Study of The Effect of Boswellia Leaf Extract on The Activity of Cardiac Enzymes (ALP, AST, ALT, LDH) Using Spectroscopic Methods

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Abstract. This study investigates the impact of Boswellia carterii leaf extracts on serum enzyme activity in myocardial infarction (MI) patients. Employing a comparative analysis between 60 healthy individuals and 40 cardiac patients, we measured the serum activity of ALP, AST, ALT, and LDH enzymes. Our findings reveal elevated enzyme activity in MI patients. We explored the inhibitory effects of aqueous, alcoholic, and ethyl acetate extracts of Boswellia carterii on these enzymes, demonstrating non-competitive inhibition. The inhibition ratio of ALT, ALP, and AST post-treatment was quantitatively assessed. Statistical analysis was conducted using SPSS-26 with independent t-tests. This research highlights the potential therapeutic role of Boswellia carterii in modulating enzyme activity associated with cardiac events, suggesting avenues for novel treatments in cardiac care.

Keywords: Enzymes, Boswellia Carterii, Inhibition, Myocardial Infarction

I. INTRODUCTION

The occurrence of a heart attack, medically known as a myocardial infarction, arises from the build-up of plaque within the arteries, resulting in a reduced blood supply to the heart. Consequently, the heart's muscles suffer from a deficiency of oxygen. Warning signs of a heart attack include shortness of breath, perspiration, nausea, vomiting, an irregular pulse, fatigue, weakness, stress, and depression. Additionally, individuals may experience chest pain that extends to the left arm and neck. It is crucial to note the significance of enzymes, high molecular weight proteins that serve as essential catalysts in the body[1]. Enzymes possess remarkable versatility and efficiency, enabling them to facilitate reactions at a significantly faster rate than chemical catalysts[2]. As described in Silverman's book, The Enzyme Revolution, enzymes exhibit exceptional proficiency in organic chemistry[3]. Notably, individuals who have suffered a heart attack display notably elevated levels of AST and ALT enzymes. Additionally, the research findings indicated that individuals with elevated AST and LDL levels had a significantly higher likelihood, approximately 24 and 7 times respectively, of experiencing a myocardial infarction compared to the control group. Furthermore, a heightened ALT level has been identified as a key factor associated with an increased risk of mortality in hospital settings for patients undergoing a heart attack.. [4]. The liver, being a vital organ with a complex circulatory system and high metabolic activity, is particularly sensitive to changes in blood flow. It has been observed that every 10 mm Hg decrease in arterial blood pressure leads to a 10% reduction in the flow of blood through the hepatic artery. Consequently, patients who have had a heart attack often show abnormal levels of transaminases in their blood. In recent times, a number of studies have discovered a connection between higher levels of transaminases in the blood and unfavorable outcomes for patients suffering from acute myocardial infarction[5]. The severity of liver damage might directly influence the prognosis for the heart, especially in individuals who have previously experienced metabolic syndrome. Numerous theories have been proposed to explain the association between elevated ALT levels and an increase in all-cause mortality during hospitalization for acute myocardial infarction. For instance, it is known that the liver exhibits a high level of metabolic activity and blood flow. Sudden changes in circulation have the potential to immediately impact the flow of blood to the liver, leading to an increase in ALT and AST levels. This can occur, for instance, when there is a sudden drop in blood pressure caused by a myocardial infarction[6]. The enzyme LDH (lactate dehydrogenase) plays a crucial role in the transfer of hydrogen atoms. Specifically, it facilitates the conversion of L-lactate to pyruvate, which represents the final step in the metabolic pathway known as anaerobic glycolysis. The equilibrium of this reaction strongly favors the reverse process, where pyruvate is reduced back to lactate. This reversibility arises from the nature of the reaction itself.. [7]

II. METHODS

Breeding the fly in the laboratory

From Company Biolabo France, all kits for enzymes used were obtained. The method discussed earlier by H. A Hashem and M Z Thani [8] was applied to this work using the extracts..

Patients and controls:

During the period from September 2022 to March 2023, blood samples were collected at the Ghazi Hariri Hospital in Baghdad, Iraq. The purpose of this study was to gather data on patients who had experienced myocardial infarctions, as confirmed by specialists. A total of 40 samples were obtained from these patients, while an additional 60 samples were taken from healthy individuals who formed the control group. The detailed information pertaining to this research can be found in Table 1.

Table 1. The data from the groups under study

Group	No. of cases	Age (year)	Females	Males	Mean±SD
Patients	40	29-70	10	30	40.17±9.81
Control	100	30-67	12	88	38.23±9.20

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Obtaining blood samples

I took five milliliters of blood from a vein using a plastic syringe with a scale of twenty-one stainless needles. To ensure cleanliness, I sterilized the area before drawing the blood. Then, I carefully placed the blood into a simple and clean gel tube. The next step was to separate the serum from the blood using a centrifuge at a temperature of 25°C for ten minutes. Once separated, I divided the serum into three portions using an exact absorber and transferred each portion into Eppendorf tubes. Finally, I stored these tubes at a freezing temperature of -20°C..

Determination of enzyme activity:

In the samples gathered, the following enzyme activities were examined: ALT, AST, ALP, and LDH.:
Using techniques, the activity of (ALT, AST, ALP, and LDH) was found. … [9],[10],[11],[12], and [13] respectively.

Determination of suppression type:

Inhibitor preparation (stock solution):

Inhibitor was taken, dissolved in Dimethyl Sulfoxide (DMSO), and prepared a five-fold dilution of stock solution with 100 mg and 100 ml..

Suppression type

All of the enzyme kits used in this experiment were from Company Biolabo France, and the extracts were prepared using the technique previously outlined by [7].

III. RESULT AND DISCUSSIONS

Chemistry Results:

Enzyme's activity:

In 40 patients with myocardial infarction and 100 healthy controls, Table 2 unveils the findings from an investigation into the levels of AST, ALT, ALP, and LDH..

Table 2. Serum enzyme levels in both the patient and the control group

Parameters	Control		Patients		P-value
	Mean	SD	Mean	SD	
N	100	-	40	-	-
LDH (U/L)	327.54	76.48	442.62	17.11	<0.001*
AST(U/L)	8.46	0.98	33.67	11.27	<0.00*
ALT(U/L)	13.91	1.66	29.52	11.40	<0.00*
ALP(U/L)	6.69	2.14	15.49	2.63	<0.00*

1- Investigation of the AST Enzyme:

The serum activity of AST in myocardial patients (33.670±11.27 U/L; P<0.001) was significantly higher than that of healthy controls (8.460±0.98 U/L), as Figure (1) illustrates.
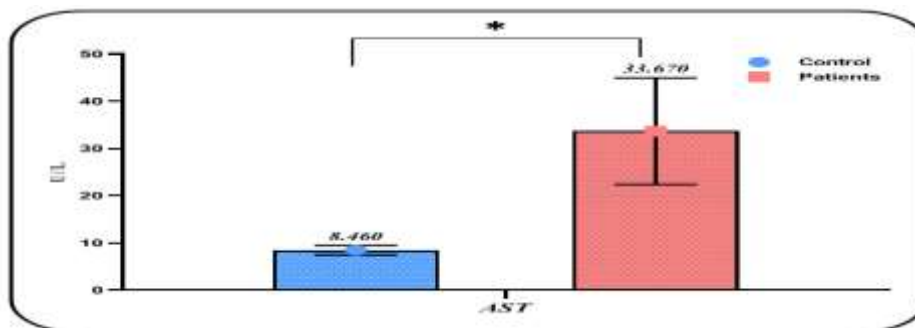

Figure (1): The average and fluctuation of AST in the groups under treatment and the control group

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While the liver produces the majority of AST, which is frequently employed as a measure of liver function, other tissues, including the heart, also produce a significant amount of AST [14]. This result is in line with numerous investigations that discovered patients with myocardial infarction had increased AST levels [15]. Moreover, it has been proposed that AST and ALT should be regarded as distinct predictors. This rise results from decreased blood flow brought on by coronary artery obstruction or constriction, which ruptures particular heart muscle cells. This causes a slow rise in the amounts of specific proteins and an enzyme called AST that is found in the cytoplasm to be released into the bloodstream [16].

2- Study of ALP Enzyme:

In Figure (2), it is observed that the ALP activity in serum of patients with myocardial issues (15.95±2.63 U/L) was markedly elevated compared to the levels in healthy controls (6.69±2.14 U/L) (p<0.001).
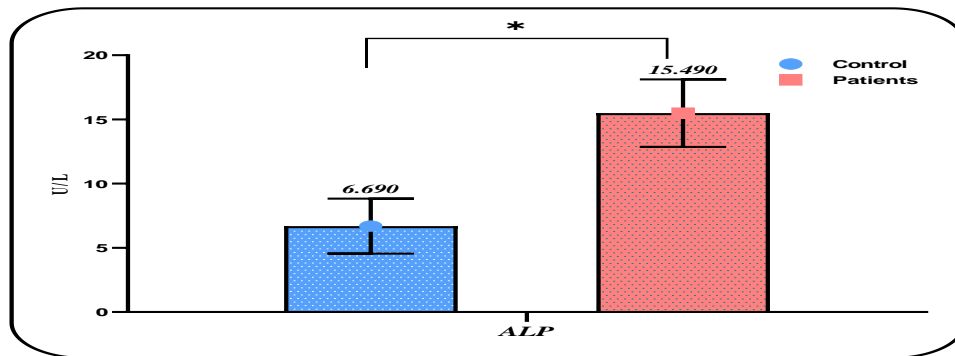.

Figure (2): ALP mean and SD for control and patients

In certain organs that have strong abilities to excrete or absorb substances, the enzyme ALP can often be found in the cell membranes. Some examples of these organs include the cells in the saliva glands, breast milk ducts, bone marrow, the border of the small intestine, cells in the placenta's villi, membranes that protect bile ducts, liver sinusoids, and kidney tubules[16]. The ALPL gene on chromosome 1 is responsible for encoding the ALP enzyme, which plays a crucial role in bone mineralization. It acts as a molecule attached to the membrane and helps in breaking down monophosphate esters before hydroxyapatite is produced. Ever since its initial discovery, alkaline phosphatase has proven to be much more than a mere enzyme associated with bones. Its functions have transcended the confines of the skeletal system, as evidenced by the identification of its presence in the intestine, placenta, and liver. Remarkably, recent research has even unveiled a connection between alkaline phosphatase and coronary artery disease (CAD). For instance, a study involving 3567 individuals who had survived a heart attack revealed that elevated levels of alkaline phosphatase were correlated with an increased risk of mortality from cardiovascular disease (CVD) and all other causes combined [17].. This particular investigation focused solely on mortality as an endpoint. Additionally, alkaline phosphatase was also evaluated as a potential prognostic indicator for patients undergoing percutaneous coronary intervention (PCI). The research discovered that ALP was found to have a separate association with the risk of heart and brain-related events, as well as non-fatal heart attacks. This connection remained even after considering all known factors that contribute to cardiovascular disease[18]. When ALP levels were higher, it indicated a lack of blood flow in the coronary arteries, which meant excluding a particular group of people who were more at risk for the effects of heart attacks. Ultimately, it was shown that ALP was linked to a higher likelihood of calcium build-up in the coronary arteries, which was observed in all stages of coronary artery disease. This connection highlights the potential for developing reliable prediction models while also demonstrating how atherosclerosis and bone remodeling intersect[19]..

3-Study of ALT Enzyme:

In myocardial patients, the serum activity of ALT was considerably higher (P<0.001) compared to healthy controls, as shown in Figure (3), with values of 29.520±11.40 U/L and 13.910±1.66 U/L, respectively..
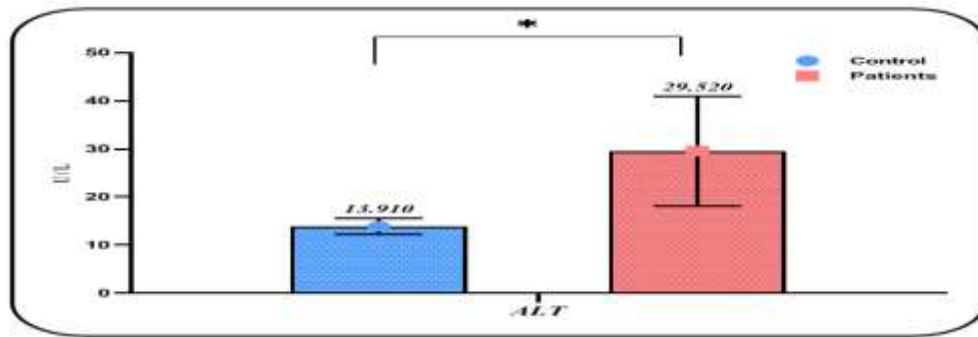
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Figure (3): ALT standard deviation and mean in both the patient and control groups

The results of the study showed that AST and ALT levels were considerably greater in those who had suffered a heart attack. Additionally, their results demonstrated that, in comparison to the control group, patients with raised AST and LDL levels had a roughly 24- and 7-fold greater risk of heart attack [20]. In hospitalized patients experiencing an acute heart attack, high ALT levels were found to be a significant signal linked to an increased risk of death. The liver is a vital organ that is highly sensitive to changes in blood flow and metabolism due to its intricate circulatory system. For every 10 mm Hg drop in arterial blood pressure, there is a 10% reduction in hepatic arterial blood flow [4]..

4- Study of LDH Enzyme:

At (442.62±17.11 U/L), the serum LDH activity of myocardial patients in Figure 5 was considerably higher (P ≤ 0.001) compared to the controls' LDH activity of (327.54±76.48 U/L).
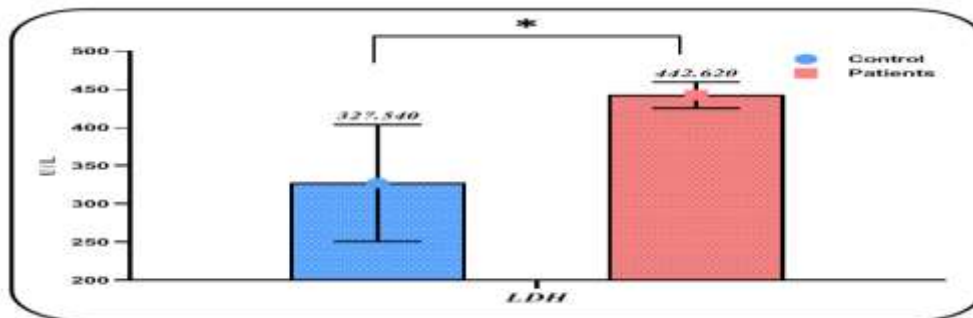

Figure (4): The LDH level mean and standard deviation of the patients compared to controls

The effectiveness of lactate dehydrogenase, a crucial enzyme, can potentially increase during a heart attack. This may be due to the damage inflicted on the heart cells, causing the release of the enzyme into the bloodstream and consequently enhancing its efficacy. According to research [21], LDH is particularly potent in the serum following a myocardial infarction, with its effects becoming noticeable after 4-12 hours and reaching their peak after 44 hours. This finding holds significant value in clinical settings, as it can serve as an important indicator for individuals seeking medical assistance for a heart attack. Given the vital role of this enzyme in carbohydrate metabolism, it is worth noting that zinc-containing compounds are widely present in various organisms such as vertebrates, invertebrates, mammals, and even microorganisms [22].. When the enzyme is present, lactate is converted to pyruvate (a "forward reaction") and DPN is decreased to DPN.On the other hand, the enzyme is responsible for the "reverse reaction," which involves the process that converts pyruvate to lactate and the oxidation of reduced DPNH to DPN. Thus, enzyme activity can be evaluated in either way depending on the starting substrates employed, appropriate pH conditions, and other factors; many clinics have chosen to use one approach over the other [23]. Additional studies showed that people with acute myocardial infarction had significantly higher serum LIDH activity. This discovery forms the basis for the broad application of this laboratory support in the identification of heart disease as it has been sufficiently verified and extended. At some point

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between 12 hours and 10 days following myocardial infarction, serum LDH activity was markedly raised above normal in an exceptionally high percentage of typical cases of myocardial infarction—up to 100% in specific series.By the eighth to fourteenth day, enzyme activity had gradually returned to normal. It was frequently elevated over the first 12 to 24 hours, peaking in 3 to 4 days. All tissues involved in glycolysis contain the cytosolic enzyme lactate dehydrogenase, which has five different isoforms called LDH1–LDH5. In cardiac tissue, LDH1 and LDH2 are the most common. Because of this, measuring elevated levels of this enzyme that are discharged into the bloodstream from damaged tissue has emerged as a reliable diagnostic and prognostic factor for a range of diseases and conditions. Additionally, studies on its isoenzymes have proven beneficial in pinpointing the location of tissue damage [6].

Types of inhibition:

Weaver Burke was intrigued by the need to understand the type of inhibition displayed by enzyme prevention. The outcomes of the models revealed that the inhibition of ALT and AST enzymes by aqueous and alcohol extracts, respectively, followed a non-competitive pattern. Non-competitive inhibition occurs when inhibitors at the allosteric site interact with substrate binding independently; this suggests that both the inhibitor and the enzyme-substrate complex converge at the same point. This convergence leads to the formation of an enzyme inhibitor compound (EI) as the non-competitive inhibitor combines with an ES chemical to create an ESI. The following elucidates the mechanism behind non-competitive inhibition[7]. The AST enzyme was inhibited by aqueous extract, while the ALP enzyme was inhibited by ethyl acetate extract. These types of enzyme inhibition are known as noncompetitive because they only bind to the complex that forms between the substrate and the enzyme, known as the E-S complex. In most processes, inhibitive behavior that is not competitive happens when there are two or more substrates or products involved. In addition, when molecules that are quite similar to the substrate molecules occur. An ALP enzyme inhibition that is competitive exists in aqueous extracts; inhibition of the ALT enzyme by ethyl acetate extract; inhibition of the ALP and AST enzymes by alcohol extract.**Table (3): The kinetic properties of ALT regarding Ethanol Extraction.**

Table (3): The kinetic properties of ALT regarding Ethanol-Extracted Alcohol

ALT patient with compound Ethanol			
conc.(mol/L)	patient without comp.	patient within comp.	%Inhibition
without Eth	89	—	—
$1*10^{-1}$	—	21	76
$1*10^{-2}$	—	33	63
$1*10^{-3}$	—	61	31

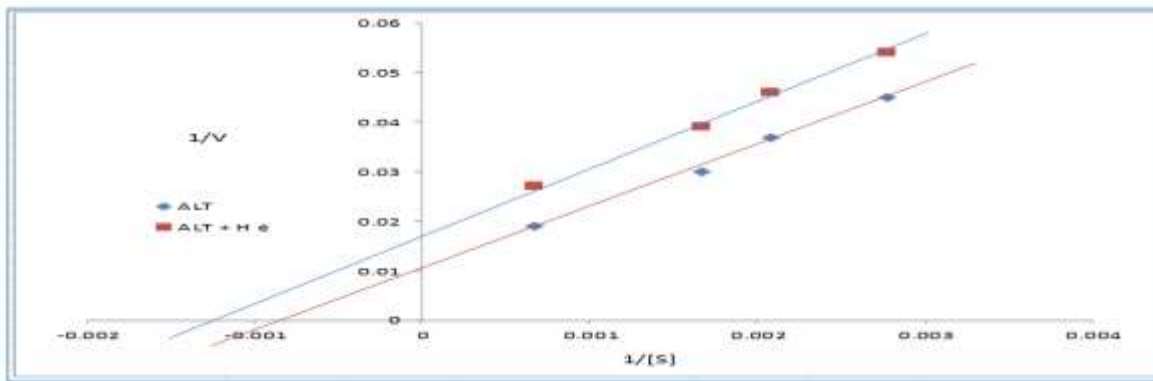| 1/[s]	ALT	ALT + Eth
0.001667	0.03	0.036
0.00067	0.019	0.029
0.002083	0.0368	0.044
0.002778	0.045	0.056

Figure (5): The effect of the ethanol extract on ALT activity in the sera of patients withMI is represented by a Line Weaver-Burk plot (Non-competitive inhibition).

Table (4): The kinetic properties of AST in relation to Ethanol-Extracted Alcohol

AST patient with compound Ethanol			
conc.(mol/L)	patient without comp.	patient within comp.	%Inhibition
without comp. Eth	90	—	—
$1*10^{-1}$	—	22	72
$1*10^{-2}$	—	40	54
$1*10^{-3}$	—	66	29

1/[s]	AST	AST+ Ethanol Ex
0.003086	0.039	0.081
0.003472	0.034	0.087
0.0053	0.051	0.121
0.0071	0.064	0.147
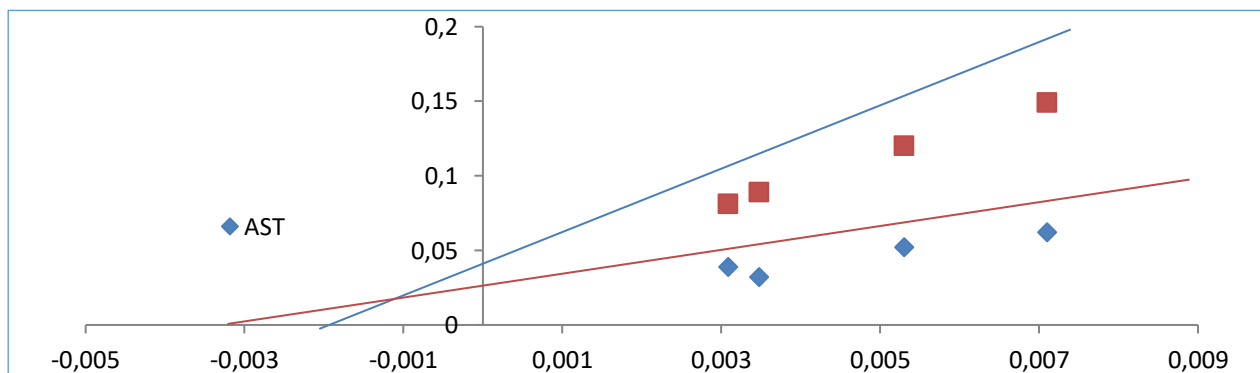
Figure (6): The effect of the ethanol extract on the AST activity in patient sera that are affected byMI is shown using a Line Weaver-Burk method

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Table (5): The kinetic properties of ALP regarding Ethanol-Extracted Aldrin

ALP patient with compound Ethanol			
conc.(mol/L)	patient without comp.	patient within comp.	%Inhibition
without Ethanol	189	—	—
$1*10^{-1}$	—	41	78
$1*10^{-2}$	—	89	53
$1*10^{-3}$	—	112	40

1/[s]	ALP	ALP + Ethanol Ex
0.006	0.0086	0.019
0.008889	0.0105	0.024
0.011429	0.0125	0.028
0.016	0.01	0.038
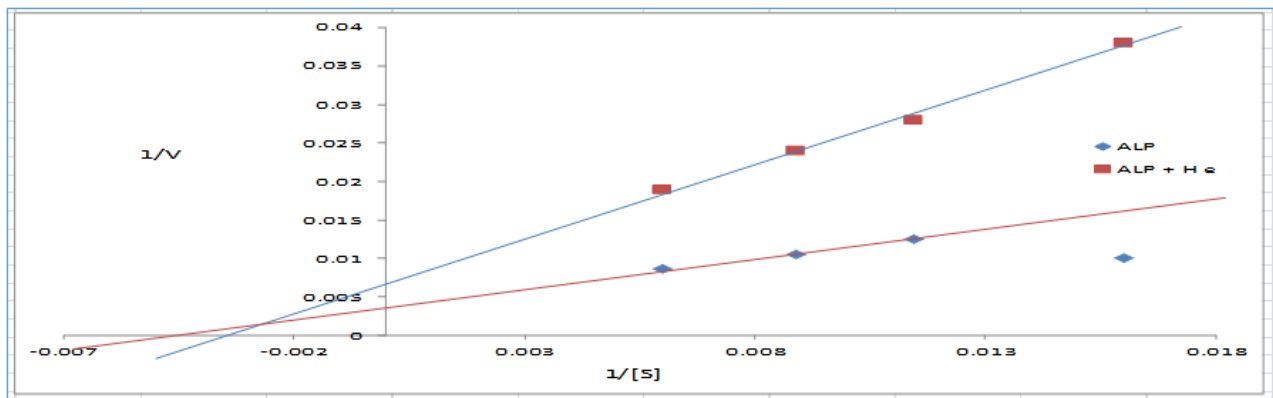
Figure (7): The effect of the ethanol extract on the activity of ALP in the serum of patients withMI was documented using a Line Weaver-Burk plot (Type of mixture inhibition)

Table (6): The kinetic properties of ALT regarding ethyl acetate extraction

ALT patient with compound Ea			
conc.(mol/L)	patient without comp.	patient within comp.	%Inhibition
without Ea	89	—	—
$1*10^{-1}$	—	34	62
$1*10^{-2}$	—	40	55
$1*10^{-3}$	—	52	42

| 1/[s]	ALT	ALT+Ea
0.0009	0.023	0.061
0.003086	0.048	0.095
0.003472	0.055	0.14
0.0053	0.071	0.17
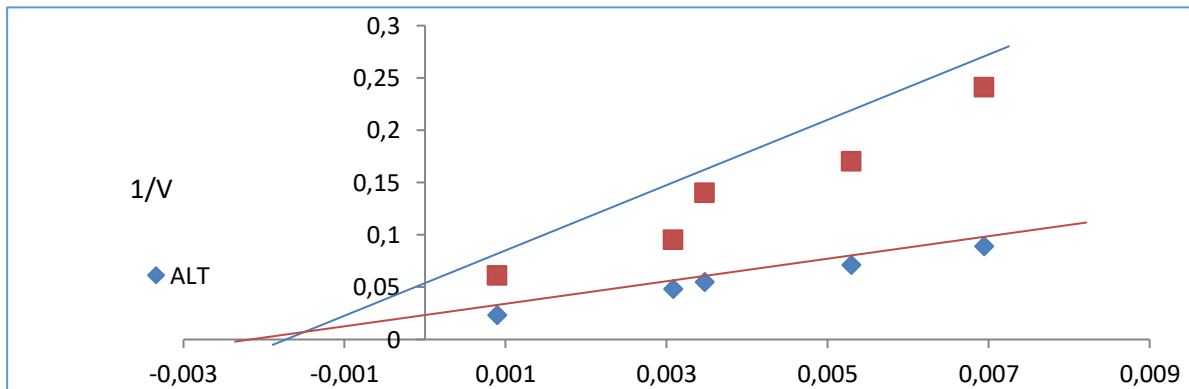
Figure (8): The line weaver-burk plot's effect on the activity of ALT in the sera of patients withMI was examined (Type of Inhibition).

Table (7): The kinetic properties of AST regarding ethyl acetate extraction

AST patient with compound Ea			
conc.(mol/L)	patient without comp.	patient within comp.	%Inhibition
without Ea	90	—	—
$1*10^{-1}$	—	36	62
$1*10^{-2}$	—	47	48
$1*10^{-3}$	—	63	33

1/[s]	AST	AST +Ea
0.0009	0.04	0.056
0.003086	0.038	0.081
0.003462	0.036	0.093
0.0054	0.044	0.14
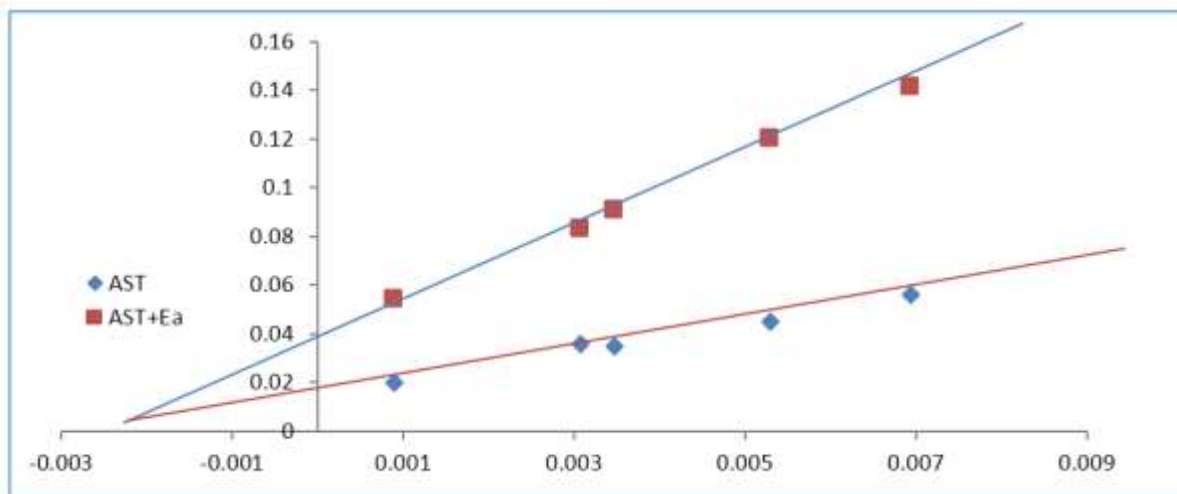
Figure (9): The line weaver-burk plot's effect on the activity of AST in the sera of patients withMI was Non-competitive (inhibition)

Table (8): The kinetic properties of ALP regarding ethyl acetate extraction

| conc.(mol/L) | ALP patient with compound Ea | | |
	patient without comp.	patient within comp.	%Inhibition
without Ea	187	—	—
$1*10^{-1}$	—	66	63
$1*10^{-2}$	—	86	57
$1*10^{-3}$	—	124	38

1/[s]	ALP	ALP+Ea
0.004	0.054	0.067
0.008889	0.075	0.092
0.011429	0.091	0.111
0.016	0.11	0.15
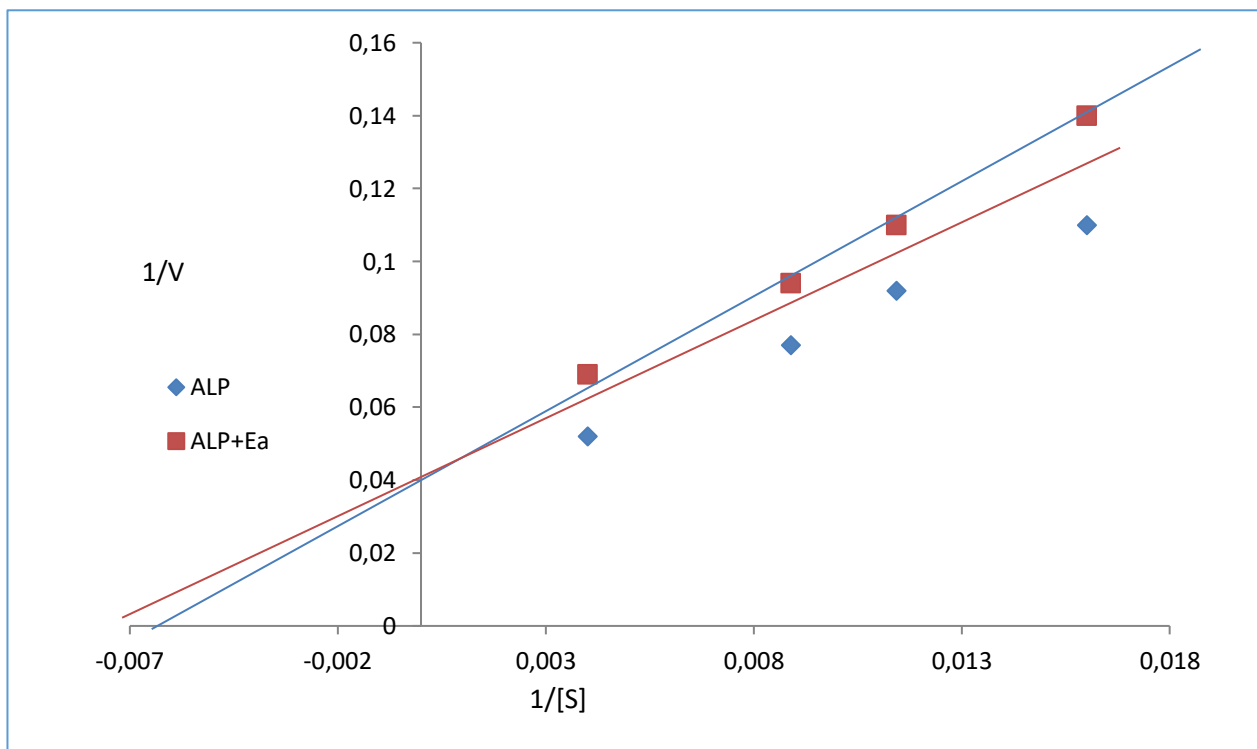
Figure (10): Line Weaver-Burk's plot of ethyl acetate's effect on ALP activity in the sera of patients withMI

(Type of Inhibition)

Table (9): The ALT's kinetic characteristics with reference to aqueous extracts

ALT patient with compound aqueous			
conc.(mol/L)	patient without comp.	patient within comp.	%Inhibition
With-out aq	87	—	—
$1*10^{-1}$	—	43	56
$1*10^{-2}$	—	56	36
$1*10^{-3}$	—	65	26

1/[s]	ALT	ALT+aq
0.001667	0.05	0.037
0.00067	0.017	0.029
0.002083	0.039	0.044
0.002778	0.047	0.056
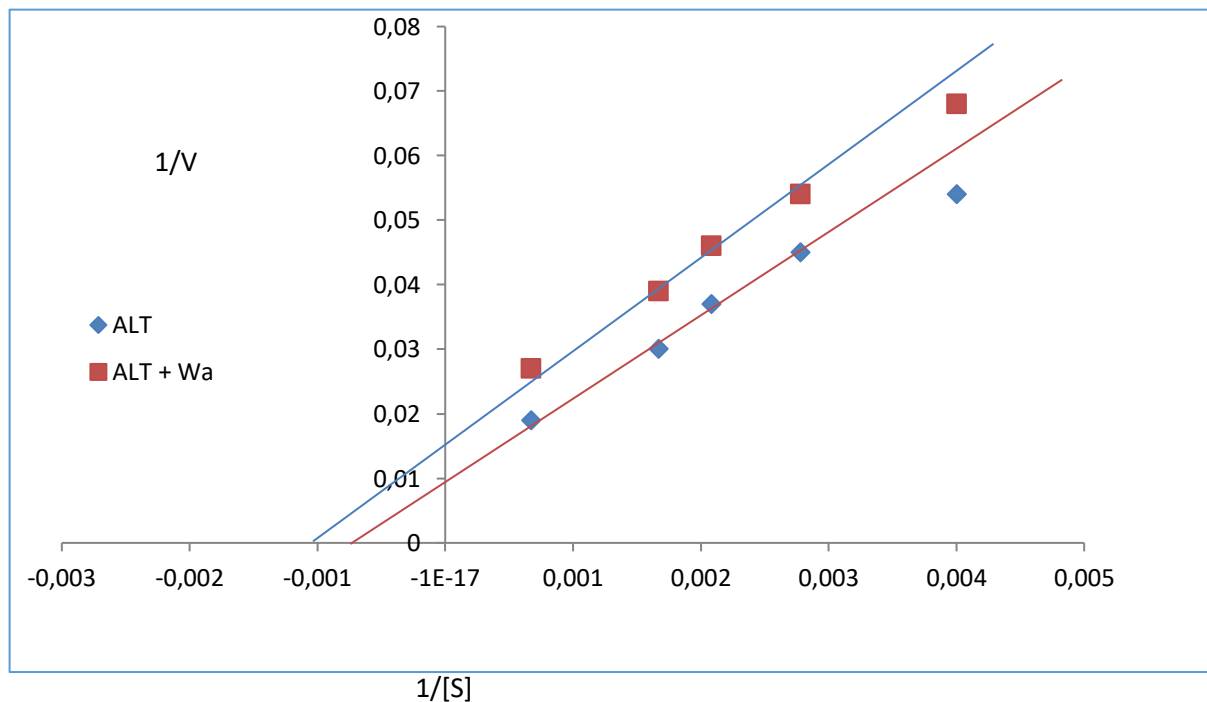
Figure (11): The line weaver-burk plot demonstrates the effect of aqueous extracts from plants on the activity of ALT in the sera of patients withMI (UN-competitive blocking).

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Table (10): The kinetic properties of AST in water are described in this section..

AST patient with compound aqueous			
conc.(mol/L)	patient without comp.	patient within comp.	%Inhibition
without aq	92	—	—
$1*10^{-1}$	—	46	50
$1*10^{-2}$	—	61	33
$1*10^{-3}$	—	71	22

1/[s]	AST	AST+aq
0.0009	0.02	0.054
0.003086	0.036	0.081
0.003472	0.032	0.089
0.0053	0.049	0.12
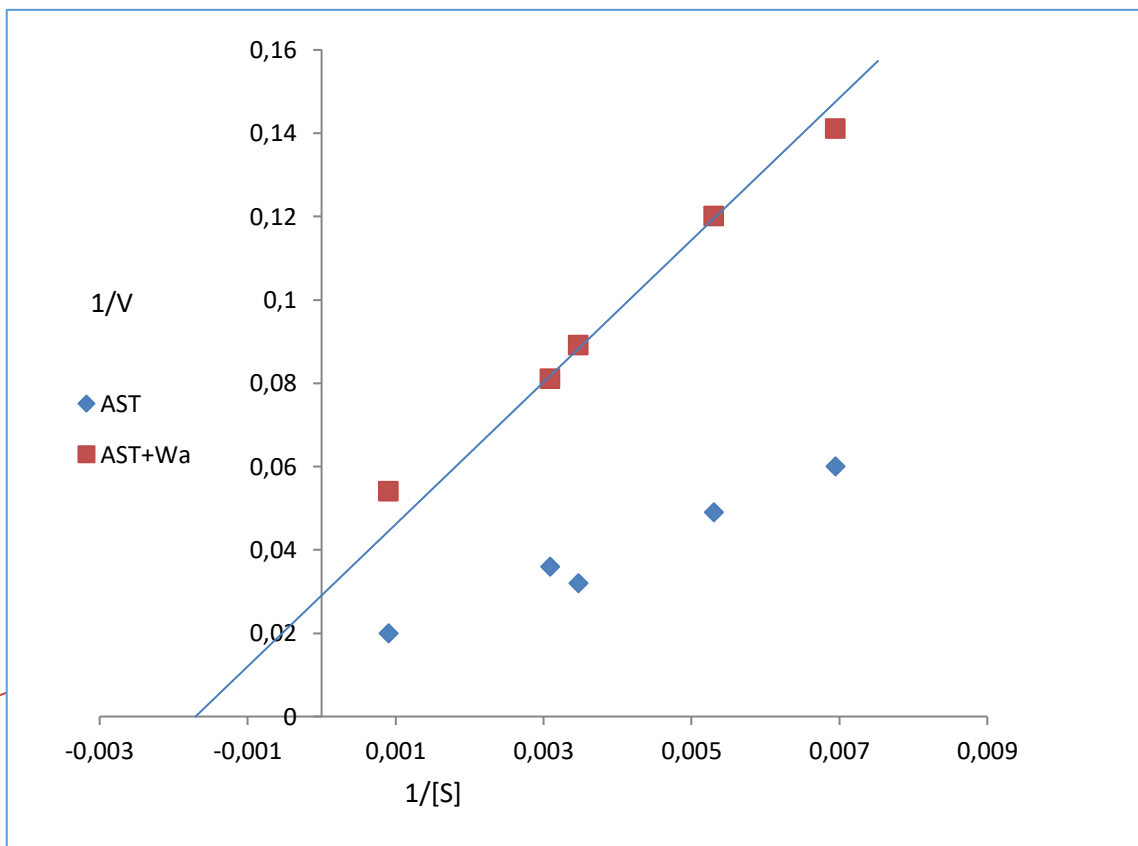
Figure (12): The effect of aqueous extracts on AST activity in the sera of MI patients (Type of Inhibition) **is** plotted using the Line Weaver-Burk method.

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Table (11): The ALP's kinetic characteristics with reference to aqueous extracts..

ALP patient with compound aqueous			
conc.(mol/L)	patient without comp.	patient within comp.	%Inhibition
without aq	187	—	—
$1*10^{-1}$	—	97	46
$1*10^{-2}$	—	125	37
$1*10^{-3}$	—	136	29

1/[s]	ALP	ALP+aq
0.006	0.009	0.03
0.008889	0.0107	0.026
0.0011429	0.013	0.028
0.016	0.01	0.37
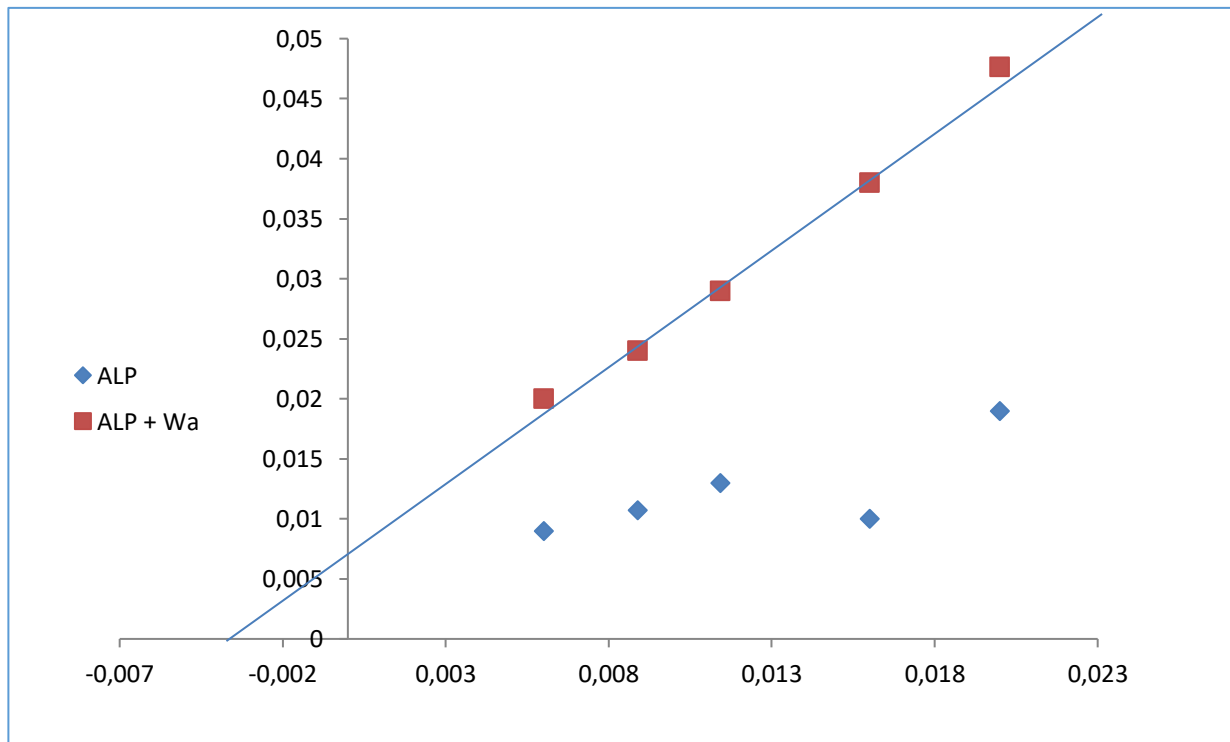
Figure (13): An illustration of the impact of plant-based aqueous extracts on the activity of ALP in the sera of MI patients using the Line Weaver-Burk plot (competitive blocking).
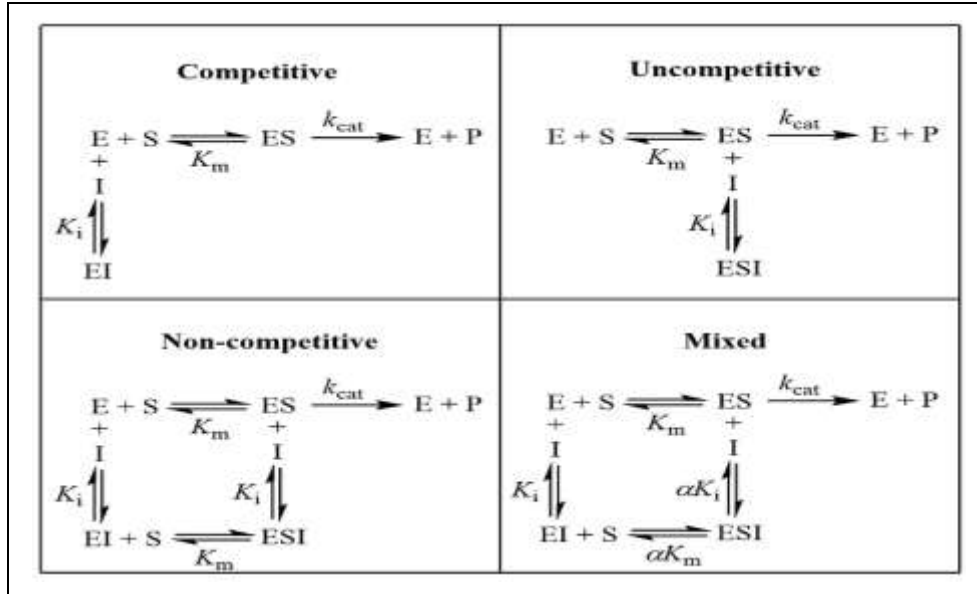
Figure (14): types of enzyme inhibition

The association between parameters (ALT, AST, ALP, LDH):

Table (12): The association between different parameters in patients

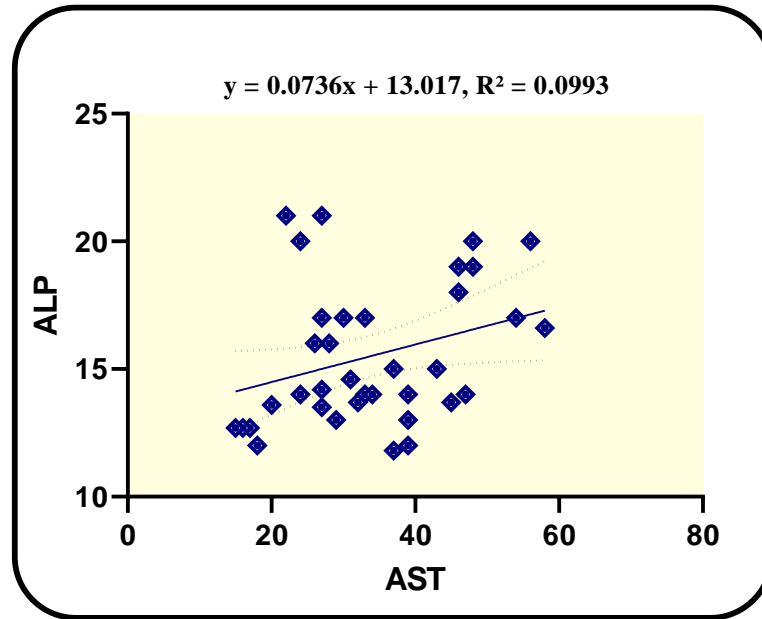|	Age	LDH	AST	ALT	ALP
Age	1	0.083	0.225	0.191	0.260
P- Value		0.609	0.162	0.239	0.105
LDH	0.083	1	-0.010	0.119	-0.070
P- Value	0.609		0.950	0.466	0.667
AST	0.225	-0.010	1	-0.062	0.315*
P- Value	0.162	0.950		0.704	0.048
ALT	0.191	0.119	-0.062	1	0.232
P- Value	0.239	0.466	0.704		0.149
ALP	0.260	-0.070	0.315*	0.232	1
P- Value	0.105	0.667	0.048	0.149	

Figure (15): The association between AST and ALP in patients

Based on the data presented in Table (11), it is evident that the enzymes AST and ALT have a strong and meaningful relationship. This connection is highly significant, with a p-value less than 0.05. The correlation between these parameters is particularly robust, measuring at $r = 0.315$. Furthermore, the p-value for this correlation was determined to be 0.048. Conversely, the other parameters examined did not demonstrate any noteworthy or substantial associations, as they were deemed non-significant...

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Table (13): The results of the ROC

Parameters	AUC	SE	*P*-value	Cut off value	Sensitivity	Specificity
LDH	0.951	0.018	<0.001	214	100 %	80 %
AST	0.951	0.018	<0.001	7.5	100 %	82 %
ALT	1.000	0.0001	<0.001	11.75	100 %	91 %
ALP	0.984	0.008	<0.001	4.5	100 %	90 %

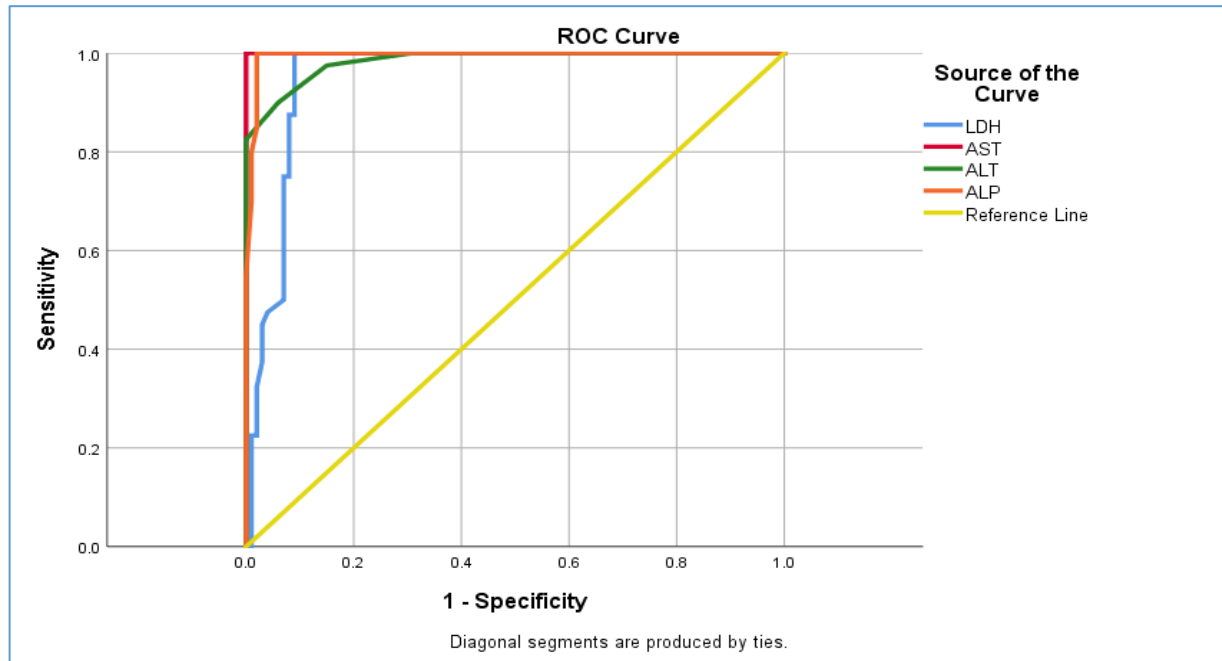* Significant at P≤ 0.05, NS: Non-Significant .

Figure (16): The predicted course of the disease based on the ROC of the tested enzymes.

The findings from the ROC analysis, as presented in Table (12), demonstrate that out of all the parameters considered ALP is the most reliable in terms of diagnostic accuracy. The enzyme ALT exhibits the largest area under the curve (AUC), with an AUC value of 1.000, indicating its exceptional sensitivity (100%) and specificity (99%). Although AST and LDH have lower AUC values, it is worth noting that LDH also has lower specificity, which contributes to its diminished diagnostic reliability..

IV. CONCLUSION

The findings of this study indicated that the water-based extract functioned as a suppressor of LDH, AST, ALT, and ALP enzymes. On the other hand, the alcoholic extract acted as a suppressor of LDH and did not have any impact on ALT and ALP enzymes. Additionally, the study revealed the specific type of inhibition exhibited by these extracts, with the results indicating a non-competitive inhibition pattern. These results were consistent with numerous previous studies that explored the utilization of Boswellia carterii for acute myocardial ischemia (IM).

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