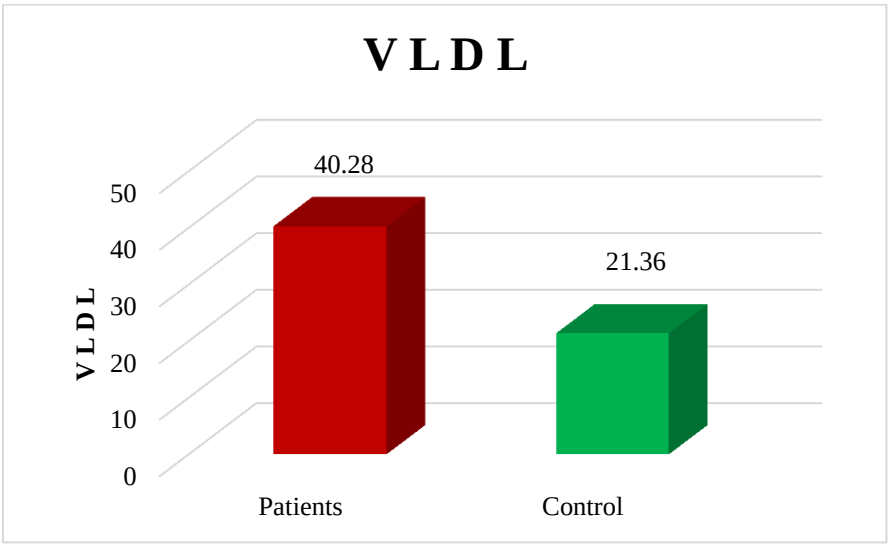


Figure (9): VLDL-C level (mg/dl) in the serums patients compared to control group.

The results of the current study agreed with the results of Khurshid and his group [37], Linton and his group [38], and Gidding and his group [39], as they indicated an increase in the levels of TC, TG, LDL-C, VLDL-C and a decrease in the level of HDL-C in patients with atherosclerosis. Cholesterol contains many lipoproteins circulating in the blood, and TC increases the risk of atherosclerosis [40]. The reason for this is that excess TC in the blood is deposited on the walls of blood vessels, which leads to their narrowing and occlusion [41]. Elevated levels of TC can be attributed to catabolism of LDL-C or a deficiency in the efficiency of LDL-C receptors in tissues as well as to the activity of the enzyme acetylcholesterol

transferase, which is responsible for the absorption of cholesterol in the intestine [42]. In addition to increasing its synthesis internally in the intestine, the consumption of meals rich in saturated fats has a role in raising levels of TC [43]. The accumulation of cholesterol under the endothelial layer of the artery eventually leads to the collapse of the inner lining, which leads to clogging of the arteries and blood clots over the ulcerated area, which leads to clogging of the coronary or cerebral vessels [44]. On the other hand, cholesterol is transported from plasma and extracellular fluids in the human body. By special transport molecules including lipoproteins, lipoproteins transport it from the original tissue to the site The target, and any imbalance in the metabolism of lipoproteins causes an increase in cholesterol concentrations, so HDL-C plays an important role in maintaining normal cholesterol levels by transferring excess concentrations in body cells to the liver, and thus reducing cholesterol in blood vessels, and its deposition is considered one of the reasons major diseases of atherosclerosis[45]. In regards to a decrease in the level of HDL-C in the blood, this could be attributed to an increase in TG and TC as an increase in these variables decreases the efficiency of HDL-C in transporting TC from tissues to the liver [46] and is often associated with low levels of HDL- C in the blood is associated with a risk of CVD [47]. The distinctive role of HDL-C molecules as anti-atherosclerotic extends to being anti-inflammatory, anticoagulant and antioxidant [48]. It is possible that the high levels of LDL-C in the blood can be attributed to an increase in the amount of TC in the diet that is eaten, which leads to a decrease in its efficiency in transporting cholesterol from the liver to the tissues, which leads to its accumulation in it. When LDL receptors are inhibited, this will lead to the accumulation of LDL particles. in a high concentration in the blood, and then deposited on the artery wall, causing atherosclerosis [49]. Oxidized LDL-C promotes the expression of pro-inflammatory genes, which leads to mobilize of monocytes in the vessel wall causing vascular endothelial cell dysfunction [50], Research indicates that oxidized LDL-C is endothelial cytotoxic, due to its generation of free radicals, and it impairs nitric oxide synthase gene expression and activity [51]. It is possible that the high levels of VLDL-C in the serum of patients with atherosclerosis can be attributed to an increase in the synthesis of this type of lipoprotein and a decrease in its removal from the plasma, and an increase in the levels of TC in the liver leads to an increase in the level of VLDL-C that affects the regulation of the secretion of this lipoprotein from The liver [52], and its high levels may be attributed to a defect in the function of LPL, which converts TG to FA, which permeates cell membranes for the purpose of creating IDL-C, which quickly turns into LDL-C, which increases the risk of CVD diseases [53].

Study of the effect of the prepared vanillin derivative on the activity of aryl esterase from the serum of patients with atherosclerosis:

The activity of aryl esterase was measured using different concentrations of the vanillin derivative, which was prepared (E)-N-(4-hydroxy-3-methoxybenylidene)-2-hydroxybenzohydrazide) after dilution, as an increase in the enzyme activity was observed with an increase in the concentration of the prepared derivative. It is shown in table (2) below, and this is consistent with previous studies that were conducted on mice with hypercholesterolemia, by treating them with a phenolic substance after diluting it, as it was observed that the activity of aryl esterase increased in the mice that were treated compared with the mice that were not treated with the substance. Phenolic compounds are considered antioxidants [54] that sweep away free radicals in the body and thus reduce the symptoms of diseases resulting from low activity of PON1, including atherosclerosis [55][56].

Table (2): Shows the effect of the prepared compound on the activity of aryl esterase (Normal Value = 130.91 U/L).

COMPOUND NAME	Concentration (mg/mL)	Activity	% of Activarion
Compound 1	500	339.34	259
	50	310.54	237
	5	278.91	213
	0.5	248.04	189
	0.05	189.47	144
	0.005	167.32	127
	0.0005	151.58	115

Conclusion

This study conclude that the increased aryl esterase activity against increasing the concentration of the prepared vanillin derivative. On the other hand, decreased level of aryl esterase, HDL and increased level of (cTnT, TG, VLDL, LDL) in patients with atherosclerosis compared to healthy people.

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