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 $1.3 \times 10^{-3}$ 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 $2.5 \times 10^{-3}$ 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 referred 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 degradation of amino acids. The Keto acids formed by the reaction are ultimately 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, pancreas 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 administrated 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-administration 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 compound 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 measurement** :-
Colorimetric determination of GOT activity according to the following reaction :-
$$\text{L- Aspartate} + \alpha - \text{Ketoglutarate} \rightarrow \text{oxaloacetate} + \text{L- Glutamate}$$

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

**Principle ALT (GPT) activity measurement** :-
Colorimetric determination of GPT activity according to the following reaction
$$\text{L- Alanine} + \alpha - \text{Ketoglutarate} \rightarrow \text{pyruvate} + \text{L- Glutamate}.$$  
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 )

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

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 pipetted 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)

Figures 2 and 3 showed Michaelis-Menten plots for GOT and GPT respectively. From Fig. 2 the Km value was found to be $1.32 \times 10^{-3}$ M, and Vmax is 195.7 U/mL for the reaction catalyzed by GOT. From Fig. 3 the km value is found to be $1.16 \times 10^{-3}$ 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 $1.3 \times 10^{-3}$ 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 $2.5 \times 10^{-3}$ 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-competitive 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 do not 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.

References
6- Schatzbery ,A.F.; (2002),pharmacological principles of antidepressant efficacy . Human psychopharmacol (suppl 2 ) : 17-22.

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

<table>
<thead>
<tr>
<th>Conc.</th>
<th>1*10^-5</th>
<th>1*10^-4</th>
<th>1*10^-3</th>
<th>1*10^-2</th>
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</thead>
<tbody>
<tr>
<td>%GOT</td>
<td>22</td>
<td>17.1</td>
<td>9.8</td>
<td>26.8</td>
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</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Conc.</th>
<th>1*10^-5</th>
<th>1*10^-4</th>
<th>1*10^-3</th>
<th>1*10^-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>%GPT</td>
<td>9.05</td>
<td>6.91</td>
<td>10.6</td>
<td>16.4</td>
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</table>

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

<table>
<thead>
<tr>
<th>Kinetic parameters</th>
<th>Before</th>
<th></th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>enzymes</td>
<td>Vmax U/mL</td>
<td>Km</td>
<td>Vmax&lt;sub&gt;app&lt;/sub&gt; U/mL</td>
</tr>
<tr>
<td>AST</td>
<td>200</td>
<td>1.3×10^-7 M</td>
<td>111.1</td>
</tr>
<tr>
<td>ALT</td>
<td>89.66</td>
<td>2.5×10^-7 M</td>
<td>56.77</td>
</tr>
</tbody>
</table>
Fig. (1A) Calibration curve of GOT

Fig. (1B) Calibration curve of GPT

Fig. (2) Michaelis-Menten plot of GOT

Fig. (3) Michaelis-Menten plot 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
تأثير [2-(9-إثنينيل)-3-(1، 3، 4-مترايازول-1-يل) -2، 3-داي هايدرو-5، 6-بين-1، 3-أوكسازين-4، 7-دايون] في فعالية إنزيمات ALT، AST

الخلاصة

تم في هذه الدراسة دراسة تأثير مركب عضوي مثل مشتقت الأوكسازين في فعالية إنزيمات إسبارتيت امينو ترانسفريز (ALT) والمثبطة، والنابين أمينو ترانسفريز (AST). الان الدراسة الحركية لهذه الإنزيمات يوجود مشتقات الأوكسازين لها أهمية خاصة لأن بعض هذه المشتقات تستعمل بصورة واسعة مثلاً مضادات للالتهاب ولكنها لأن عملية إزالة هذه الفعالية يتم من خلال عمليات أخرى من الكبد، لذا فإن تأثير هذه الفعالية على كفاءة عمل الكبد تحتل اهتمامًا خاصًا.

كما أظهرت نتائج الدراسة أن المركب العضوي مثبطًا لـ AST لإنزيم غير المثبط في Vmax 200 U/mL، والإنزيم المثبط في 56.77 U/mL و Vmax 89.66 U/mL، لإنزيم غير المثبط والمثبط على التوالي.