Aspartate aminotransferase [AST] activity exposed to selected cyclic organic compounds in liver diseases sera[+]

Atheer Awad Mehdi[*] Dr.Wesen Adel Mehdi[**]

Abstract

Human serum AST activity was measured in vitro in sera for 45 normal persons,their age ranged between [9-35] years which showed mean about 8 U/L.

The AST activity in 40 patients with liver diseases shows the activity ranged between [30-83]U/L, total serum bilirubin ranged between [1.6-7.5]mg/dl.

The present study aims to examine the effect of different new organic compounds on the activity of AST in sera from normal and patients with liver diseases.AST activity was inhibited by four of these compounds however the other compounds affected as activator.

The inhibitory effect of two different concentrations of the four compounds were studied, using five different concentrations of the substrate.

The type of inhibition, V_{mapp} and k_i were determined. The recovery percentage and inhibition percentage were calculated with the absence and with the presence of different concentrations of these compounds.

المستخلص:

تضمنت الدراسة الحالية قياس فاعلية إنزيم Aspartate aminotransferase (AST) في مصول الأصحاء الذين بلغ عددهم ٤٥ شخص وقد تراوحت أعمارهم بين year (9-35) وقد بلغ معدل فعالية الإنزيم AST حوالي U/L 8.0 وقد تم حساب الفعالية الإنزيمية لـ ٤٠ حالة مرضية تم تشخيصهم بإصابات مختلفة لأمراض الكبد وقد بلغت قيم الـ Total serum bilirubin لهم بين mg/dl[1.6-7.5] بينما تراوحت فعالية إنزيم AST بين U/L[30-83]. هدف الدراسة هو دراسة تأثير عدد من المركبات العضوية حيث تبين إن من مجموع ثماني مركبات عضوية مختارة تبين ان لأربعة مركبات منها فقط لها اثر تثبيطي على فعالية إنزيم AST في حين سلكت المواد الأربعة المتبقية سلوك مواد منشطة. تم تحديد نوع التثبيط للمواد العضوية المثبطة وباستعمال تركيزين مختلفين من المواد المثبطة وباستعمال خمسة تراكيز مختلفة من المادة الأساس، تم حساب قيم ثابت التثبيط k_i و V_{mapp}.

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

Introduction

Aspartate aminotransferase [AST] also known as Glutamic-oxaloacetic transaminase [GOT] [1] . AST is one of two enzymes that catalyze the conversion of the nitrogenous portion of an amino acid to an amino acid residues [2]. It is essential to energy production in the Krebs cycle [3] . AST is found in the cytoplasm and mitochondria of many cells [4] , liver,

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[*] Assist Lecturer / / Institute of Health and Medical Technology Baghdad
[**] Lecturer / College Science for Women / University of Baghdad

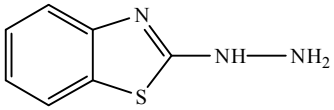
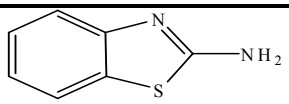
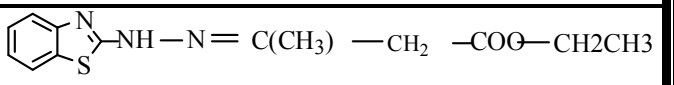
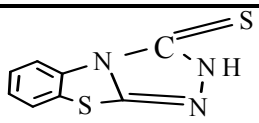
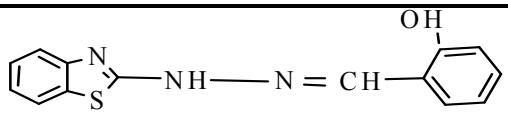
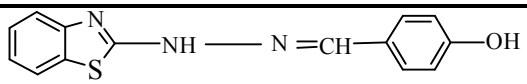
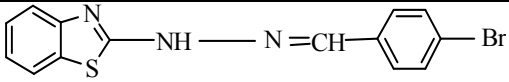
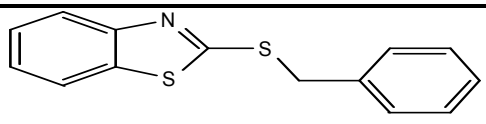
hearts, skeletal muscles, kidneys, pancreas, RBCs [5]. The evaluation of AST activity is a basic procedure for diagnosis and the monitoring of hepato cellular disorders or muscle damage [6][7]. Increased activities of AST in plasma are of considerable diagnostic help in the recognition of myocardial infarction and other conditions associated with myocardial damage [8][9][10].

In liver damage, plasma AST activity is usually more than five times the upper reference value[11][12] if the damage is severe enough to case jaundice, the peak may occur in the prodromal stage [13][14].

In chronic liver disease much smaller elevations are often observed[15], these may indicate containing hepato cellular damage [16][17]. High plasma AST activities may also be found in patients with acute renal disease [18][19] .

Aspartate aminotransferase [AST] catalyzes the transfer of an amino group from specific amino acid [L-aspartate] to specific keto acids [oxaloacetate]. Although at physiological pH the reaction is energetically favored toward the formation of L-aspartate and α-ketoglutarate [20][21]. In vivo the reaction goes to the right to provide a source of nitrogen for urea cycle [22][23].

Table 1 :Name and chemical structure of compounds

Comp.No.		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	
8	2-(benzylthio)-1,3-benzothiazole	

Objective

The aim of this study is to show the effect of some organic compound on AST activity, the literature preview did not contain any studies on the biological effect on AST activity.

Materials and methods

A. Patients :

Forty patients with liver diseases; aged (9-35) years [M±SD: 28.45 ± 6.65]. The medical history was taken concerning the illness.

The sample was taken from specialized center of gastroenterology and hepatology in Baghdad .

B. Healthy Control :

For comparison ,forty five apparently healthy control who were matched for age, [n=45; age=24.87± 5.27 (years); mean ± SD].

C. Reagents :

In vitro the activity of transaminases is determined by measuring the colour of the reaction between 2,4 dinityroplenyl hydrazine (DNPH) and the keto acid which one of the products of transaminase reaction [24][25].The DNPH reacts with all oxoacids [26].

1.Enzyme activity:

AST activity was measured in human serum using colorimetric method [Rittman – Frankel Method][26][27] .

2.Organic compounds:

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

D. Statistical Methods:

Descriptive analysis was used to show the mean and standard deviation of variables. The data were processed with the software package SPSS[statistical package for social sciences) Ver.11 [SPSS Inc. Chicago IL]and Microsoft Excel XP version.

Results

AST activity in human serum was measured according to Reitman – Frankel. Table (2) shows that two groups were chosen, group 1 consists of 45 health persons their age ranged between [9-35] years of age. Group 2 consists of 45 patients with hepatic diseases their age ranged between[8-30]. Total serum bilirubin was estimated for each sample of patient and shows a wide range in patient between [1.6-7.5] mg/dl.

Table (2):showed the AST activity in sera of normal persons and in patients.

Groups of sample	Number	Age (year)		AST activity [U/L]		Normal value [U/L]
		Mean±SD	Range	Mean±SD	Range	
Normal	45	28.45 ± 6.65	9-35	8.00±3.85	6-16	3.85-19.3
Patient	40	24.87± 5.27	8-30	48.80±11.67	30-83	3.85-19.3
Total	85					

The purpose for studying the selected compounds is to find the biological effectiveness of AST activity. The present work shows that some of these compound No. [1, 2, 3, 4] have inhibitory effects, while compounds No. [5, 6, 7, 8] having activator effects.

The inhibitory effect of the DMSO solvent on the activity of AST was [1.65 ± 0.5] %. Table (3) and (4) shows the degree of inhibition of AST 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. We found that [1→4] compounds are a good inhibitor at the used concentrations. The inhibition percentage was [40.67-71.25] % at $5x10^{-2}$ M in patient while in normal the inhibition percentage was [43.48-68.18] %.

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

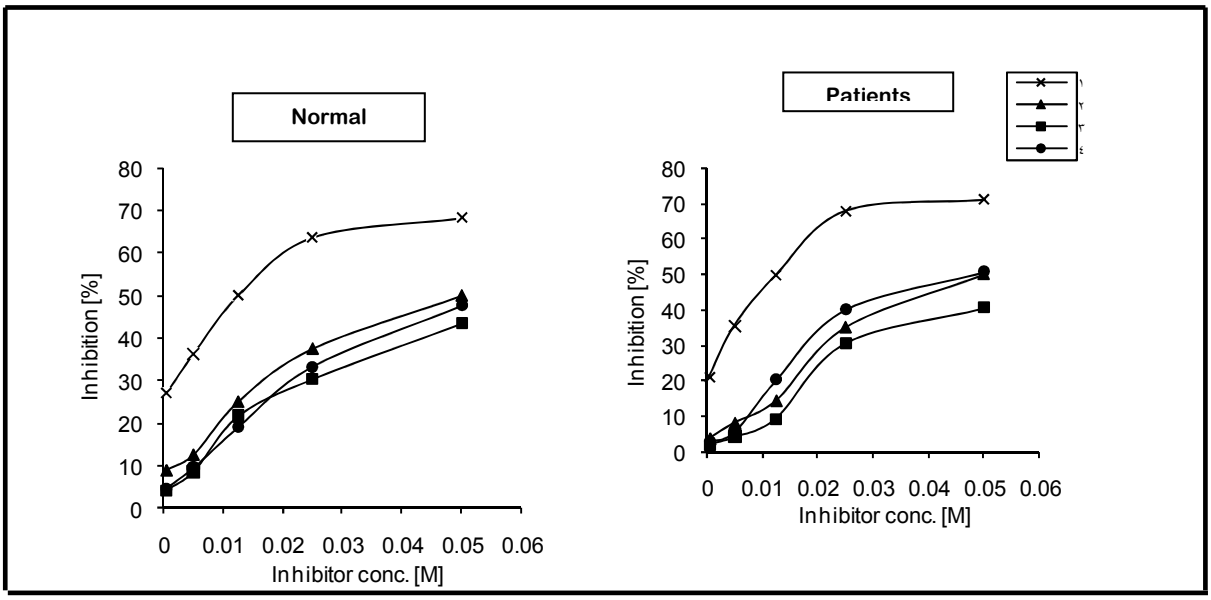


Figure (1) :The inhibitory effect in normal persons and patients

Table (3) : the degree of inhibition of AST activity at [$5x10^{-2}$, $2.5x10^{-2}$, $1.25x10^{-2}$, $5x10^{-3}$, $5x10^{-4}$] M of the compounds using 10 normal samples for all 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	80±0.82	23.00±0.66	71.25	28.75	25.50±0.70	68.12	31.88	40.00±0.50	50.00	50.00	51.60±0.6	35.62	64.38	63±0.5	21.25	78.75
2	80±0.93	40.00±0.80	50.00	50.00	52.00±0.6	35.00	65.00	68.50±0.60	14.38	85.62	73.50±0.7	8.12	91.88	77.00±0.55	3.75	96.25
3	75±0.78	44.5±0.8	70.67	57.33	52.00±0.7	30.67	69.33	68.00±0.5	9.33	90.67	72.00±0.5	4.00	96.00	73.50±0.6	2.00	98.00
4	75±0.84	37.00±0.70	50.67	49.33	45.00±0.8	40.00	60.00	60.0±0.5	20.0	80.00	71.00±0.5	5.33	94.67	73.00±0.5	2.67	97.33

Table (4) : The degree of inhibition of AST activity at [$5x10^{-2}$, $2.5x10^{-2}$, $1.25x10^{-2}$, $5x10^{-3}$, $5x10^{-4}$] M of compounds, using 10 normal samples for all 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	11.00±0.5	3.50±0.5	68.18	31.82	4.00±0.70	63.64	36.36	5.50±0.70	50.00	50.00	7.00±0.60	36.36	63.64	8.00±0.60	27.27	72.73
2	12.00±0.65	6.00±0.7	50.0	50.00	7.50±0.60	37.5	62.50	9.00±0.70	25.00	75.00	10.50±0.50	12.50	87.50	11.00±0.62	8.33	91.67
3	11.50±0.80	6.50±0.6	43.48	56.52	8.00±0.65	30.43	69.52	9.00±0.80	21.74	78.26	10.50±0.60	8.70	91.30	11.00±0.75	4.35	95.65
4	10.5±0.70	5.50±0.5	47.61	52.38	7.00±0.60	33.33	66.67	8.50±0.70	19.05	80.95	9.50±0.50	9.52	90.48	10.00±0.60	4.76	95.24

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

Compounds 2 was found to have higher inhibition effect in sera AST in patients than in normal at [$1.25x10^{-2}$] M while compound No. 3 was found to have higher inhibition effect in sera AST in patients than in normal only at [$2.5x10^{-2}$] M. Compound No. 4 was found to have higher inhibition effect in patient than in normal at [$5x10^{-2}$, $2.5x10^{-2}$, $1.25x10^{-2}$] M.

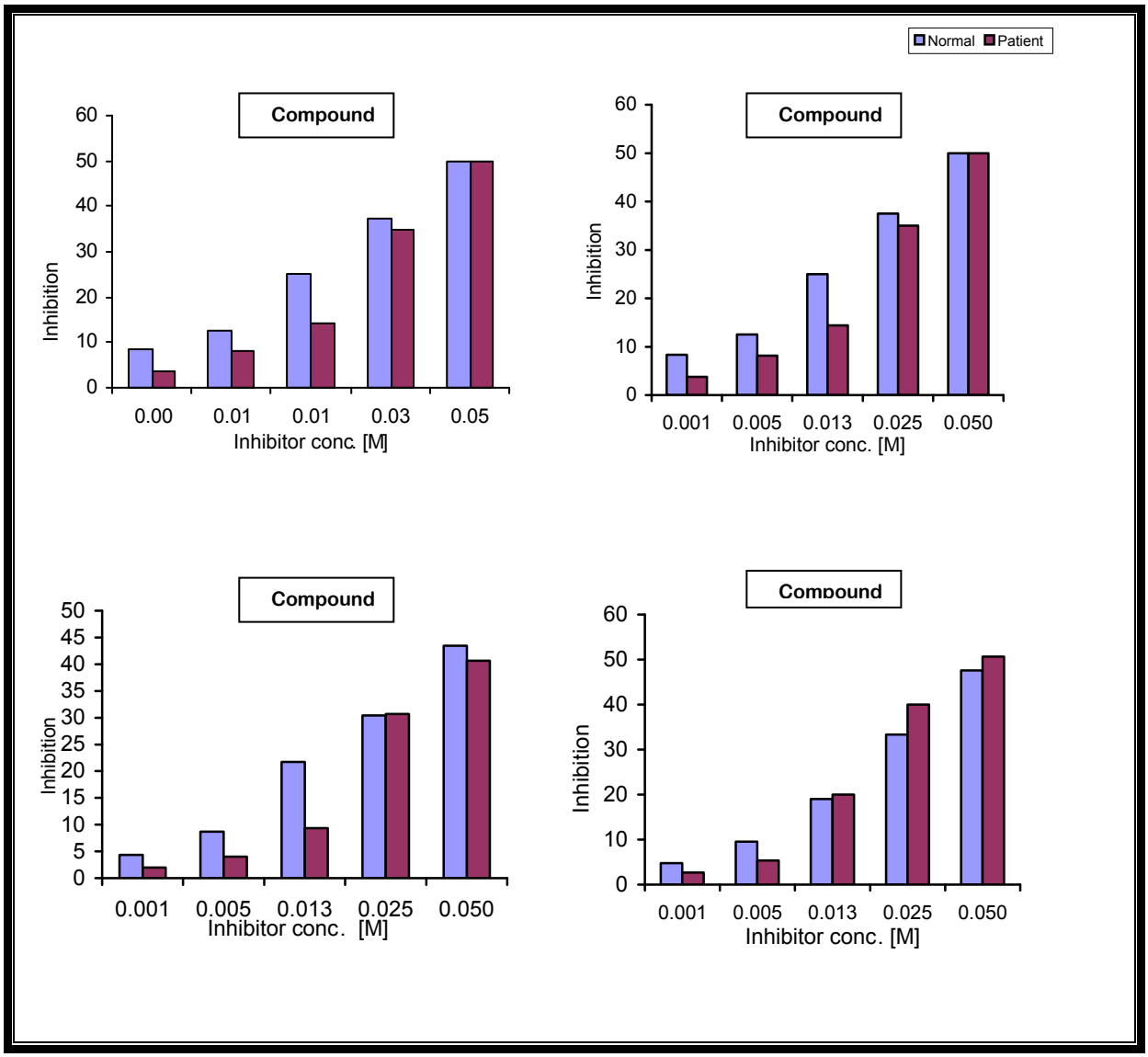


Figure (2) :The inhibitory effect of compounds [1→4] concentration on the inhibition %.

Type of inhibition, V_{mapp} and k_i were estimated by measuring the enzyme activity with absence and presence of inhibitor at different concentrations of substrate under the optimum conditions using Lineweaver Burk equation and plot[28] as shows in table (6) and figure (3).

Table (5): Kinetic parameters and kind of inhibition of AST.

No. of compound	Kind of inhibition	k_m [mM]	$1.25x10^{-2}$ M of inhibitor		$5.0x10^{-2}$ M of inhibitor	
			V_{mapp}	k_i	V_{mapp}	k_i
1	Un competitive	1.33×10^{-1}	21.28	6.615×10^{-1}	19.05	5.27
2	Non competitive	1.34×10^{-1}	22.99	1.59×10^{-1}	22.22	1.54
3	Un competitive	1.33×10^{-1}	20.83	7.06×10^{-1}	18.52	2.826
4	Non competitive	1.34×10^{-1}	22.99	2.917×10^{-1}	22.22	1.893

The results suggest that compounds No. 3 acted as un-competitive inhibitior [29].V_{max} was calculated directly from the graph figure (3) (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 using line weaver Burk equation [29][30].

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

Compounds No.2and No.4 acted as non competitive inhibition, k_m remained constant[29][30][31] .

V_{mapp} was calculated directly from the graph figure 4 [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 AST by organic compounds: compound No. [5, 6, 7, 8] acted as activator to enzyme. Compound No. 6 was chosen to reactivate the activity of inhibited AST. Different concentration of compound No.6 were prepared [$5x10^{-2}$, $2.5x10^{-2}$, $1.25x10^{-2}$, $5x10^{-3}$, $5x10^{-4}$] M.

Table (7) shows the effect of compound No. 6 at different concentrations on the activity of inhibited AST by using compound [1, 2, 3, 4] as inhibitor [using 5 sample for all compound individually.

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

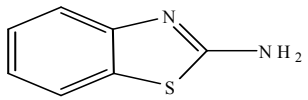
Inhibitor No.	V[U/L] without inhibitor	V with inhibitor $5*10^{-2}$M			Activitor $5x10^{-4}$M with inhibitor $5*10^{-2}$M			Activitor $5x10^{-3}$M with inhibitor $5*10^{-2}$M			Activitor $1.25x10^{-2}$M with inhibitor $5*10^{-2}$M			Activitor $2.5x10^{-2}$M with inhibitor $5*10^{-2}$M			Activitor $5x10^{-2}$M 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	11.50±0.6	3.80±0.4	67.00	33.00	4.0±0.5	65.21	34.78	5.0±0.3	56.52	43.48	7.0±0.4	39.13	60.87	9.0±0.4	21.73	78.27	10.4±0.3	9.57	90.43
2	11.0±0.5	5.4±0.5	51.00	49.00	6.0±0.3	45.45	54.55	7.0±0.4	36.36	63.64	8.4±0.3	23.69	76.36	9.5±0.5	13.64	86.36	10.0±0.4	9.09	90.91
3	12.0±0.5	6.8±0.4	43.33	56.67	7.5±0.2	37.5	62.50	8.0±0.4	33.33	66.67	9.4±0.5	21.67	78.33	10.0±0.3	16.67	83.33	10.8±0.5	10.0	90.0
4	10.5±0.6	5.4±0.5	48.57	51.43	6.00±0.3	42.86	57.14	6.8±0.5	35.24	64.76	7.5±0.4	28.57	71.43	8±0.5	23.81	76.19	10.0±0.5	4.76	95.24

%Inhib. : % Inhibibition.

%Reco. : %Recovery.

Discussion

This research addresses investigation of the effects of eight new derivatives of benzothiozole on human serum AST activity *in vitro*.The differences in potency of inhibition from any compound to another are due to the difference in nature of groups substituted instead of hydrogen atom in benzothiozole compounds .



The present work is the first study that demonstrates the effect of these kind of compounds on the activity of AST enzyme.

The present work shows that some of these compound [2-hydrazino –1,3-benzothiozole**;** 1,3- benzothiozole-2-amine **;** Ethel(4E)-4-(1,3)-benzothiozole-2-yl hydrazino-3-methyl butan-oate **;**and1,9 a-dihydro(1,2,4) trizolo (3,4-b) (1,3) benzothiozole-3(2H)-thione]have inhibitory effects, while compounds [2-hydroxybenzaldehyde-1,3- benzothiozole-2-yl hydrazone **;** 4-hydroxybenzaldehyde-1,3- benzothiozole-2-yl hydrazone **;** 4 - bromobenzaldehyde -1, 3- benzothiozole-2-yl hydrazone**;**and 2-(benzothio**)-**1,3- benzothiozole**]** having activator effects , this may be due to the effect of the compensatory groups on the benzothiozole derivatives**.**

The influence of compound [4-hydroxybenzaldehyde-1,3- benzothiozole-2-yl hydrazone] as reactivator is mainly attributed to the ability of these compounds to raise coordinating number by forming a new coordinating bond to the inhibitory molecule after breaking the bound between these molecule and enzyme in E-inhibitor complex.

References

1- Braunwald E.& et al, *Harrison's principles of internal medicine*, McGraw-Hill, New York, *2001*.
2- Edwards C. & et al ,*Davidson's principles and practice of medicine,* person professional limited, London ,17th edition ,1995
3- Bennett J. & Plumof T. ,*Cecil textbook of medicine* ,W.B. Saunders company, Philadelphia, ,20th edition, 1996.
4- Wilkinson J. & et al,"Liver function test " *J. Clin. Pathol*.,Vol. 25 , pp. 940-944, 1972.
5- Davidson I. & Henty J., *Clinical diagnosis by laboratory methods*, Sanders company, Philadelphia, 1st edition, 1994.
6- Gum portR. &et al , *Student companion for Stryer s Biochemistry* , WH.Free man and company ,New York, 4th edition,1995.

7- Robert A. & et al , *Clinical biochemistry*, Harcourt Brace and company limited, China,1999.
8- Marshall W. ,*Clinical chemistry,*Harcourt Barce and company limited, Spain,2000.
9- Spath L. , Gerstein D. & Finland M. ,"Liver Enzyme" *New Emg. J. Med*., Vol. 21,pp. 1137, 1968.
10- Dennis E., Lwavelle M., *Illustrated Guide to diagnostic tests* ,United States of America.USA.,1993.
11- Whitby L., Peicy I. Robb A,*Lecture Notes on clinical chem*. ,Black Weel scientific Publications,1984.
12- Fischbach F, *A manual of laboratory diagnostic test,* Lippincott company, Philadelphia,1992.
13- Walker C. & et al, *Clinical Laboratory test* , springhouse corporation, Pennsylvania,1991.
14- BurtisC. A. & Ashwood E. R., *Tietz fundamentals of clinical chemistry,*W.B. Saunders company, Philadelphia,1996.
15- Zhu K. & et al, "The relationship of hepatitis and pathologcal dignosis of primary liver cancer" *J. Clin. Epidemiol* ,Vol. 50,PP.297-301,1997.
16- Katsura T. & Nojiri M., Arai H., "liver diseases –especically alcoholic disease" *Jpn. J. Public Health*,Vol. 41,pp. 208-218,1994.
17- Saito Y. & et al, "Health examination " *Jpn. J. Hug*,Vol. 44,pp. 953-961,1989.
18- Walmsleg R. & WhiteG., *A Guide to diagnostic clinical chemistry*, Blackweel scientific publications, USA.,1988.
19- TakahashiA., sekiyaC. & et al, "Hepatic GOT and GPT activities in patients with various liver diseases " *Hokkaido Igaku Zasshi* , Vol. 61 , No.3 , pp.431-6 ,1986.
20- Reitman S. & Frankel S,"Liver enzyme Am. " *J. Clin. Path*.,Vol.28 , No. 1, pp.56,1957.
21- RanikSood M., *Medical Labororatory Technology – methods and interpretations,* Brthers medical publishers, Ltd, New Delhi,1994.
22- Rbert K.& et al, *Harpers Biochemistry,*McGraw-Hill , New York , 2000.
23- Robinson D., "Effect of body mass and other factors on serum liver enzyme levels in men attending for well population screening" *Ann. Clin. Biochem*.,Vol.26,pp. 393-400 ,1989.
24- Henry J. & et al, *Clinical diagnosis and management by laboratory method,*W.B. Saunders company; Philadelphia,1990.
25- DavidC.; Rex Mo. ,*Biochemistry – A case orinted Approach,*Mosby-yearbook, Inc.,USA, 1996.
26- Burtis C. & Ashwood R., *Tietz textbook of clinical chemistry,*W.B. Saunders company, Philadelphia,1994.
27- Schmidt E., "Liver enzyme" *Enzymology Biol. Clin*,Vol. 3,No. 1pp.11-14,1963.
28- David N. & Micheaal M.,*Lehninher principles of Biochemistry,*worth publishers, USA,1994.
29- SeigleI.,*Biochemical Calculations ,* John Wiley & Sons, Inc. New York ,1976.
30- Rowan J.,*Biochemistry,* Carolina Biological Supply company; North Carolina., 1989.
31- Stryer L., *Biochemistry,*WH. Freeman and company, New York, 1988.