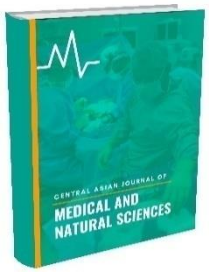
CENTRAL ASIAN JOURNAL OF MEDICAL AND NATURAL SCIENCES



Volume: 04 Issue: 05 | Sep-Oct 2023 ISSN: 2660-4159

http://cajmns.centralasianstudies.org

Study of the Effect of Ginkgo Biloba Leaf Extracts on the Activity of Cardiac Enzymes LDH, CK-MB using Spectroscopic Methods

1. **Heyam A. Hashim**
2. **Aseel Khalil Ibraheem**
3. **Shaymaa Saadi Abbood**

Received 2nd Aug 2023,
Accepted 19th Sep 2023,
Online 27th Oct 2023

[1,2,3] Iraqi Atomic Energy Commission, Central Laboratories Directorate, Iraq

Abstract: Acute cessation of coronary blood flow leads to myocardial necrosis, which is referred to as myocardial infarction (MI). Myocardial infarction always occurs due to atherosclerosis. The present study is designed to investigate cardiac effects from aqueous and alcoholic ginkgo leaf extracts on human serum in patients with myocardial infarction. This study was conducted between December 2021 and March 2022. Clinical tests included the inclusion of (60) patients diagnosed with myocardial disease and (40) others who were in good health as an officer group. The serum of these samples was analyzed for activity (ALP, AST, ALT, LDH, and CK-MB) using spectroscopy methods. Then the extracts of Ginkgo Biloba leaves (aqueous and alcoholic) were applied to each patient's serum and the LDH, and the CK-MB inhibition ratio was measured to verify their effects, and the results showed a rise in the activity of the five enzymes of myocardial infarction patients, with good inhibition rates for both extractors on the effectiveness of enzymes (LDH, CK-MB) and the type of inhibition was non-competitive. SPSS-26 was used for intermediate comparisons by independent t-test.

Key words: Enzymes, Ginkgo Biloba, Inhibition, Myocardial infarction, LDH, CK-MB.

Introduction:

Myocardial infarction (MI) is a leading cause of the worldwide rate of conditions and deaths caused by the death of myocardial cells caused by oxygen deprivation and heart muscle death begins 20 minutes after oxygen interruption[1]. The most common cause of MI cardiac infarction is the rupture of the fatty plaque with the exposure of the basal membrane leading to platelet accumulation, thrombosis formation, and the accumulation of fibrin, this causes partial or complete blockage of the blood vessels followed by myocardium ischemic embolism and then leads to irreversible necrosis when the blockage in the vessels occurs for more than 4-6 hours but during this period the heart muscle can be saved and reduce medical conditions and deaths[2]. Proteins with a high molecular weight called enzymes serve

as catalysts in the body. When enzymes are used as biological catalysts in a natural setting, reaction rates are substantially higher than when chemical catalysts are used[3], this is due to their uniqueness, adaptability, and efficacy. In his book The Enzyme Revolution, Silverman defines enzymes as "very effective organic chemists" [4]. Enzymes minimize the activation energy of the process because they are complementary in form and electrostatic properties to the rate-limiting transition state, which explains the tremendous rate acceleration witnessed when compared to uncatalyzed processes[5]. Heart Enzymes, Creatine Kinase MB (CK) (EC.2.7.3.2) Called Creatine Phosphokinase, Creatine kinase Adenosine Triphosphate: Creatine N phosphotransferase (CK) is a cytosolic enzyme essential for energy transfer during muscle metabolism [6], It is made up of the amino acids arginine, glycine, and methionine and is found in the liver and pancreas. It is essential for the transfer of energy [7]. Creatine Phosphokinase (KC) is found in the cytoplasm, And the symmetry of enzymes is a dichotomy consisting of a combination of B, M subunits, the M of muscles, and the B of the brain, leading to the formation of three isotopes, the two diodes formed B2, M2 (CK-MM and CK-MB) are only found in trace amounts in the brain and muscles and elsewhere in the body, and in the natural heart the rate of 15-20% of CK is CK-MB as its distribution is not homogeneous, The percentage in the right of the heart is greater than the heart's left[8]. Lactate dehydrogenase LDH (EC 1.1.1.27) is a hydrogen transfer enzyme that uses nicotine amide adenine dinucleotide (NAD) as the hydrogen acceptor to catalyze the conversion of L-lactate to pyruvate, the last step of anaerobic glycolysis' metabolic chain, reaction equilibrium strongly favors the reverse reaction because the reaction is reversible, namely the reduction of pyruvate (P) to lactate (L)[9]. Ginkgo Biloba leaves have a wide spectrum of pharmacological actions and are abundant in a variety of natural active substances [10], which play an important role in food[11], health care[12], medicine[13], supplements[14], anti-inflammatory, anti-cancer, antioxidant properties[15], and other fields. Ginkgo leaves practiced antioxidant mechanisms by suppressing free radicals and reactive oxygen produced during oxidative stress [16].

Materials and Methods:

The extracts used in this work by the method previously described by AK Ibraheem, MZ Thani [17], and all kits for enzymes used were from Company Biolabo France.

Patients and controls:

Blood samples were collected at Ghazi Hariri Hospital from November 2021 to March 2022. The study included the collection of 60 samples of patients with myocardial infarction diagnosed by specialist doctors, and 40 healthy people were also included in the control group, full the information is available in the Table 1.

Table 1. The information of the studied groups.

Group	No. of cases	Age (year)	Females	Males	Mean±SD
Patients	60	30-70	30	30	50.15±11.66
Control	40	29-67	20	20	45.90±11.52

Collection of blood samples:

A 10 ml of intravenous blood was withdrawn after the area was sterilized through a vein hole using a plastic syringe with a scale of 21 stainless needles and transferred to a simple clean gel tube. The serum was separated using a centrifuge for 10 minutes at room temperature. The separated serum was then precisely split into three parts using an absorber in Eppendorf tubes and stored at -20°C prior to the biochemical analysis.

Determination of enzymes activity:

The activity of ALT, AST, ALP, LDH, and CK-MB enzyme activity was studied in the samples collected as follows :

The activity of (ALT, AST, ALP, LDH and CK-MB) were determined by using method …[18], [19],[20],[21]and[22] respectively.

Determining the type of inhibition:

Preparation Inhibition (Stock solution):

100 mg of inhibitor was taken, then dissolved in 100 ml of Dimethyl Sulfoxide (DMSO), and prepared a three-fold dilution of stock solution.

Type of inhibition:

A. Reading without inhibitor). (Take two stocks), one for dilution and the second for reaction.

1. Take 800 µL of reagent (A).

2. Add 200 µL of reagent (B).

3. Make a two-fold dilution.

B. Reading with inhibitor.

Re-read the absorbance using an inhibitor.

Note: In this case, we use the dilution (1) on the first page as an inhibitor, which means taking 100 µL of this dilution and completing the procedure

Results and Methods:

Chemistry Results:

There are non-significant (P>0.05) differences in age between control (45.90±11.52 years) and patients (50.15±11.66 years), as shown in Table 2.

Table 2. Age and Gender.

Parameters	Control	Patients	*p*-value
N	40	60	-
Gender M (F)	20 (20)	30 (30)	-
Age (year)	45.90±11.52	50.15±11.66	0.076

M: Male
F: Female

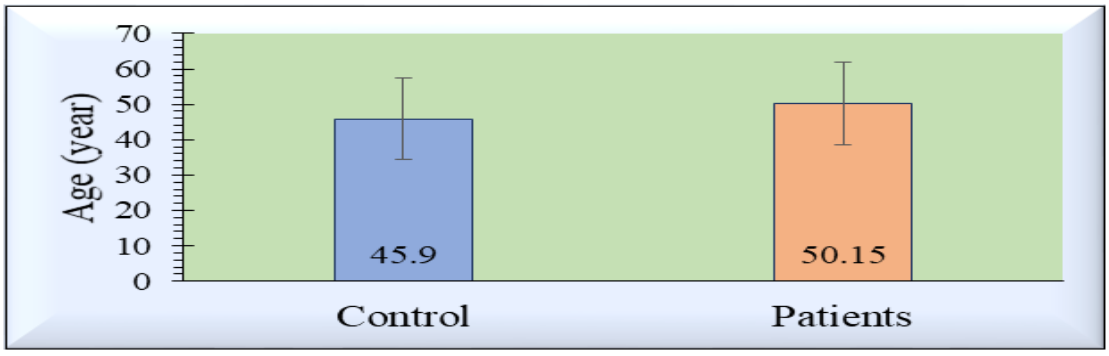


Figure 1. The mean and SD of age in control and patients.

Enzyme's activity:

The activity of enzymes (AST, ALT, ALP, LDH, and CK-MB) was studied on 60 patients with myocardial infarction and 40 as healthy controls, and it is shown in Table 3.

Table 3. The activity of serum enzymes in patients and control.

Parameters	Control	Patients	*p*-value
AST (U/L)	24.58±7.68	29.92±16.71	0.034
ALT (U/L)	20.93±7.85	31.15±21.37	0.001
ALP (U/L)	122.24±32.41	193.63±84.89	<0.001
LDH (U/L)	340.80±62.40	464.27±140.67	<0.001
CK-MB (U/L)	14.80±4.16	21.03±6.01	<0.001

Study of AST Enzyme:

The activity of AST was increased significantly *(P<0.05)* in myocardial patients' serum (29.92±16.71 U/L) compared to that in healthy controls (24.58±7.68 U/L) as shown in Figure 2.

This finding is consistent with several studies that have reported elevated AST activities in patients with heart disease. They also stated that ALT and AST could be considered independent predictions[23]. This rise is caused by rupture of heart muscle cells in the affected area due to low blood circulation resulting from blockage or narrowing of the coronary arteries and thus the release of proteins in the cytoplasm and enzymes (AST, CK-MB, LDH) gradually increasing their concentration in the circulatory system[24].

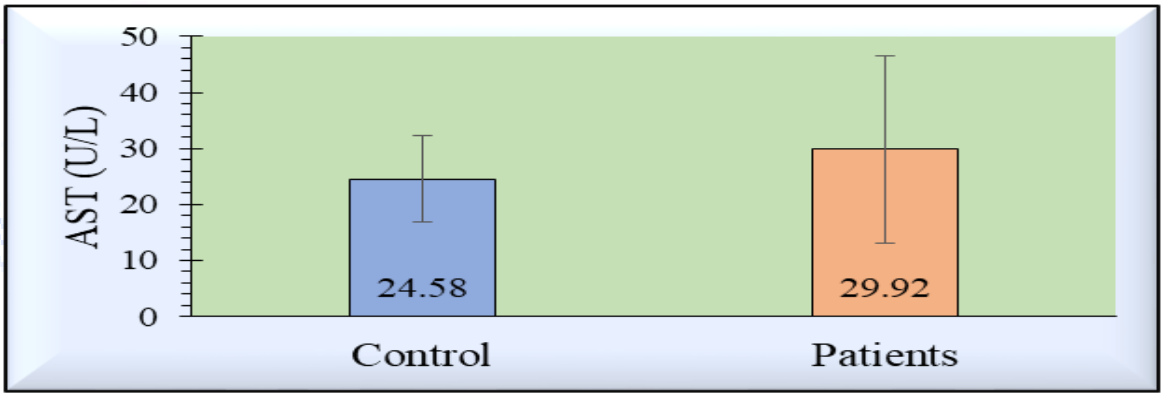


Figure 2. The mean and SD of AST in control and patients.

Study of ALT Enzyme:

The activity of ALT was increased highly significantly ($P \leq 0.001$) in myocardial patients' serum (31.15±21.37 U/L) compared to that in healthy controls (20.93±7.85 U/L) as shown in Figure 3.

The results were spent with Kyung Mook Choi and his co-workers [25]. Liver markers can also predict patient outcomes after acute myocardial infarction. Increased serum levels of ALT and AST are correlated with an increased risk of MI events [26]. On the other side, a study conducted by Dodji Kossi Djakpo and his assistants [27], showed that one of the reasons for the increased activity of ALT is acute myocardial ischemia or necrosis of myocardial cells that occur in the acute myocardial infarction model causes increased ALT activity in the serum.

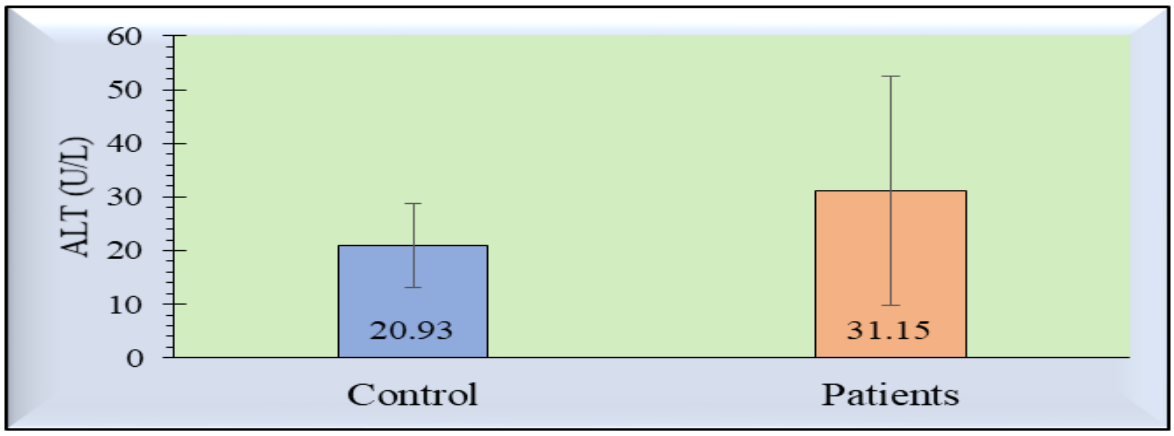


Figure 3. The mean and SD of ALT in control and patients.

Study of ALP Enzyme:

The activity of ALP was increased highly significantly ($P \leq 0.001$) in myocardial patients' serum (193.63±84.89 U/L) compared to that in healthy controls (122.24±32.41 U/L) as shown in Figure (4.

Excessive vascular calcification is linked to increased ALP activity. This ultimately results in early atherosclerosis and cardiovascular incidents, this is seen in the progeria syndrome Hutchinson-Gilford or the widespread arterial calcification of infancy syndrome [28]. Serum ALP is also a good prognostic factor in MI patients[29], One of the reasons for the increase in the enzyme ALP is oxygen-hungry heart muscle tissue after restoring blood supplies that have undergone oxidative stress that generate reactive oxygen types that cause damage to proteins and DNA and increase membrane permeability [30].

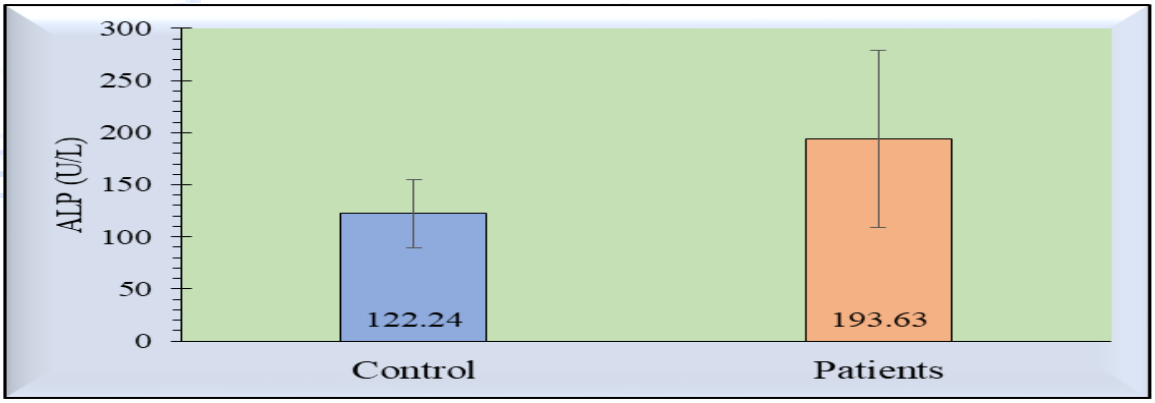


Figure 4. The mean and SD of ALP in control and patients.

Study of LDH Enzyme:

The activity of LDH was increased highly significantly ($P \leq 0.001$) in myocardial patients' serum (464.27±140.67 U/L) compared to that in controls (340.80±62.40 U/L) as shown in Figure 5.

LDH is more effective in the serum after myocardial infarction and begins after 4 -12 hours the infection appears and reaches its maximum height after 44 hours and is a useful clinical sign for people entering the Hospital in myocardial infarction [31], the high effectiveness of lactate dehydrogenase may increase in the infarction of the heart and maybe because the condition has damaged the heart cells, causing the release of the enzyme and thus the high effectiveness of the enzyme in the serum [32].

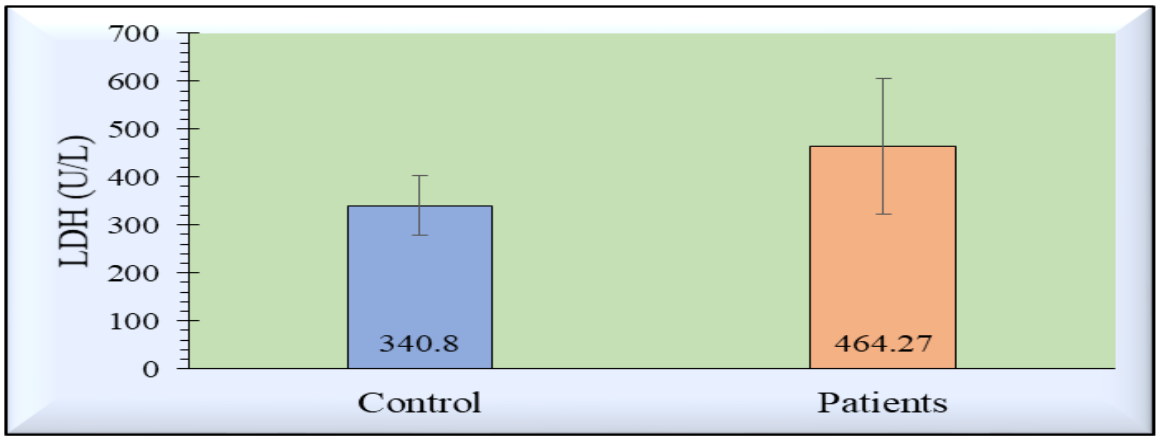


Figure 5. The mean and SD of LDH in control and patients.

Study of CK-MB Enzyme:

The activity of CK-MB was increased highly significantly ($P \leq 0.001$) in myocardial patients' serum (21.03±6.01 U/L) compared to that in controls (14.80±4.16 U/L) as shown in Figure 6. This study has indicated a high level of effectiveness of CK-MB enzyme in heart patients and our current study is consistent with this study, as most heart diseases are accompanied by a high level of effectiveness of this enzyme, and this rise is caused by the presence of this enzyme with high concentrations in the cells of the heart muscle leads to the destruction of these cells and then release their content from this enzyme to the bloodstream with certain concentrations close to the defect that occurred in the heart tissue[24].

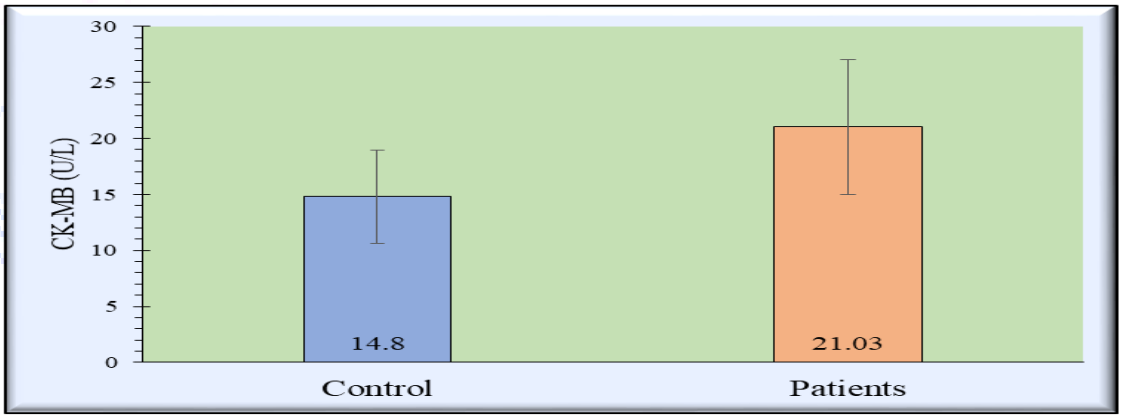


Figure 6. The mean and SD of CK-MB in control and patients.

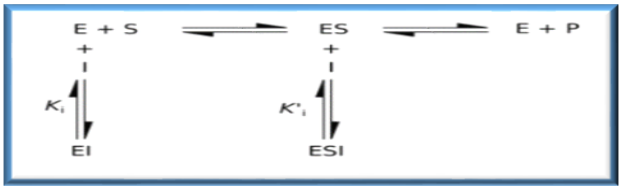
Effect of GBLEs on Enzymes activity (LDH and CK-MB):

When examining the effect of these extracts with a different focus on enzymes in serum and patient controls, it was found that the water extract acts as an inhibitor for both enzymes, and this was demonstrated by the decrease in readings resulting from the addition of this extract and increased inhibition with increased concentration of the extract. The alcohol extract acts as an inhibitor of LDH, while it does not affect the CK-MB enzyme where the same readings appeared in all the concentrations used. The results obtained were identical to several studies that have shown that ginkgo extractors have the potential to inhibit the effectiveness of enzymes (LDH and CK-MB) [25] [26] [27].

Types of inhibition:

To determine the type of inhibition of extracts for enzyme prevention, Weaver Burke was drawn. The results shown in the models indicated that the type of aqueous inhibition of LDH and CK-MB, and

alcohol extract from the LDH enzyme, was non-competitive. In non-competitive inhibition, inhibitors at the allosteric site are independently associated with substrate binding; this means that the inhibitor shares the same convergence of both the enzyme and the enzyme-substrate compound. The non-competitive inhibitor is associated with the enzyme to form an enzyme inhibitor compound (EI), with a compound (ES) to form (ESI). The non-competitive inhibition mechanism can be explained as follows [33] :



Tables 4, 5, 6, and Figures 7, 8, and 9 demonstrate the application of the Lineweaver-Burk equation on each extract to predict the quality parameters, Vmax and Km, which give an impression of the type of inhibitor.

Table 4. The kinetic parameters of LDH for Ethanol Extract.

LDH						
Concentration [mM]	**Activity (Normal)**	**Activity (with Inhibitor)**	**Km [Mm]**	**Vmax UI/L**	**Vmax $^{-1}$ UI/L**	**Type**
0.36	0.000921888	0.001470631	0.4	142.8	714	Non-competitive
0.67	0.001038093	0.001764758				
1.00	0.0015	0.002330812				

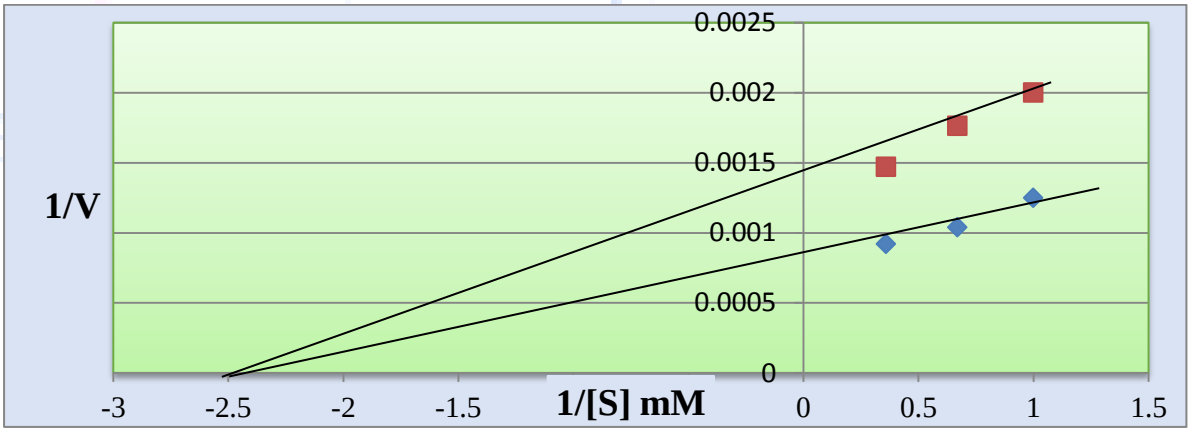


Figure 7. Line Weaver-Burk plot for Ethanol Extract effect on LDH activity in sera of MI patients.

Table 5. The kinetic parameters of LDH for Aqueous Extract.

LDH						
Concentration [Mm]	**Activity (Normal)**	**Activity (with Inhibitor)**	**Km [mM]**	**Vmax UI/L**	**Vmax $^{-1}$ UI/L**	**Type**
0.36	0.000921888	0.010535	0.4	142.8	107	Non-competitive
0.67	0.001038093	0.011522				
1.00	0.001583757	0.0127849				

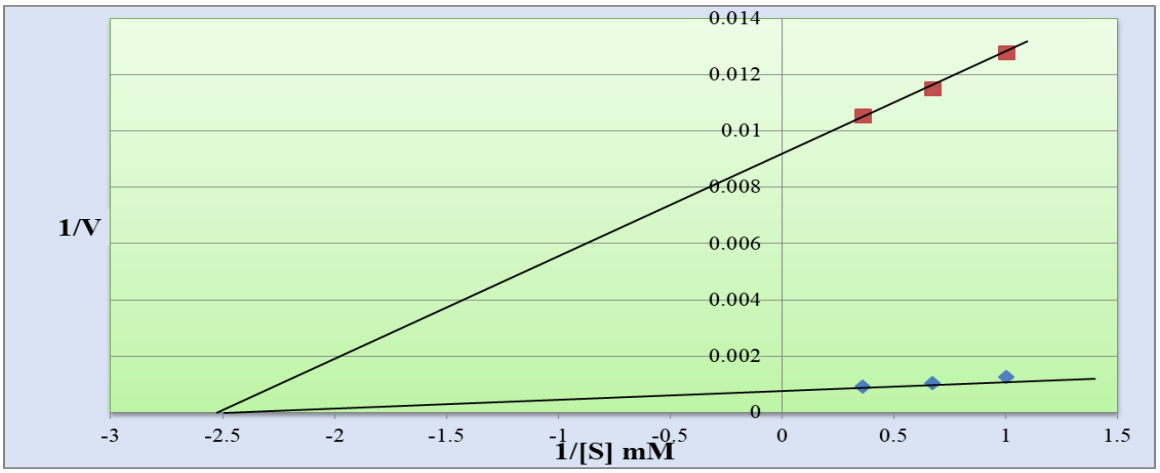


Figure 8. Lineweaver Burk Aqueous Extract Effect on LDH activity in sera of MI patients.

Table 6. The kinetic parameters of CK-MB for Aqueous Extract.

CK-MB						
Concentration [mM]	**Activity (Normal)**	**Activity (with Inhibitor)**	**Km [mM]**	**Vmax UI/L**	**Vmax $^{-1}$ UI/L**	**Type**
15.2	0.07571169	0.091527646	5.26	100	76.923	Non-competitive
10.2	0.055069352	0.06729928				
6.00	0.035934501	0.04097446				

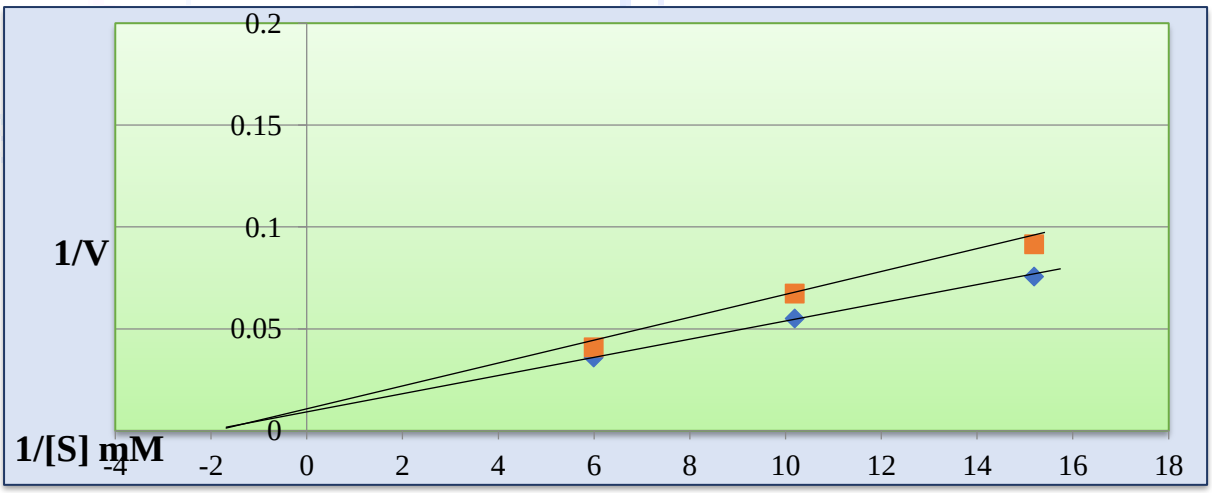


Figure 9. Lineweaver Burk Aqueous Extract effect on CK-MB activity in sera of MI patients.

Table 7. Correlation among parameters in patients.

Parameters	AST		ALT		ALP		LDH		CK-MB	
	r	***p***	**r**	***p***	**r**	***p***	**r**	***p***	**r**	***p***
AST (U/L)	-	-	0.383*	0.003	-0.041	0.753	0.197	0.131	-0.009	0.944
ALT (U/L)	0.383*	0.003	-	-	0.015	0.912	0.202	0.122	0.045	0.733
ALP (U/L)	-0.041	0.753	0.015	0.912	-	-	0.050	0.703	-0.100	0.446
LDH (U/L)	0.197	0.131	0.202	0.122	0.050	0.703	-	-	-0.090	0.496
CK-MB (U/L)	-0.009	0.944	0.045	0.733	-0.100	0.446	-0.090	0.496	-	-
Age (year)	0.112	0.392	0.141	0.282	-0.120	0.361	-0.033	0.802	-0.015	0.907

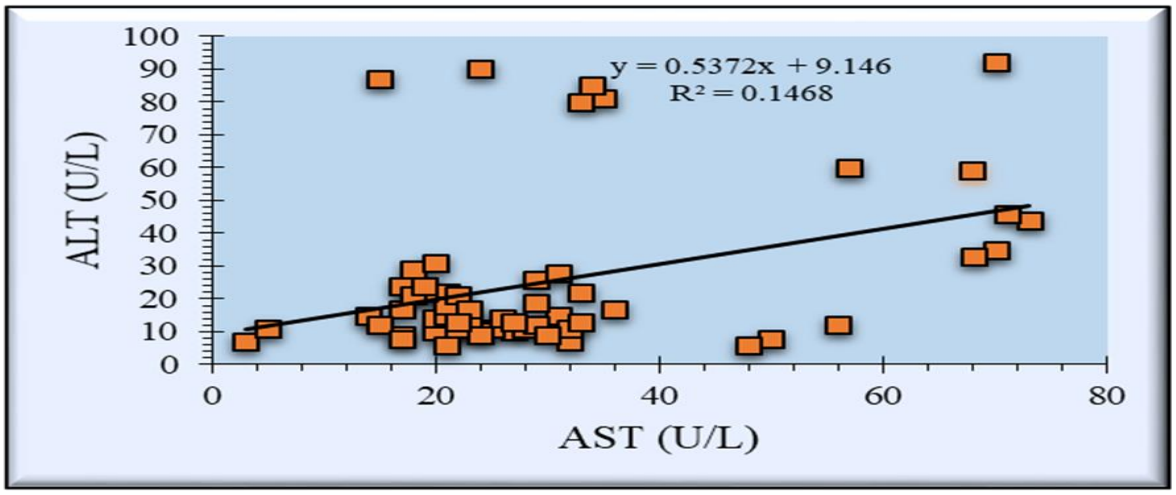


Figure 10. Correlation between AST and ALT in patients.

Table (7) shows that there was a strong and influential association (highly significant) ($P < 0.05$) between the enzyme AST and ALT where (r = 0.383) and ($p = 0.003$), is the highest correlation between measured parameters, while there was no influential association (non-significantly) between the rest of the parameters.

Table 8. ROC outcomes.

Parameters	AUC	SE	*p*-value	Cut-off value	Sensitivity	Specificity
AST (U/L)	0.524	0.058	0.683	-	-	-
ALT (U/L)	0.638	0.057	0.019	21.50	70%	60%
ALP (U/L)	0.884	0.033	<0.001	152.17	78.3%	75%
LDH (U/L)	0.772	0.046	<0.001	328.0	61.7%	70%
CK-MB (U/L)	0.799	0.043	<0.001	17.50	73.3%	65%

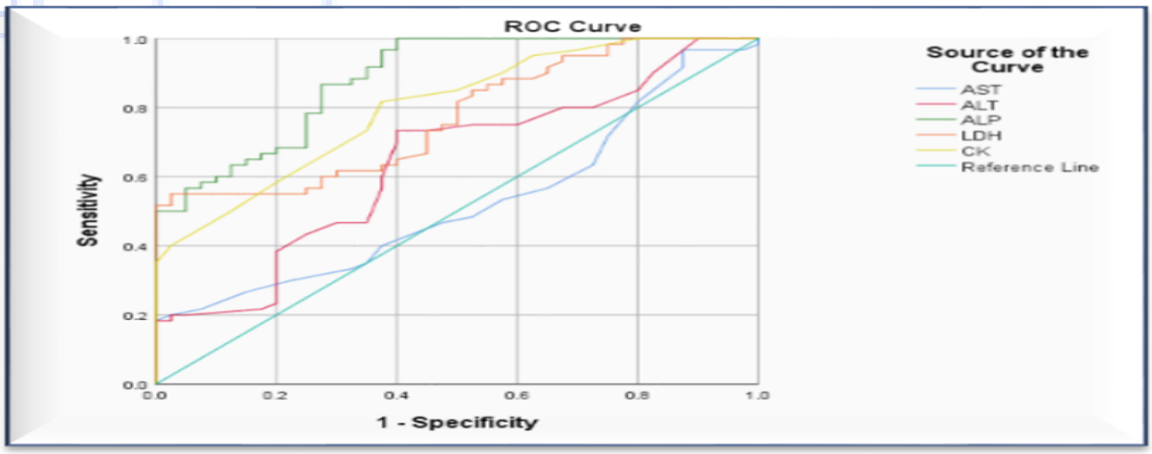


Figure 11. The ROC curve of the tested enzymes in the prognosis of the disease.

Results ROC outcomes in Table (8) showed that the largest area under the curve (AUC) was for the enzyme ALP where (AUC = 0.884) thus has the highest sensitivity (78.3%) and highest specificity (75%), thus considering ALP has the highest adoption in diagnosis among other parameters. While it was less (AUC) for AST and less sensitive and less specificity, AST is considered to have less reliability in diagnosing.

Conclusion:

This study showed that the aqueous extract acted as an inhibitor of LDH and CK-MB, while the alcoholic extract acted as an inhibitor of LDH and showed no effect on CK-MB, and the study also showed the type of inhibition of these enzymes where the results showed non-competitive inhibition of the extracts. The results obtained were identical to several studies that showed that ginkgo extracts were used in acute myocardial ischemia (IM).

Acknowledgment:

The authors are grateful to the Department of Chemistry, College of Science, University of Mustansiriyah University, Department, Baghdad, Iraq for their support in completing this work.

References:

1. M. O. A. M. M. Al-shawk and H. Y. Ali, "Echocardiographic Estimation of Infarct Size By Using Cardiac Biomarkers (Troponin I, CK And CK-MB) And Some Hematological Changes in Patients With STEMI," vol. 11, no. 3. pp. 528–538, 2014.
2. G. L. Koerbin, "High Sensitivity Troponin - Its Use in Diagnosis of Cardiac Dysfunction," pp. 1–342, 2014, [Online]. Available: papers3://publication/uuid/309B358B-BA7D-46DC-8F16-3451074CDC8B%0Afile:///Users/tomkaier/Backup/Papers Library/Files/54/54227CF9-F40D-4E4D-BB22-BACCC25ABA50.pdf.
3. S. Bhatia, *Introduction to enzymes and their applications*, no. October. 2018.
4. D. Herries, "Enzymes as catalysts in Organic Synthesis," *Biochemical Education*, vol. 13, no. 1. p. 42, 1985, doi: 10.1016/0307-4412(85)90151-7.
5. C. Whittington, J. Latham, and A. R. Offenbacher, "Tunneling through the Barriers: Resolving the Origins of the Activation of C-H Bonds Catalyzed by Enzymes," *ACS Symp. Ser.*, vol. 1357, no. February 2021, pp. 139–160, 2020, doi: 10.1021/bk-2020-1357.ch007.
6. M. J. Ivars, "No Titleトランス・トランスレーションの分子メカニズム," 生化学, vol. 7, no. 3, pp. 213–221, 2007.
7. D. A. Bonilla *et al.*, *Metabolic basis of creatine in health and disease: A bioinformatics-assisted review*, vol. 13, no. 4. 2021.
8. S. Aydin, K. Ugur, S. Aydin, İ. Sahin, and M. Yardim, "Biomarkers in acute myocardial infarction: Current perspectives," *Vasc. Health Risk Manag.*, vol. 15, pp. 1–10, 2019, doi: 10.2147/VHRM.S166157.
9. M. Drent, N. A. M. Cobben, R. F. Henderson, E. F. M. Wouters, and M. Van Dieijen-Visser, "Usefulness of lactate dehydrogenase and its isoenzymes as indicators of lung damage or inflammation," *Eur. Respir. J.*, vol. 9, no. 8, pp. 1736–1742, 1996, doi: 10.1183/09031936.96.09081736.
10. R. Li *et al.*, "Advances in supercritical carbon dioxide extraction of bioactive substances from different parts of Ginkgo biloba L.," *Molecules*, vol. 26, no. 13, pp. 1–16, 2021, doi: 10.3390/molecules26134011.
11. C. W. Zhang, C. Z. Wang, and R. Tao, "Characterization and antioxidant activities of polysaccharides extracted from enzymatic hydrolysate of Ginkgo biloba leaves," *J. Food Biochem.*, vol. 41, no. 3, pp. 1–7, 2017, doi: 10.1111/jfbc.12352.
12. H. M. R. Abdel-Latif, B. M. Hendam, M. I. Nofal, and M. A. M. El-Son, "Ginkgo biloba leaf extract improves growth, intestinal histomorphometry, immunity, antioxidant status and modulates

transcription of cytokine genes in hapa-reared Oreochromis niloticus," *Fish Shellfish Immunol.*, vol. 117, no. March, pp. 339–349, 2021, doi: 10.1016/j.fsi.2021.06.003.

13. G. Achete De Souza, S. V. De Marqui, J. N. Matias, E. L. Guiguer, and S. M. Barbalho, "Effects of Ginkgo biloba on Diseases Related to Oxidative Stress," *Planta Med.*, vol. 86, no. 6, pp. 376–386, 2020, doi: 10.1055/a-1109-3405.
14. T. K. Mohanta, Y. Tamboli, and P. K. Zubaidha, "Phytochemical and medicinal importance of Ginkgo biloba L.," *Nat. Prod. Res.*, vol. 28, no. 10, pp. 746–752, 2014, doi: 10.1080/14786419.2013.879303.
15. L. Zhang, T. Wu, W. Xiao, Z. Wang, G. Ding, and L. Zhao, "Enrichment and Purification of Total Ginkgo Flavonoid O-Glycosides from Ginkgo Biloba Extract with Macroporous Resin and Evaluation of Anti-Inflammation Activities In Vitro," *Molecules*, vol. 23, no. 5, 2018, doi: 10.3390/molecules23051167.
16. J. Kobus-Cisowska, E. Flaczyk, M. Rudzińska, and D. Kmiecik, "Antioxidant properties of extracts from Ginkgo biloba leaves in meatballs," *Meat Sci.*, vol. 97, no. 2, pp. 174–180, 2014, doi: 10.1016/j.meatsci.2014.01.011.
17. A. K. Ibraheem, M. Z. Thani, and M. T. Mohammed, "Determination of Vitamins, Trace Elements, and Phytochemical Compounds in Ginkgo Biloba Leaves Extracts," *Egypt. J. Chem.*, 2022.
18. I. P. Ezeugwunne, "Activities of Pancreatic Enzymes and Anthropometric Indices in Type 2 Diabetic Patients Attending Nnamdi Azikiwe University Teaching Hospital Nnewi, South Eastern, Nigeria," *Int. J. Curr. Sci. Res. Rev.*, vol. 04, no. 08, 2021, doi: 10.47191/ijcsrr/v4-i8-16.
19. C. Biolabs, "Aspartate Aminotransferase (AST) activity assay kit (colorimetric)," *Aspartate Amin. Act. assay kit*, pp. 1–8, 2019.
20. A. Names, N. M. Aziz, and A. History, "Effect of postmenopausal on some liver enzymes in Kirkuk women ' s Authors Names," no. 26, pp. 17–23, 2021.
21. N. A. Khadse, M. P. Deshmukh, and A. M. Wankhade, "Cardioprotective Activity Of Herbal Formulation In Experimental Animal," *Am. J. PharmTech Res.*, vol. 10, no. 5, pp. 15–27, 2020, doi: 10.46624/ajptr.2020.v10.i5.002.
22. D. S. Nirwan and U. Kumar, "International Journal of Medical Science and Education An official Publication of Association for Scientific and Medical Education (ASME) Original Research Article COMPARATIVE STUDY OF SERUM AST , CK-MB , IN MYOCARDIAL INFARCTION WITH SURVIVAL AND MYOCA," vol. 5, no. June, pp. 182–187, 2018.
23. M. Cheraghi, H. Ahmadvand, A. Maleki, E. Babaeenezhad, S. Shakiba, and F. Hassanzadeh, "Oxidative Stress Status and Liver Markers in Coronary Heart Disease," *Reports Biochem. Mol. Biol.*, vol. 8, no. 1, pp. 49–55, 2019.
24. J. A. Reyes-Retana and L. C. Duque-Ossa, "Acute Myocardial Infarction Biosensor: A Review From Bottom Up," *Curr. Probl. Cardiol.*, vol. 46, no. 3, p. 100739, 2021, doi: 10.1016/j.cpcardiol.2020.100739.
25. X. J. Chen, S. M. Ren, J. Z. Dong, C. G. Qiu, Y. W. Chen, and H. L. Tao, "Ginkgo biloba extract-761 protects myocardium by regulating Akt/Nrf2 signal pathway," *Drug Des. Devel. Ther.*, vol. 13, pp. 647–655, 2019, doi: 10.2147/DDDT.S191537.
26. J. Ding *et al.*, "Ginkgo biloba extract alleviates liver fibrosis induced by CCl4 in rats," *Liver Int.*, vol. 25, no. 6, pp. 1224–1232, 2005, doi: 10.1111/j.1478-3231.2005.01169.x.

27. T. Baars *et al.*, “Liver parameters as part of a non-invasive model for prediction of all-cause mortality after myocardial infarction,” *Arch. Med. Sci.*, vol. 16, no. 1, pp. 71–80, 2020, doi: 10.5114/aoms.2018.75678.

28. D. K. Djakpo, Z. Q. Wang, and M. Shrestha, “The significance of transaminase ratio (AST/ALT) in acute myocardial infarction,” *Arch. Med. Sci. – Atheroscler. Dis.*, vol. 5, no. 1, pp. 279–283, 2020, doi: 10.5114/amsad.2020.103028.

29. M. Amin, “No Title終末論的永劫回帰とモダニズムの弁証法,” *日本ワーグナー協会編『年刊ワーグナー1990』*, vol. 168, no. 3, p. 東京：音楽之友社：pp. 56-79, 1990.

30. S. K. Kunutsor, S. J. L. Bakker, J. E. Kootstra-Ros, R. T. Gansevoort, J. Gregson, and R. P. F. Dullaart, “Serum alkaline phosphatase and risk of incident cardiovascular disease: Interrelationship with high sensitivity C-reactive protein,” *PLoS One*, vol. 10, no. 7, pp. 1–16, 2015, doi: 10.1371/journal.pone.0132822.

31. Y. Wu *et al.*, “Serum lactate dehydrogenase activities as systems biomarkers for 48 types of human diseases,” *Sci. Rep.*, vol. 11, no. 1, pp. 1–8, 2021, doi: 10.1038/s41598-021-92430-6.

32. O. A. Khalil, K. S. Ramadan, A. H. Hamza, and S. E. El-toukhy, “Association of plasma protein C levels and coronary artery disease in men,” vol. 12, no. 50, pp. 6986–6991, 2013, doi: 10.5897/AJB12.2013.

33. F. Song, H. Li, J. Sun, and S. Wang, “Protective effects of cinnamic acid and cinnamic aldehyde on isoproterenol-induced acute myocardial ischemia in rats,” *J. Ethnopharmacol.*, vol. 150, no. 1, pp. 125–130, 2013, doi: 10.1016/j.jep.2013.08.019.