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RESEARCH ARTICLE
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Purification of alkaline phosphatase and measurement of some antioxidants in the serum of patients with high blood cholesterol

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ABSTRACT

This study included the purification of the alkaline phosphatase and the estimation of the level of antioxidants in the serum of people .Those with high cholesterol. 33 blood samples were collected from people with high cholesterol in the blood of both sexes, with ages ranging between (60-42 years), and 20 blood samples were collected from people with normal cholesterol levels for both sexes.

The alkaline phosphatase was also purified, as the degree of purification of the enzyme in the purification stage reached 2.7 times, with an enzymatic yield of 42.2%, and then using the gel filtration chromatography method using Sephadex G100 gel, as a single peak was obtained for the purified enzyme as ,with a degree of purification that reached 3.8 times and an enzymatic yield of 22%.

High cholesterol leads to cardiovascular disease. The results a Significat increase in activity of ALP ,Cholestrol ,Lipoprotiens, catales,uric acid.

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Keywords: *ALP, Hypercolestrolemia ,Antioxadinat*

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INTRODUCTION

Alkaline phosphatase [EC.3.1-3.1] belongs to the class of hydrating enzymes, as it has the ability to degrade many different types of organic phosphate esters to produce inorganic phosphates, alcohol, phenol or sugar in the alkaline environment (pH=9-10) (1). The alkaline phosphatase enzyme is one of the unspecialized enzymes as it reacts with several basic substances, and it is a very stable enzyme (2), as it maintains its activity for six months at 4 °C and in the presence of the buffer solution mM Tris-HCl, but when stored at 25 °C, It maintains its activity for ten (3) days. The basal phosphatase varies in molecular weight (4). The alkaline phosphatase enzyme is present in high concentrations in the liver, bones, intestines, placenta, kidneys, spleen, and white blood cells, but the main source for it is the liver and bones (5).

The increase in the level of cholesterol in the blood leads to the possibility of being exposed to oxidation inside the body, where it is deposited on the inner walls of the arteries, causing damage to the endothelial cells of the arteries as well as to other tissue cells (6). The role of oxidative stress in the occurrence and progression of cardiovascular disease has been proven. As the free radicals are released in quantities that exceed the body's ability to neutralize them, this leads to the oxidation of fatty substances, mainly cholesterol, and its deposition in the lining of the arteries, which leads to the emergence of many diseases related to the heart and blood vessels, including myocardial infarction (7) and antioxidants work It protects the body from oxidative stress by reducing the concentrations of free radicals and scavenging them, such as glutathione and super-oxide dismutase, or by preventing the formation of chain reactions that form free radicals, in addition to stopping their spread such as vitamin E and vitamin C (8). Studies have indicated the role of antioxidants in the treatment or prevention of heart disease and atherosclerosis. The researcher (9) Voutilainen and his group indicated that antioxidants work to prevent the oxidation of cholesterol and its deposition in the lining of the arteries, thus reducing the risk of coronary heart disease.

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MATERIALS AND METHODS

Determination of ALP concentration in the blood serum

I used the (method10). In estimating the activity of the base phosphatase enzyme in the serum, which includes the addition of phenyl phosphate, which is the basis for the enzyme's action, to release phenol as a result of the enzyme's effect on the base substance, and thus the red-colored quinone complex is formed, as the intensity of the color is directly proportional to the activity of the enzyme.

Separation and partial purification of ALP enzyme from serum of hyper cholesterol emia patients ALP enzyme was purified from serum of hyper cholesterol emia patients using the following steps (11)

Saturation of ammonium sulphate

Saturation of ammonium sulphate until reaching a saturation rate of 55%, as 2.75 gm of ammonium sulfate was added to 5 ml Serum proteins were precipitated using gradual concentrations of ammonium sulfate of serum during a period of (60 - 45) minutes by placing the serum in an ice bath with continuous stirring, and then it was Dissolve the precipitate in 4ml of 0.1M Tris-HCl buffer solution pH 7.2

Dialysis

It is one of the most important and oldest methods used in the purification of enzymes, and its purpose is to remove the residual ammonium sulfate added to precipitate the proteins by placing the dissolved protein in the above step in a dialysis bag after measuring the activity of the ALP enzyme and the concentration of the protein. The bag is immersed in 0.1M Tris buffer solution. HCl pH 7.2 The buffer solution was changed from time to time for a whole night. This step was performed at a temperature of 4°C to maintain the activity of ALP. After the membrane separation process, the activity of ALP and protein concentration were measured.

Gel filtration

The basis of the work of this technique is the difference in molecular weight, as it was used to purify the separated symmetry by gel filtration chromatography using Sephadex -G100 gel filtration column, collecting 20 Fractions, each Fraction 5 ml.

The level of cholesterol in the blood serum was estimated using a ready-made kit (Kit) supplied by the Syrian company Syrbio at a wavelength of 500 nm (12).

Also, the concentration of glutathione (GSH) Glutathione in the blood serum was measured using the modified method by (13) Sedlak. The method depends on the use of Almane solution, as it reacts with 5,5-dithio bis(2-Nitrobenzoic acid) DTNB (Ellmans reagent) to produce glutathione as a component. The absorbance is read at 412nm.

The amount of albumin was also estimated by using the Bromo cresol green method to be a colored precipitate on the albumin(14).

Estimation of the level of total protein concentration in the blood serum The Lowry method (15) was used to estimate the protein concentration in the blood serum.

The concentration of uric acid in the blood serum was estimated by using ready-made kits by enzymatic colorimetric method Where the mean and standard deviation were found between the study groups, and the t-test was used to compare the results between the groups.

RESULTS AND DISCUSSION

The figure below shows an increase in the activity of ALP enzyme in the blood of people with hyper cholesterolemia compared to the control group at the level of probability ($P \geq 0.01$) and as in the figure below.

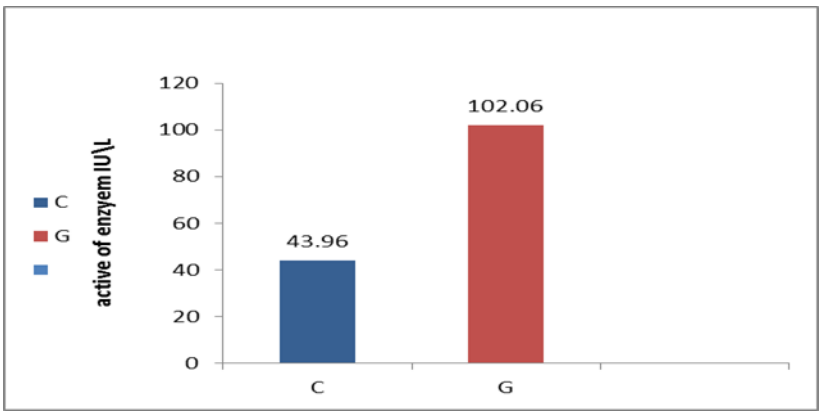

FIG 1: The ectivetty of ALP enzyme in the serum of hypercholesterolemia patients compared to the control group.

The results of the current study are in agreement with what was reached by (Snoring and Abbas) (17), where elevated ALP enzyme was found in the serum of patients with high lipoproteins. This increase in the effectiveness of ALP is usually due to many pathological conditions such as diseases of the liver or bile ducts or the presence of gallstones, and high ALP may indicate bone diseases such as Osteopenia or Osteoporous Blood disorders also cause an increase in ALP (18).

partical purification

The process of partial separation and purification of ALP enzyme from patients' sera was carried out in several stages. In the first purification steps, the enzyme was precipitated using ammonium sulfate salt with a saturation concentration of 55% to concentrate the enzyme and obtain a degree of purity. Excess salt was removed during the Dialysis process by Tris-HCl with pH 7.2 The degree of enzyme purification at this stage was 2.7 times, with an enzymatic yield of 42.2% as shown in Table (1). Then the purification of the enzyme was completed using the gel filtration chromatography method using Sephadex- G100, as a single peak was obtained for the purified enzyme as shown in Figure (2). , with a degree of purification that reached 3.8 times and an enzymatic yield of 22% as shown in Table (1)

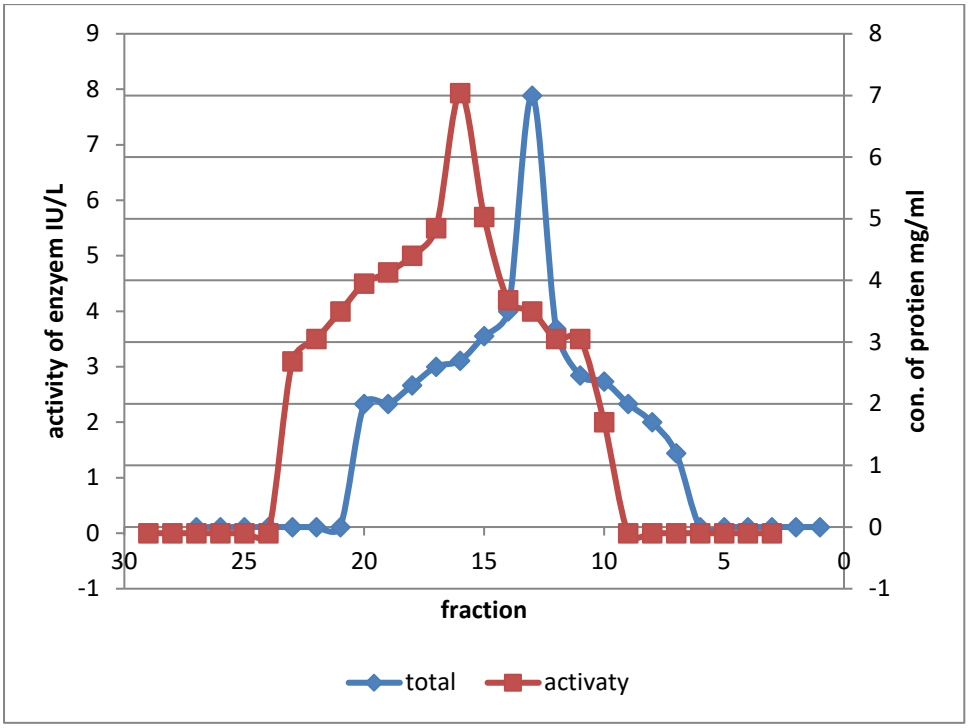

FIG 2: Purification of ALP by gel filtration chromatography

TABLE 1: Partical purification of ALP from serum of hypercholesterolemia patients

Step	Elute (ml	Activity (I U/L	Total activity I U)	Protein conc. (mg/ml	protein mg)	Specific activity I U/mg	Purification)Fold	Yield %
Crud serum	6	360	2.16	73.6	284.48	0.00198	-	100
Ammonium sulphate	5	75	0.375	23.54	117.7	0.00318	1.6	66.4
Dialysis	4	59.6	0.238	14.32	57.3	0.0041	2.07	42.2
(Gel filtration) Sephadex G100	5	24.9	0.124	3.24	16.2	0.0076	3.8	22

TABLE 2: the concentration of cholesterol and some values of antioxidants in the serum of patients with hyper cholesterolemia compared to the control group

Parameter	Control	Pat.	p≤
Total cholesterol mg/100ml	138.33±5.35	329.34±49.28	0.05
CAT (U/mg)	28.34 ± 2.67	37.37 ± 1.78	0.05
GSH µmoll/L)	5.29±1.05	3.62±0.95	0.05
Total protein(mg\L)	5.1±1.79	9.7±1.7	0.05
Albumin (mg\L)	5.46±1.1	3.9±1.4	0.05
Uric acid(mg\dl)	3.82±0.59	4.12±1.18	0.05

The above table shows an increase in cholesterol in the group of patients in comparison with the healthy subjects at the probability level p≤0.05, as well as the increase in the total protein and uric acid in the group of patients compared with the healthy ones at the probability level p≤0.05. From the above table, glutathione and albumin decreased in the group of patients compared with the healthy ones at the probability level p≤0.05.

Decreased glutathione in the blood serum of these people compared to the control group, this decrease In glutathione means an increase in the state of oxidative stress and free radicals for these patients and this increase works to destroy and break down cell membranes (19) .

As for the low concentration of glutathione in the blood serum of people with high blood cholesterol, it is due to the effective participation of glutathione in preventing oxidation in case of oxidative stress, either by direct removal of free radicals or by being a basis for some antioxidant enzymes such as glutathione peroxidase(20).

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