STUDY THE EFFECT OF SELECTIVE ORGANIC COMPOUNDS ON THE ACTIVITY OF ALANINE AMINO TRANSFERASE [ALT] IN SERA OF NORMAL AND PATIENTS WITH LIVER DISEASES[+]

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Abstract

Human serum ALT activity was measured in vitro in sera for 40 normal persons, their age ranged between [10-25] years which showed a wide activity range between (6-14) U/L.

The ALT activity in patients with different liver diseases, total serum bilirubin ranged between [1.5-7.0]mg/dl which showed the ALT activity range between [28-88]U/L.

Different organic compounds were examined regarding their effects on the activity of ALT in sera from normal and patients with liver diseases.

ALT activity was inhibited by four of these compounds, and the other compounds affected as activators, one of them was chosen to reactivate the activity of inhibited ALT.

The inhibitory effect of two different concentrations of the four compounds were studied , using five different concentrations of the substrate. The percentage type of inhibition and the recovery percentage were calculated with the absence and with the presence of different concentrations of these compounds.

المستخلص:

تم في هذا البحث قياس فعالية أنزيم ALT [Alanine Amino transferase] في مصل دم الإنسان لـــ ٤٠ شخص طبيعي تراوحت أعمارهم بين [١٠-٢٥] سنة وقد تراوحت الفعالية الإنزيمية بين U/L [6-14] . قيست الفعالية الإنزيمية أيضا لـــ ٤٠ شخص مصاب بأمراض الكبد المختلفة وقد تراوحت قيم البيلروبين المصلي الكلي لهم بين mg/dl. [1.5-7.0] أما فعالية الأنزيم [ALT] فقد كانت ما بين U/L [٨٨-28] . تم دراسة تأثير عدد من المركبات العضوية حيث أظهرت النتائج أن أربعة منها ذات اثر تثبيطي على فعالية أنزيم آل [ALT] في حين أن المركبات الثلاثة الأخرى لم يكن لها تأثيرا يذكر بل على العكس فقد تبين إنها تنشط الأنزيم . تم استخدام أحد هذه المركبات الثلاثة لإعادة تنشيط الأنزيم المثبط وان المواد العضوية الأربعة السابقة الذكر تم التوصل إلى تحديد نوع التثبيط باستعمال تركيزين مختلفين من المواد المثبطة باستعمال خمسة تراكيز مختلفة من المادة الأساس وكذلك تم حساب قيم ثابت التثبيط K_i و V_{mapp} .

كذلك تم حساب النسبة المئوية لتثبيط المواد العضوية المثبطة وبعدها تم حساب النسبة المئوية لإعادة التنشيط بوجود تراكيز مختلفة من المثبطات.

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Introduction

Alanine amino transferase [ALT] or serum glutamate pyruvate transaminase [S.GPT] [1], uses alanine as a substrate [2]. ALT exists primarily in the liver [3] .

Alanine amino transferase [ALT], one of the two enzyme that catalyses irreversible amino group transfer reaction in the Krebs cycle[4], is necessary for tissue energy production[5].

Very high ALT levels [up to 50 times of the normal level] suggests viral or severe drug induced hepatitis or other hepatic disease with extensive necrosis[6].

Moderate – to high level may indicate infectious mononucleosis, chronic hepatitis, intra hepatic cholestasis or cholecystitis or severe hepatic congestion due to heart failure[7][8].

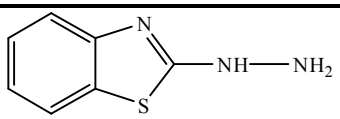
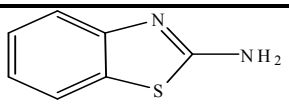
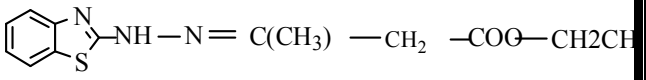
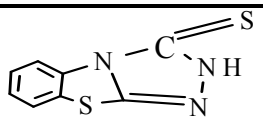
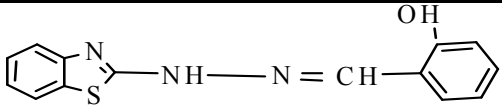
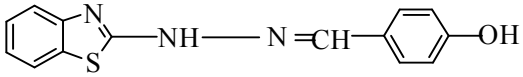
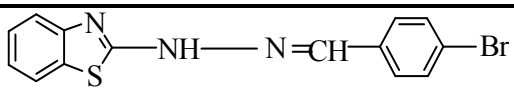
Slight to moderate elevations of ALT [usually with higher increases in aspartate trance aminase levels] may appear in any condition that produces acute hepatocellular injury – such as active cirrhosis and drug – induced or alcoholic hepatitis[9][10][11][12].

Marginal elevations occasionally occurs in acute myocardial infarction reflecting secondary hepatic congestion or the release of small amounts of ALT from myocardial tissue[12][13]. Level of up to 2 x U/L of normal, sometimes found in patients who have no clinical evidence of tissue damage.

Alcohol abuse should be considered as a possible cause in such cases[14].Hepatic GPT activity was markedly decreased in liver tissue of patient with alcoholic liver cirrhosis[15][16] [17] .There have been a few studies on sub – clinical or normal range liver function decreases and their causal risk factors[18][19][20].

In this study the effect of some organic compounds on the activity of ALT as described in table (1) were regarded. The literature contain many studies on the biological activity but there was no study on their effects on enzyme activity is involved.

Table 1 : Name and chemical structure of compounds

Comp.No.	Name of Compound	Chemical Structure
1	2-hydrazino –1,3- benzothiozole	
2	1,3-benzothiozole-2-amine	
3	Ethel(4E)-4-(1,3)-benzothiozole-2-yl hydrazino-3-methyl butanoate	
4	1,9 a-dihydro(1,2,4) trizolo (3,4-b) (1,3) benzothiozole-3(2H)-thione	
5	2-hydroxybenzaldehyde-1,3-benzothiozole-2-yl hydrazone	
6	4-hydroxybenzaldehyde-1,3-benzothiozole-2-yl hydrazone	
7	4 - bromobenzaldehyde -1, 3-benzothiozole-2-yl hydrazone	

Experimental

The activity of transaminase was determined by measuring the absorption of the colour of hydrazone which is formed by the reaction between 2,4 di ntrophenyl hydrazine [DNPH] and keto acid as a product of transaminase activity[21][22].

DNPH reacts with all oxoacids. [23]. In ALT reaction the substrate are sub optimal, to reduce the back ground colour given by the alpha keto glutarate in the reaction with DNPH[24][25].

Reagents and Procedure

ALT activity was measured in human serum using colorimetric method [Reitman – Frankel Method][1][21][26][27].

A- Reagents:

1- Phosphate buffer [pH] 7.4 , 0.1 M]

a- [0.1 M] of Di sodium hydrogen phosphate dehydrate [$Na_2HPO_4.2H_2O$]: Dissolve 8.9 gm of $Na_2HPO_4.2H_2O$ in 500ml distilled water.

b- [0.1 M] of Monopotassium phosphate anhydrous [KH_2PO_4]: Dissolve 1.36 gm of KH_2PO_4 in 100ml distilled water

c- Mix 420ml of $Na_2HPO_4.2H_2O$ with 80ml of KH_2PO_4 check the pH and adjust to pH 7.4.

2- ALT substrate [alanine 200 m mole/L + alpha – ketoglutrate 2 m mole/L]: Dissolve 1.78gm of alanine and 30mg of oxoglutaric acid in 20 ml of phosphate buffer containing 1.25ml of 0.4 N NaOH and make up to 100ml with buffer.

3- Pyruvate standard solution :

a- Stock standard [20 mmole/L]: Dissolve 220mg sodium pyruvate in 100 phosphate buffer.

b-Working standard [2 mmole/L] : Dilute 10ml of the stock standard to 100 ml with phosphate buffer. [keep freezed] .

4- 2,4- dinitrophenyl hydrazine [1mmole/L]: Dissolve 200mg in 1000ml 1N HCl.

5- Sodium hydroxide solutions [1N and 0.4N]:

a- NaOH 1N: Dissolve 40gm of NaOH in 1000ml distilled water.

b- NaOH 0.4 N: Dilute 40ml of [1N] NaOH to 100ml with distilled water.

6- Organic compounds:

The effect of the organic compounds shown in table (1) on ALT was studied by preparing stock solutions of [$5x10^{-2}$M] in Dimethylsulfoxide [DMSO] solvent. The inhibition effect was calculated for the different inhibitors concentrations[$5x10^{-2}$,$2.5x10^{-2}$,$1.25x10^{-2}$,$5x10^{-3}$,$5x10^{-4}$] M. Concentrations were prepared by serial dilutions with DMSO from the stock solution [$5x10^{-2}$]M.

B- Procedure

Table (2) : Illustrates the procedure which describes the simultaneous determination of ALT:

Reagent	Test without organic comp.	Test with organic comp.	Blank
Substrate	0.5ml	0.4ml	0.5ml
Pre incubate for 5 min. at 37°C			
Distilled water	-	-	0.1ml
Organic comp.	-	0.1ml	-
Serum	0.1ml	0.1ml	-
Mix return to bath for 30 min. at 37°C			
DNPH	0.5ml	0.5ml	0.5ml
Mix – allow to stand for 20 min. at 25°C			
NaOH 0.4N	5ml	5ml	5ml
- Mix, let stand for 5 min. at 25°C - Read at 505nm against a water blank the [colour is stable at least 60 min].			

Preparation of calibration curve:

Reagent	1	2	3	4	5	6
Water	0.2	0.2	0.2	0.2	0.2	0.2
GPT substrate	1.0	0.9	0.8	0.7	0.6	0.5
Pyruvic standard	-	0.1	0.2	0.3	0.4	0.5
DNPH	1.0	1.0	1.0	1.0	1.0	1.0
Mix. Allow to stand for 20 min . at 25°C						
NaOH	10	10	10	10	10	10
Mix. Allow to stand for at least 5 min. Read against water blank at 505 nm. Plot a calibration curve of the absorbance found vs. the corresponding units on a graph paper according to the following concentrations.						
ALT U/L	0	12	24	40	62	90

Results and Discussion

ALT activity in human serum was measured in vitro according to Reitman – Frankel method[1][21][26][27] .

Table (3) shows that two groups were chosen, group 1 consists of 40 healthy persons their age ranged [10-25] years. Group 2 consists of 40 patients with hepatic diseases their age ranged between [10-35] years. Total serum bilirubin was estimated for each sample which showed a wide range in patients between [1.5 -7] mg/dl.

Table (3): GPT activity in sera of normal person and patients

Groups of sample	Number	Age (years)	ALT activity [U/L]		Normal value [U/L]
			Mean	Range	
Normal	40	10-25	9.50	6.00-14.00	2.40-14.50
Patient	40	10-35	50.40	28.00-88.00	2.40-14.50
Total	80				

The purpose for studying the selected compounds is to find the biological effectiveness of ALT activity. The present work shows that some of these compounds has inhibitory effects compounds No. [1, 2, 3, 4] while [5, 6, 7] having activator effects. The inhibitory effect of the DMSO solvent on the activity of ALT was [1.8 ± 0.4] %.

Table (4) and (5) shows the degree of inhibition of ALT activity at [$5x10^{-2}$, $2.5x10^{-2}$, $1.25x10^{-2}$, $5x10^{-3}$, $5x10^{-4}$] M of the compounds [1→4] using 10 normal and 10 patients samples for [1→4] compounds individually. It has been found that [1→4] compounds were good inhibitors at the used concentrations. The inhibition percentage was [30.77-69.23] % at $50x10^{-2}$ M in normal while in patients the inhibition percentage was [49.37-74.42] % respectively .

The inhibitory effect found to increase as the concentration of the compounds increases as shown in figure (1) and (2) for normal and the patients respectively.

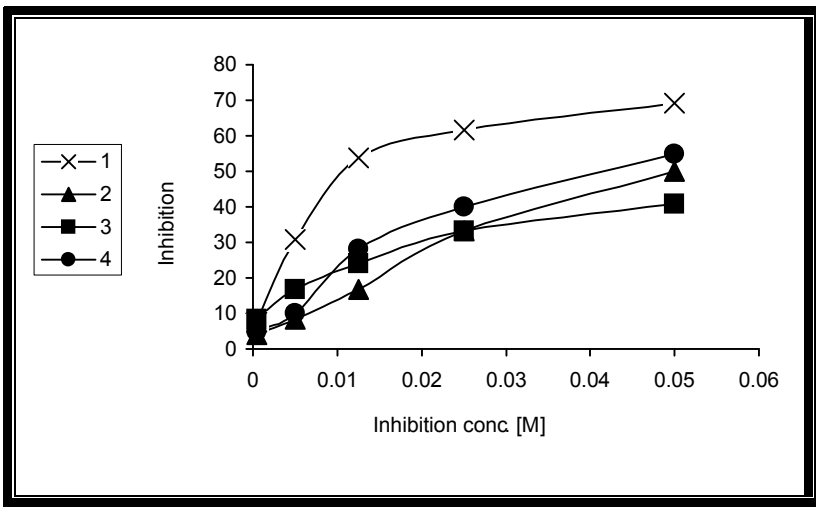


Fig (1) :The inhibitory effect of compounds (1-4) for normal persons.

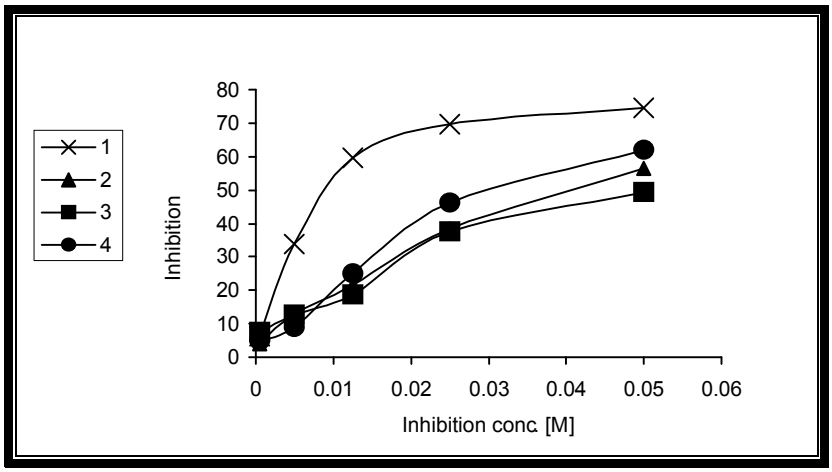


Fig (2) :The inhibitory effect of compounds (1 - 4)for patients

Figure (3) shows the inhibition effect of compounds [1→4] at $5x10^{-2}$ M in sera of patients and normal. Compound (1) shows higher activity against enzyme than other studied compounds in patients and in normal.

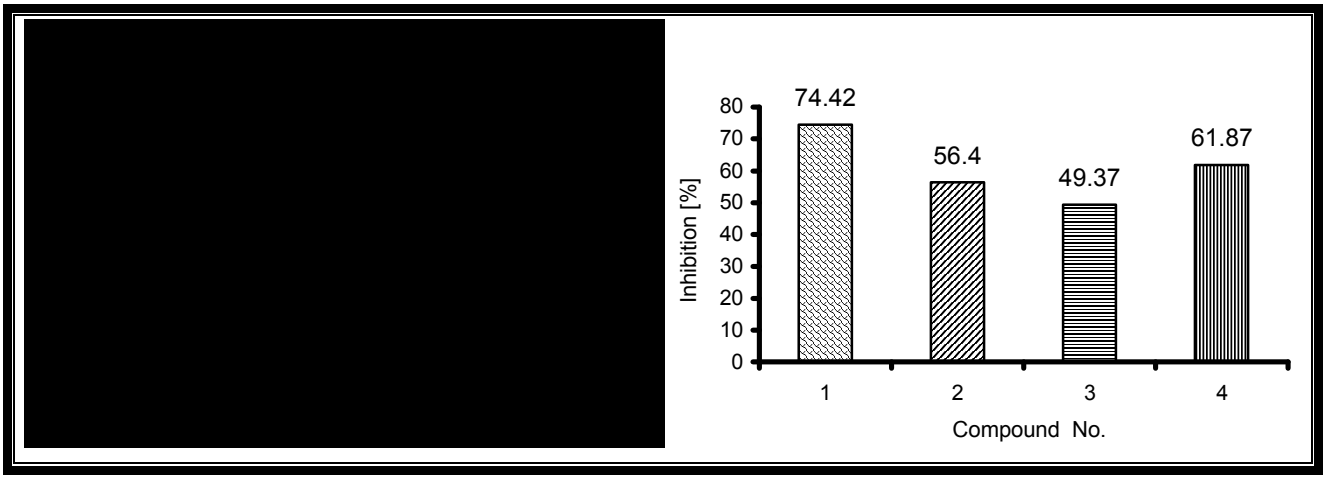


Fig (3) :The inhibitory effect of compounds(1-4) at $5*10^{-2}$M in sera of normal persons and patient

Figure (4) shows the variable effect of compounds [1→4] concentration in normal and patients as follows: compound 1 and 2 were found to have higher inhibition effect on sera ALT in patients than in normal at [$5x10^{-2}$, $2.5x10^{-2}$, $1.25x10^{-2}$, $5x10^{-3}$, $5x10^{-4}$] M of these compounds ; while at $5x10^{-4}$ M of these compounds, the inhibitory effect is higher in normal than in patients.

Compounds 3 and 4 were found to have higher inhibitory effect on serum ALT in normal than in patients at [$5x10^{-2}$, $2.5x10^{-2}$] M of these compounds, the inhibitory effect is higher in normal than in patients.

Table (4): The degree of inhibition of GPT activity at [$5x10^{-2}$, $2.5x10^{-2}$, $1.25x10^{-2}$, $5x10^{-3}$, $5x10^{-4}$] M of compounds, using 10 normal samples for (1-4) compounds individually

Inhibitor No.	V[U/L] without inhibitor	[I] = $5x10^{-2}$ M			[I] = $2.5x10^{-2}$ M			[I] = $1.25x10^{-2}$ M			[I] = $5x10^{-3}$ M			[I] = $5x10^{-4}$ M		
		V[U/L]	% Inhib.	% Reco.	V[U/L]	% Inhib.	% Reco.	V[U/L]	% Inhib.	% Reco.	V[U/L]	% Inhib.	% Reco.	V[U/L]	% Inhib.	% Reco.
1	13.00±0.85	4.00±0.8	69.23	30.77	5.00±0.80	61.54	38.46	6.00±0.75	53.85	46.15	9.00±0.73	30.77	69.23	12.00±0.75	7.7	92.3
2	12.00±0.92	6.00±0.82	50	50	8.00±0.68	33.33	66.67	10.00±0.80	16.67	83.33	11.00±0.85	8.33	91.67	11.50±0.35	4.17	95.83
3	12.00±0.88	7.10±0.55	40.93	59.17	8.00±0.60	33.3	66.67	9.10±0.5	24.17	75.83	10.00±0.61	16.67	83.33	11.0±0.7	8.33	91.67
4	10.00±0.91	4.50±0.6	55.00	45.00	6.00±0.70	40.00	60.00	7.20±0.7	28.00	72.00	9.00±0.5	10	90	9.50±0.6	5.0	9.5

Table (5) : The degree of inhibition of GPT activity at [$5x10^{-2}$, $2.5x10^{-2}$, $1.25x10^{-2}$, $5x10^{-3}$, $5x10^{-4}$] M of compounds, using 10 patient samples for (1-4) compounds individually

Inhibitor No.	V[U/L] without inhibitor	[I] = $5x10^{-2}$ M			[I] = $2.5x10^{-2}$ M			[I] = $1.25x10^{-2}$ M			[I] = $5x10^{-3}$ M			[I] = $5x10^{-4}$ M		
		V[U/L]	% Inhib.	% Reco.	V[U/L]	% Inhib.	% Reco.	V[U/L]	% Inhib.	% Reco.	V[U/L]	% Inhib.	% Reco.	V[U/L]	% Inhib.	% Reco.
1	86.00±0.95	22.00±0.82	74.42	25.58	26.00±0.81	69.76	30.23	35.00±0.85	59.30	40.70	57.00±0.80	33.72	66.28	80.50±0.70	6.40	93.60
2	86.00±88	37.5±0.9	56.40	43.60	53.00±0.82	38.37	61.62	67.50±0.85	21.51	78.49	75.00±0.88	12.79	87.20	82.5±0.81	4.07	95.93
3	80.00±0.81	40.5±0.90	49.37	50.63	50.00±0.79	37.50	62.50	65.00±0.8	18.75	81.25	70.00±0.81	12.50	87.50	74.00±0.60	7.50	92.50
4	80.00±0.83	30.5±0.86	61.87	38.13	43.00±0.81	46.25	53.75	60.00±0.79	25.00	75.00	73.00±0.70	8.75	91.25	76.00±0.65	5.00	95.00

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Type of inhibition, V_{mapp} and k_i were estimated by measuring the enzyme activity with the absence and with the presence of inhibitor at different concentrations of substrate under the optimum conditions using Lineweaver Burk equation and plot [28] as shown in table (6), and figure (5).

Table (6): Kinetic parameters and kind of inhibitions of ALT.

No. of comp.	Kind of inhibition	k_m [mM]	$1.25x10^{-2}$ M of inhibitor		$5x10^{-2}$ M of inhibitor	
			V_{mapp}	k_i	V_{mapp}	k_i
1	Un competitive	2.0×10^{-1}	14.71	1.25×10^{-2}	12.20	4.99×10^{-2}
2	Non competitive	2.1×10^{-1}	76.92	1.25×10^{-2}	55.55	4.99×10^{-2}
3	Un competitive	2×10^{-1}	38.46	0.77×10^{-2}	23.81	3.81×10^{-2}
4	Non competitive	1.9×10^{-1}	55.55	0.56×10^{-2}	40.00	3.00×10^{-2}

Result suggests that compound 1 and 3 acted as un-competitive inhibitiors .V_{max} was calculated directly from the graph figure (5) (a,c) as the point of intercept with 1/V axis – the value of K_i was calculated from V_{mapp} according to the velocity equation [29][30][31].

$$\frac{1}{v} = \frac{k_m}{v_{max}} \frac{1}{[s]} + \frac{1}{v_{max}} \left[1 + \frac{[I]}{k_I}\right] \quad , \quad \frac{1}{v_{mapp}} = \frac{1}{v_{max}} \left[1 + \frac{I}{k_I}\right]$$

Compounds [2, 4] acted as non competitive inhibitors, k_m remained constant[29][30][31]. V_{mapp} was calculated directly from the graph figure 5 [b, d] as the point of intercept with 1/v axis. The value of k_i was calculated from v_{mapp} according to the velocity equation[29] .

$$\frac{1}{v} = \frac{k_m}{v_{max}} \left[1 + \frac{[I]}{k_I}\right] \frac{1}{[s]} + \frac{1}{v_{max}} \left[1 + \frac{[I]}{k_I}\right] \quad , \quad \frac{1}{v_{mapp}} = \frac{1}{v_{max}} \left[1 + \frac{I}{k_I}\right]$$

Reactivate inhibited ALT by organic compounds compound No. [5, 6, 7] acted as activator on enzyme activity. Compound No. 5 was chosen to reactivate the activity of inhibited ALT, different concentration of compound 5 were prepared [$5x10^{-2}$, $2.5x10^{-2}$, $1.25x10^{-2}$, $5x10^{-3}$, $5x10^{-4}$] M. Table (7) shows the effect of compound 5 at different concentrations on the activity of inhibited ALT by using compound [1→4] inhibitor [using four samples for compounds individually.

This table shows firstly the velocity without inhibitor or activator, secondly velocity with inhibitor only in compound [1→4] and velocity with the presence of compounds and recovery percentage were calculated with presence and absence of compounds No. 5. The reactivation effect was found to be increased as the concentration of compound No. 5 increases.

The influence of compound No. 5 as reactivator is mainly attributed to the ability of this compound to raise coordinating number by forming a new coordinating bound to the inhibitory molecule after breaking the bound between these molecule after breaking the bound between these molecule enzyme in Enzyme-inhibitor complex.

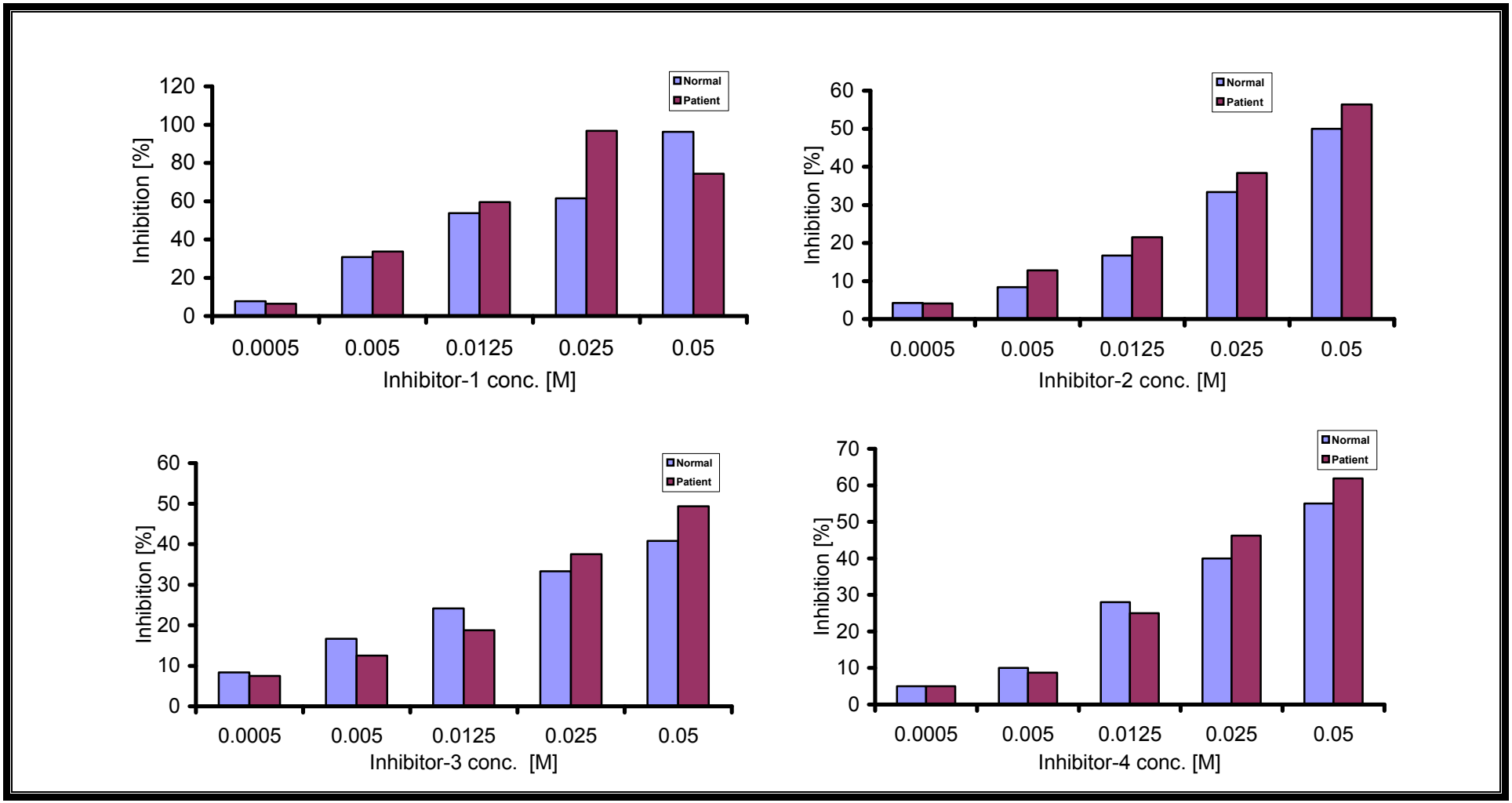

Fig (4):The variable effect of compounds conc. On the inhibition percentage.

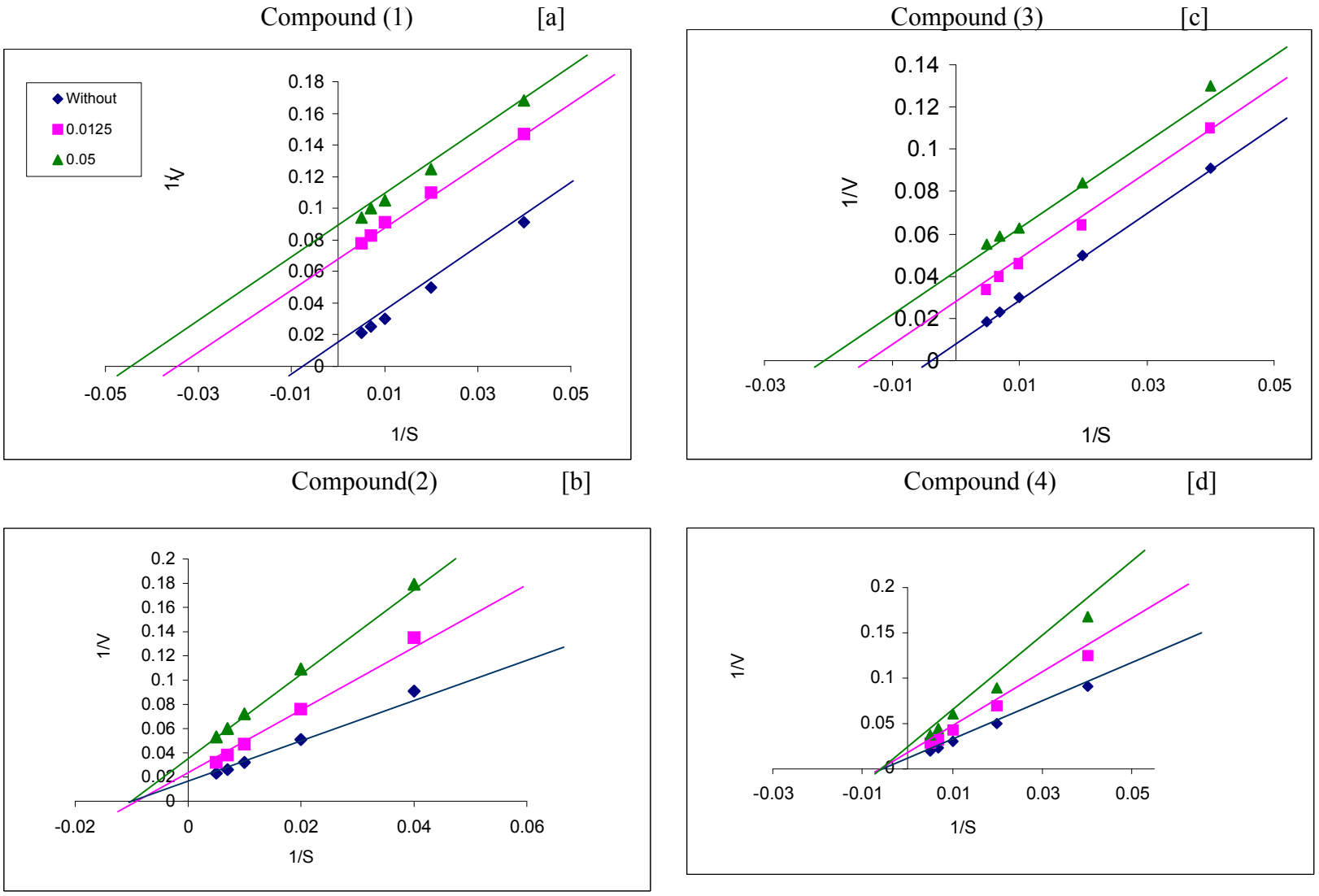

Fig (5): Lineweaver-Burk plot of ALT in the presence and absence of inhibitiors

Table (7): Effect of compound No. 6 at different concentration on the activity of inhibited ALT using 5 normal sample for all compounds individually.

Inhibitor No.	V[U/L] without inhibitor	V with inhibitor $5*10^{-2}$M			Activitor $5x10^{-2}$M with inhibitor $5*10^{-2}$M			Activitor $2.5x10^{-2}$M with inhibitor $5*10^{-2}$M			Activitor $1.25x10^{-2}$M with inhibitor $5*10^{-2}$M			Activitor $5x10^{-3}$M with inhibitor $5*10^{-2}$M			Activitor $5x10^{-4}$M with inhibitor $5*10^{-2}$M		
		V[U/L]	% Inhib.	% Reco.	V[U/L]	% Inhib.	% Reco.	V[U/L]	% Inhib.	% Reco.	V[U/L]	% Inhib.	% Reco.	V[U/L]	% Inhib.	% Reco.	V[U/L]	% Inhib.	% Reco.
1	12.0±0.3	3.70±0.3	69.17	30.83	10.5±0.4	12.5	87.5	8.0±0.3	33.33	66.67	7.0±0.4	41.67	58.33	5.6±0.6	53.33	46.67	4.5±0.4	62.9	37.5
2	10.0±0.5	5.0±0.4	50.00	50.00	9.0±0.5	10.0	90.0	8.3±0.45	16.7	83.30	7.0±0.3	30.0	70.0	6.5±0.3	35.0	65.0	5.80±0.4	42.0	58.0
3	12.2±0.2	7.0±0.3	42.62	57.38	10.8±0.3	11.48	88.52	9.5±0.50	22.13	77.87	8.5±0.3	30.33	69.67	7.8±0.4	36.07	63.93	7.5±0.5	38.52	61.48
4	10.3±0.4	4.5±0.5	56.31	43.69	9.5±0.3	7.77	92.23	8.0±0.40	22.33	77.66	7.5±0.4	33.98	66.02	6.5±0.3	36.89	63.11	5.0±0.3	51.46	48.54

%Inhib. : % Inhibibition.

%Reco. : %Recovery.

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