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The Effect of [2-(9-anthryl) -3- (1,3,4- Triazole -1- yl) -2,3- Dihydro -5,6- ene – 1,3- Oxazepine- 4,7-Dione] on Serum AST and ALT Activities

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Abstract

In this study , the effect of an organic compound prepared as derivative of oxazepine tested on the activities of aspartate amino trasferase (AST) and alanin amino transferase (ALT). The kinetic study of such enzymes is in the presence of oxazepine derivative.

The results revealed that the organic compound is a non competitive inhibitor for both enzymes.

The Km value for AST is 1.3×10^{-3} M and Vmax for the uninhibited is 200 U/mL and for the inhibited is 111.1 U/mL while Km value for ALT is 2.5×10^{-3} M and Vmax are 89.66 U/mL and 56.77 U/mL for the uninhibited and inhibited enzyme respectively.

Introduction

Aspartate amino transferase (AST) is an enzyme belonging to the class of transferases. It is commonly reffered to as a transaminases and is involved in the transfer of an amino group between aspartate and α- keto acids. The older terminology, serum glutamic oxaloacetic transaminases (SGOT or GOT), may also be used. The transamination reaction is important in intermediary metabolism because of its function in the synthesis and degredation of amino acids. The Keto acids formed by the reaction are altimatly oxidized by the tricarboxylic acid cycle to provide a source of energy [1,2]. Aspartate amino transferase is widely distributed in human tissue. The highest concentrations are found in cardiac tissue, liver and skeletal muscle, with smaller amounts found in the kidney, pancrease and erythrocytes.The clinical use of AST is limited mainly to the evaluation of hepato cellular disorders and skeletal muscle involvement[3].

Alanin amino transferase (ALT) is a transferase with enzymatic activity similar to AST. Specifically, it catalyzes the transfer of an amino group from alanin to α- Keto glutarate with the formation of glutamate and pyruvate. The older terminology was serum glutamic pyruvic transaminase (SGPT or GPT).It is distributed in many tissues , with comparatively high concentrations in the liver. It is considered more liver specific enzyme of the transferases.Clinical applications of ALT assays are confined mainly in evaluation of hepatic disorders. Higher elevations are found in hepato cellular disorder, then in extra hepatic or intrahepatic obstructions of the liver.The elevation of ALT activity are frequently higher than those of AST and tend to remain elevated longer as a result of the longer half-life of ALT in serum[1,2,4].

Derivative of oxazepine organic compound was used to study its effect on liver enzyme's function (i.e AST and ALT). The compound [2- (9-anthryl)-3-(1,3,4- triazole -1-yl) -2,3-dihydro – 5,6-ene -1,3-oxazepine -4,7- dione] is among tricyclic antidepressant compounds. Many of these compounds are adminstrated orally for the treatment of depression as well as anxiety or agitation associated with depression , through blocking post synaptic dopamine receptors in the central nervous system. Many of the metabolic products formed have therapeutic actions. The

rate of metabolism of these agents is variable and influenced by a wide variety of factors. As a result, the half-life of tricarboxylic acid varies considerably among patients. The rate of elimination can also be influenced by the Co adminstration of other drugs that are eliminated by hepatic metabolism. The toxicity of tricarboxylic acids are dose dependant[5,6] .

Experimental

Preparation of organic solutions :-

Thirty seven mg of the organic compomd was dissolved in 10 mL absolute ethanol to prepare 10^{-2} M stock solution .

Series of dilutions were made to prepare [10^{-3} , 10^{-4}, 10^{-5} and 10^{-6} M] solutions.

The enzyme activities were measured according to Biomghreb Kit No. 20039 France.

Principle of GOT (AST) activity measurment :-

Colorimetric determination of GOT activity according to the following reaction :-

L- Aspartate + α - Ketoglutarate oxaloacetate + L- Glutamate

The oxaloacetate formed is measured from derivative with 2,4 Dinitrophenyl hydrazone at 505nm [7].

Principle ALT (GPT) activity measurment :-

Colorimetric determination of GPT activity according to the following reaction

L- Alanine + α- Ketoglutarate $\overset{\text{GPT}}{\rightleftharpoons}$ pyruvate + L- Glutamate.

The pyruvate formed was measured from derivative with 2,4 – Dinitrophenyl hydrazone at 505 nm [6].

The effect of ethanol used as a solvent and diluent was determined by adding a quantity equivalent to the sample and all steps completed as in the procedure used for the determination of GOT and GPT activities [6].

Determination of the percentage of inhibition :-

Using the series of dilutions prepared (10^{-6} -10^{-2} M) of the organic compound, while the concentration of the substrate was kept fixed to get the percentage of inhibition according to the equation :- (The [S] according to the kit is)

$$\% \text{ Inhibition} = 100 - \left(\frac{\text{Activity with inhibitor}}{\text{Activity without inhibitor}} \times 100\right)$$

The inhibitor concentration which is closer to the Km value obtained from the Michaelis – Menten plot of the uninhibited enzyme is used for determination of the type of inhibition which was performed by using different concentrations of substrate with the fixed concentration of the organic compound. The same method of GOT and GPT,activities used by utilizing the same concentrations of substrate without the addition of 10^{-3} M organic compound as inhibitor.

Results and Discussion

The Conc. of substrate was obtained by multiplying the volume pipetted times the total Conc. (202 mmole/L)divided by total volume (24.4).The factor of conversion from ml pipeted to Conc.is 8.28(202/24.4)

For obtaining the activities of GOT and GPT in U/mL , a standard curve was drawn according to the instruction in the Kit. The y- axis is the absorbance and the x- axis for the activity in U/mL.

Figures 1A and 1B represented the GOT and GPT calibration curves respectively.

To obtain U/mL from calibration curve the following equation was applied

Slope =Abs/(U/mL)

Slope=434.78 for GOT ; Slope=170.36 for GPT

Figures 2 and 3 showed Michaelis-Menten polt for GOT and GPT respectively. from Fig.2 the Km value was found to be 1.32×10^{-3} M , and Vmax is 195.7 U/mL for the reaction catalyzed by GOT. From Fig.3 the km value is found to be 1.16×10^{-3} M and Vmax 68.1 U/mL for GPT. Table 1 and 2 showed the effect of different concentrations of the organic compound on GOT and GPT activities respectively.

Figures 4 and 5 were the lineweaver-burk plot for the effect of organic compound on GOT and GPT activities. It is clear that the organic compound has a non-competitive inhibitory effect on both enzymes.

The Km value for GOT is 1.3×10^{-3} M and the Vmax for the uninhibited GOT is 200 u/mL. $Vmax_{app.}$ for the inhibited GOT is 111.1 U/mL in a non-competitive inhibitors.

The Km value for GPT is 2.5×10^{-3} M for both uninhibited and inhibited enzyme and the Vmax is 89.66 U/mL for the uninhibited enzyme. $Vmax_{app.}$ for the inhibited GPT is 56.77 U/mL in a non – competitive inhibitors, the Vmax for a reaction catalyzed by the enzyme is reduced in the presence of inhibitor even if substrate were saturating ($S >> Km$),the observed Vmax will be lower than it would be in the absence of the inhibitor because the non-competitative inhibition, the inhibitor binds to Enzyme E and (ES) enzyme substrate complex[8].

Non- competitive inhibition occurs when the inhibitor and substrate bind at different sites on the enzyme. Non –competitive inhibition can not be overcome by increasing the concentration of substrate thus non-competitive inhibitors decrease the apparent Vmax of the reaction , Since non-competitive inhibitors donot interfere with the binding of substrate to enzyme.

Thus the enzyme shows the same Km in the presence or absence of the non-competitive inhibitor[9,10].

There are no studies in the literature about the effect of oxazepine derivatives on AST and/or ALT.

The variable inhibitory effect of the synthesized derivative under study on serum AST and ALT may be due to the change in the stereostructure of the enzyme in the presence of such derivative or the binding of this derivative to some side chains of the amino acids present in the active site.

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Table: (1) Effect of different concentrations of the organic compound on GOT activity

Conc.	$1*10^{-5}$	$1*10^{-4}$	$1*10^{-3}$	$1*10^{-2}$
%GOT	22	17.1	9.8	26.8

Table: (2) Effect of different concentrations of the organic compound on GPT activity

Conc.	$1*10^{-5}$	$1*10^{-4}$	$1*10^{-3}$	$1*10^{-2}$
%GPT	9.05	6.91	10.6	16.4

Table: (3) kinetic parameters of AST and ALT before and after the addition of oxazepine derivative.

Kinetic parameters / enzymes	Before		After	
	Vmax U/mL	Km	$Vmax_{app.}$ U/mL	$Km_{app.}$
AST	200	1.3×10^{-3} M	111.1	1.3×10^{-3} M
ALT	89.66	2.5×10^{-3} M	56.77	2.5×10^{-3} M

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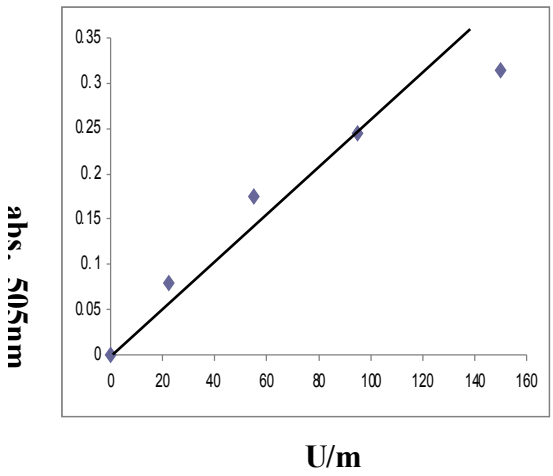

Fig.(1A)Calibration curve of GOT

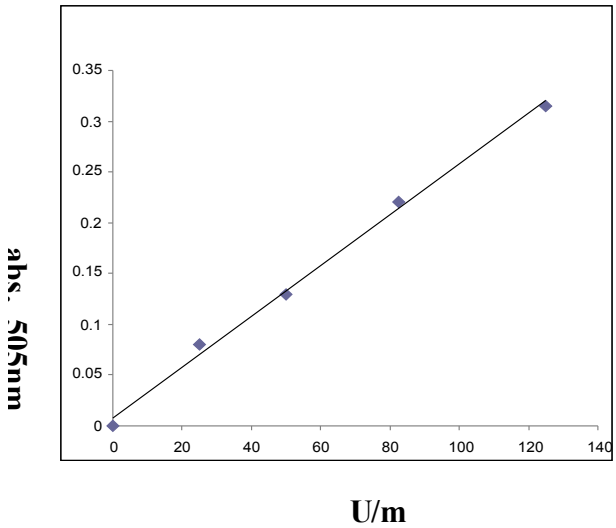

Fig. (1B)Calibration curve of GPT

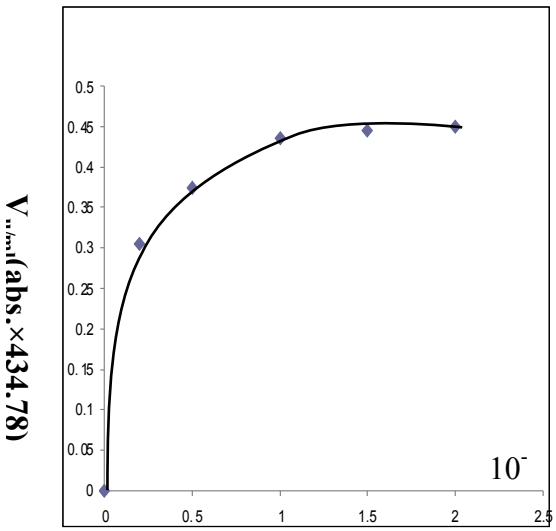

Fig.(2) Michaelis-Menten polt of GOT

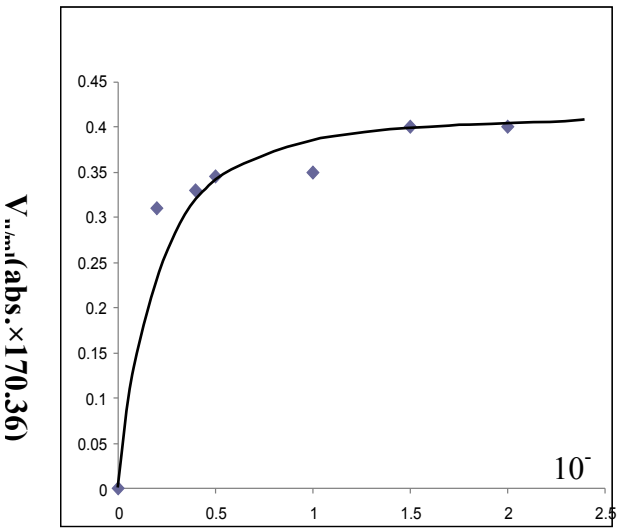

Fig.(3) Michaelis-Menten polt of GPT

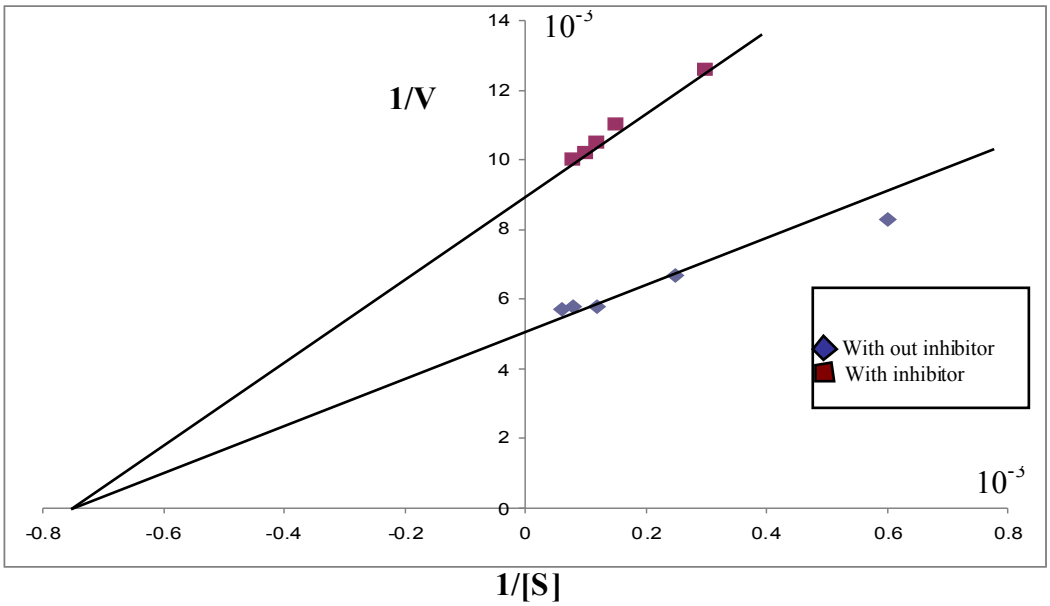

Fig.(4) Lineweaver-burk plot for the effect of organic compound on GOT activity

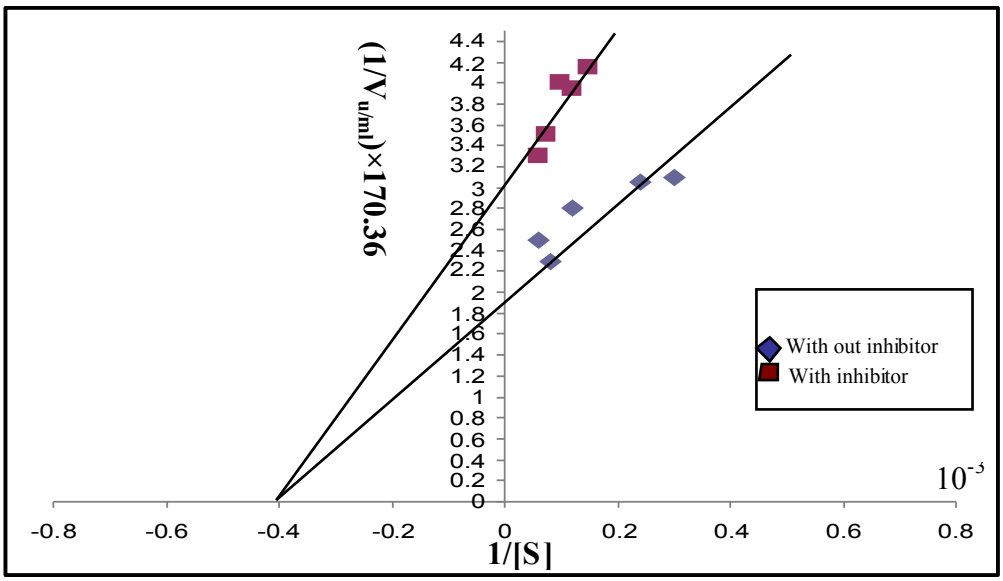

Fig.(5) Lineweaver-burk plot for the effect of organic compound on GPT activity

مجلة ابن الهيثم للعلوم الصرفة والتطبيقية المجلد23 (2) 2010

تأثير [2-(9- انثريل)-3- (1 ، 3 ، 4 -ترايازول – 1 – يل) – 2 ، 3 –داي هايدرو – 5 ، 6 – يين – 1 ، 3 – اوكسازبين – 4 ، 7 – دايون] في فعالية انزيمات AST ، ALT في مصل دم الانسان

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الخلاصة

تم في هذه الدراسة دراسة تأثير مركب عضوي مثل مشتق الاوكسازبين في فعالية انزيمات اسبارتيت امينو ترانسفريز (AST)، والانين امينو ترانسفريز (ALT) . ان الدراسة الحركية لهذه الانزيمات بوجود مشتقات الاوكسازبين لها اهمية خاصة لان بعض هذه المشتقات تستعمل بصورة واسعة مضادات للكآبة وبما ان عملية ازالة هذه العقاقير يتم من خلال عمليات أيض الكبد لذا فأن تأثير هذه العقاقير على كفاية عمل الكبد تحتل اهتمام خاص .

أظهرت نتائج الدراسة ان المركب العضوي مثبطاً لا تنافسياً بالنسبة الى الانزيمين . وان قيمة ثابت مايكلس Km لانزيم AST هي M 1.3×10^{-3} وان السرعة القصوى Vmax للانزيم غير المثبط هي 200 U/mL والانزيم المثبط هي 111.1 U/mL بينما كانت قيمة Km لانزيم ALT هي 2.5×10^{-3}، و Vmax 89.66 U/mL و 56.77 U/mL للانزيم غير المثبط والمثبط على التوالي .