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By Salma Abdul Rudhaabbass , Manalsadeqhammood , Wafa Raji Mohammed & Amer Hasan Abdullah

Al-Mustansiryh University

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Antagonist Effect of Theophylline and Caffeine on Some Transaminase Enzymes Activities

Salma Abdul Rudhaabbass [α], Manalsadeqhammood [σ], Wafa Raji Mohammed [ρ] & Amer Hasan Abdullah [ω]

Abstract - The purpose of this study is to show the effect of caffeine and theophylline on the activities of aspartate aminotransferase AST and alanine aminotransferase ALT, in the human sera. Serum AST and ALT were activated by caffeine, while inhibiting by theophylline, this effect increased with increasing the concentration of theophylline and caffeine. Kinetic properties of AST and ALT activities revealed by theophylline and that non-competitive inhibition type, and non-competitive activation by caffeine.

I. Introduction

Theophylline and caffeine are natural compounds that are made by plants. They are classified as a member of the xanthine family alkaloid[1]. Figure (1) shows their structure:-

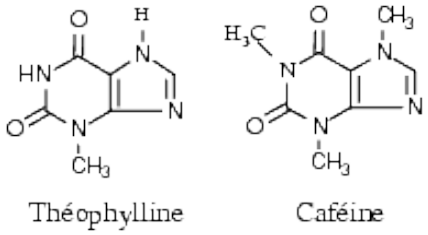

Figure (1) : Theophylline and caffeinestructure[2]

Coffee consumption is worldwide spread with few side effects. Interestingly, coffee intake has been inversely related to the serum enzyme activities gamma glutamyltransferase, and ALT in studies performed in various countries. In addition, epidemiological results, taken together, indicate that coffee consumption is inversely related with hepatic cirrhosis; however, they cannot demonstrate a causative role of coffee with prevention of liver injury figure (2) shows the negative and positive effect of caffeine [3,4].

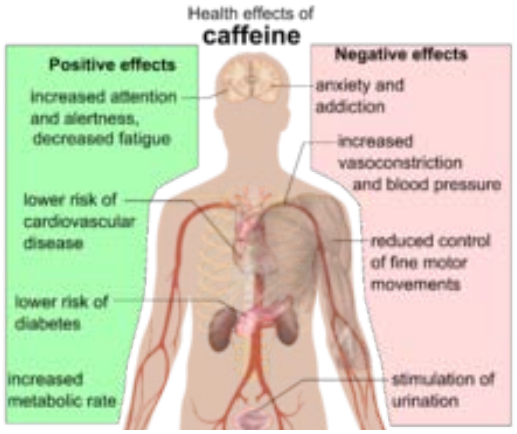

Figure (2) : Positive and negative effect of caffeine[5]

Theophylline also known as dimethylxanthine, it a beans structural and trace amounts (~1 mg/L) ,significantly less than therapeutic doses .It is found also in cocoa beans. Amounts as high as 3.7 mg/g have been reported in Criollo cocoa beans pharmacological similarity to caffeine. It is naturally found in tea, although in [6]. Theophylline and caffeine from coffee or other beverages are easily absorbed by the stomach and small intestine of ingestion, and it is rapidly distributed throughout all tissues of the body. The volume of distribution may increase in neonates and those suffering from cirrhosis or malnutrition, whereas the volume of distribution may decrease in those who are obese.[7]

Theophylline and caffeine are metabolized in the liver, through demethylation and oxidation, it forms three dimethylxanthines, and each of these metabolites is further metabolized and then excreted in the urine. Methylation caffeine is also important in the infant population. Smokers and people with hepatic impairment metabolize it differently. Caffeine causes an increase in blood flow to the kidneys and an increase in the production of urine. It also decreases the tubular reabsorption of sodium and water, resulting in more dilute urine. Caffeine stimulates skeletal muscle by increasing the strength of contraction and decreasing fatigue. It also stimulates the breakdown of glycogen and lipids to enhance endurance [8]. In view of the importance of transferees enzymes reactions like GOT, and GPT which form links between the metabolism of amino acids, carbohydrates and fats [9].

Author α σ ω : Department of Chemistry; College of Science; Al-Mustansiryh University.
Author ρ : Nursing department; Technical Medical Institution; Technical Medical Foundation.

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a) How Caffeine Work

Adenosine helps prepare the body for sleep by curbing the chatter between nerve cells and by widening blood vessels to increase the flow of oxygen. Receptors on the surface of brain cells can't tell the difference between adenosine and caffeine. So when you consume caffeine, it attaches itself to the receptors and adenosine is shut out. Without adenosine to make you sleepy, your brain activity perks up and you're more alert. By blocking adenosine, caffeine also constricts your blood vessels, which makes your headache disappear [10].

b) Aim of Study

Peoples in the world consume large quantities of theophylline and caffeine through having main sources of theophylline and caffeine in general life which are: coffee, tea, chocolate, drugs…etc, therefore This study was designed to show the effects of caffeine and theophylline on some transaminase enzymes such as GOT and GPT.

II. Materials and Methods

a) Effect of theophylline and Caffeine on GOT and GPT activities

Colorimetric determination of GOT or GPT activity according to the following reactions:-

$$\text{L-Aspartate} + \alpha\text{-ketoglutrate} \xrightarrow{\text{GOT}} \text{Oxaloacetic} + \text{glutmate}$$

$$\text{Alanine} + \alpha\text{-ketoglutrate} \xrightarrow{\text{GPT}} \text{pyruvate} + \text{glutmate}$$

The pyruvate or oxaloacetate formed was measured in its derivates form 2, 4-dinitropheny-lhydraone, which was absorbed at wave length 505 nm [11] .

A-.A stock solution (0.1M) of caffeine and theophylline compounds was prepared and the following concentration of ($1x10^{-2}$, $1x10^{-3}$, $1x10^{-4}$, $1x10^{-5}$, $1x10^{-6}$,$1x10^{-7}$,$1x10^{-8}$) M were prepared by diluting with distilled water.

The enzymes GOT and GPT activities were measured in human serum by using the same methods of these enzymes with replace 100μl of buffer with 100μl of compound.

The inhibition percentage was calculated by comparing the activity with and without the compound and under the same conditions, according to the equation:-

$$\% \text{ Inhibition} = 100 - 100 \times \frac{\text{The activity in the presence of inhibitor}}{\text{The activity in the absence of inhibitor}}$$

The activation percentage was calculated by comparing the activity with and without the activator and under the same conditions, according to the equation:-

$$\% \text{ Activation} = 100 \times \frac{\text{The activity in the presence of activator}}{\text{The activity in the absence of activator}} - 100$$

B- A constant concentration of compound (10^{-1}, $10^{-2}$and 10^{-3}) M were used with different substrate concentrations of (40, 80,120,160, 200) mmol/L for GOT and GPT, to study the type of inhibition .Buffers were used to prepare different substrates concentrations of these enzymes, (phosphate buffer pH 7.4, 100 mmol/L)

The enzymes activities were determined with and without compound, by using the Lineweaver-Burk equation and plotting 1/v against 1/[s] were evaluated values [12]:-

a) ki, b) Apparent v_{max} (v_{mapp}), c) Apperent k_m (k_{mapp}),

d) Type of inhibition.

III. Results and Discussion

This research addresses investigation of the effects of caffeine and theophylline on GOT and GPT enzymes. The biochemical tests revealed that activatory effects of caffeine on GOT and GPT enzymes activities, while theophylline caused inhibitory effects on GOT and GPT enzymes activities ,Figures 3A, 3B, 5A,and 5B respectively .

The normal value of the GOT enzyme activity was (77 U/L). The relationship between caffeine concentration versus and the activity of this enzyme as shown in figure 3A, these results observed that any increase in compound concentrations caused increase

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in percentage of activation of enzyme. The greater activation of caffeine was demonstrated at concentration (0.1M) (20.78 %) .

While the normal value of the GOT enzyme activity was (105 U/L). to theophylline results observed that any increase in theophylline concentrations caused increase in percentage of inhibition of GOT enzyme ,this relationship illustrated in figure 5A . The greater inhibition was demonstrated at concentration (0.1M) (40.9 %) .

The normal value of the GPT enzyme activity was (55 U/L). The relationship between caffeine concentration versus and the activity of enzyme as shown in figure 3B, these results observed that any increase in compound concentrations caused increase in percentage of activation of enzyme. The greater activation of caffeine was demonstrated at concentration (0.1) M was (22.72 %).

The normal value of the GPT enzyme activity was (33 U/L). The relationship between theophylline concentration versus and the activity of enzyme, these results observed that any increase in theophylline concentrations caused increase in percentage of inhibition of GPT enzyme illustrated in figure 5B. The greater inhibition was demonstrated at concentration (0.1M)(54.5 %) .

Competitive, noncompetitive and uncompetitive inhibition can be easily distinguished with the use of double reciprocal plot of the Lineweaver-Burk plot. Two sets of rate determination in which enzyme concentration was held constant, were carried out. In the first experiment the velocity of enzyme without inhibitor was established, in the second experimental constant amount of inhibitor is included in each enzyme assay. Varieties of substances have the ability to reduce or eliminate the catalytic activity of specific enzyme [13-16].

Table (1) and figure 6A, showed that the type of enzyme activation using Lineweaver-Burk plot for caffeine on serum GOT activity. The V_{max} and K_m with (10^{-1} and 10^{-8})M of caffeine and without it, V_{max} and K_m without caffeine was 67 U/L, 200 M respectively. A liquate 10^{-1} and 10^{-8} M of caffeine were non-competitive activation for enzyme activity. Non-competitive activation changed the V_{max} of the enzyme but not the K_m. When concentration of caffeine (10^{-1}, 10^{-8}) M the V_{max} were (200,100) M respectively. By using Lineweaver-Burk equation was calculated the Ki values of enzyme for compound which was studied in different concentration. The Ki of caffeine in (10^{-1}, 10^{-8}) M were (0.15, 3 x 10^{-8}) M respectively.

Table (2) and figure 7A showed that the type of enzyme inhibition using Lineweaver-Burk plot for theophylline on serum GOT activity. The Vmax and Km with (10^{-1}, 10^{-2} and 10^{-3}) M of theophylline and without it , Vmax and Km without theophylline was 125 U/L ,20 M respectively. A liquate 10^{-1}, 10^{-2} and 10^{-3}M of theophylline were non-competitive inhibition for enzyme activity. Non-competitive inhibition changed the Vmax of the enzyme but not the Km. When concentration of theophylline (10^{-1}, 10^{-2} and 10^{-3})M the V max were (76.9,90.9,111.1) U/L respectively. By using Lineweaver-Burk equation was calculated the Ki values of enzyme for theophylline which was studied in different concentration. The Ki of theophylline in (10^{-1},$10^{-2}$and 10^{-3}) M were (16x10^{-2}, 26 x 10^{-4} , 8x10^{-4}) M respectively.

Table (1) and figure (4) B1 showed that the type of enzyme inhibitor using Lineweaver-Burk plot for caffeine on serum GOT, GPT activity. The V_{max} and K_m with (10^{-1} and 10^{-8}) M of caffeine and without it, V_{max} and K_m without caffeine was 37.037U/L, 0.40 M respectively. A liquate 10^{-1} and 10^{-8} M of caffeine were competitive inhibtion for enzyme activity. Competitive inhibition changed the K_m of the enzyme but not the V_{max}. When concentration of caffeine (10^{-1}, 10^{-8}) M the K_m were (1.429, 0.714) M respectively. By using Lineweaver-Burk equation was calculated the Ki values of enzyme for compound which was studied in different concentration. The Ki of caffeine in (10^{-1}, 10^{-8}) M were (0.0388, 1.27 x 10^{-8}) M respectively.

Table (2) and figures 7A and 7B showed that the type of enzyme inhibition using Lineweaver-Burk plot for theophylline on serum GOT, GPT activity. The Vmax and Km with (10^{-1}, 10^{-2} and 10^{-3}) M of theophylline and without it, the GOT Vmax and Km without theophylline was (76.9 ,90.9 ,111.1) U/L, and (16x10-^{2},26x10^{-3},8x10^{-4}) M respectively. A liquate 10^{-1}, 10^{-2} and 10^{-3}M of theophylline were non-competitive inhibition for GPT enzyme activity, when concentration of theophylline (10^{-1},10^{-2} and 10^{-3})M the Vmax were (27.7,29.4,30.3,) U/L respectively. By using Lineweaver-Burk equation was calculated the Ki values of enzyme for theophylline which was studied in different concentration. The Ki of theophylline in (10^{-1},10^{-2} and 10^{-3}) M were (62x10-^{2},10 x10^{-2},15x10^{-3}) M respectively.

The enzymes play important role in amino acid metabolism and in the urea and tricarboxylic acid cycles. Therefore activation or inhibition of GOT and GPT enzymes may affect of metabolism of carbohydrates, proteins and lipids. So it was useful to study effect of any compound which was intake by foods or drugs on these enzymes and other enzymes that related to the metabolism. We suggested that theophylline molecule has (N-and O=) groups by which, it inhibits the active sides of GOT and GPT enzymes by decreasing affinity of active sides of enzymes to react with the substrate. The results of our study is in agreement with before studies of same enzymes[17-20], and the results is in disagreement with before study of same enzymes[21],which was study the effect of caffeine on GOT and GPT enzymes activities, the study showed activation of GOT and GPT by caffeine. Theophylline and caffeine exist in plants such as coffee, tea,cocoa beans. Theophylline and caffeine are rapidly and completely absorbed, distributedin the extracellular fluid, in the central nervous system and metabolized in the

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liver [22]. Theophylline was inhibition of GOT and GPT, caffeine was activation of these enzymes in the same plant and in the same time. Therefore we suggest that the theophylline and caffeine have no effect on GOT and GPT when the plants (coffee, tea, cocoa beans) are intake.

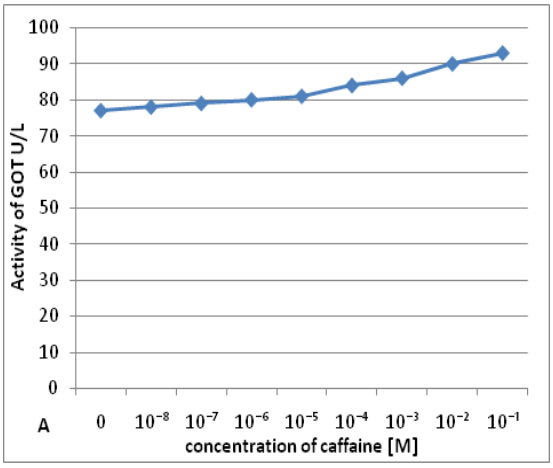
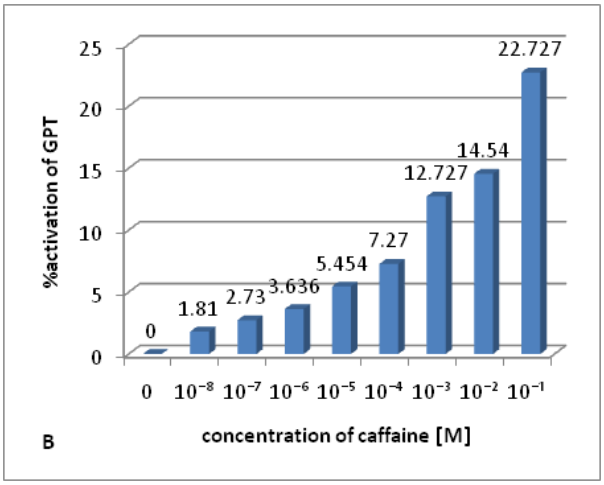
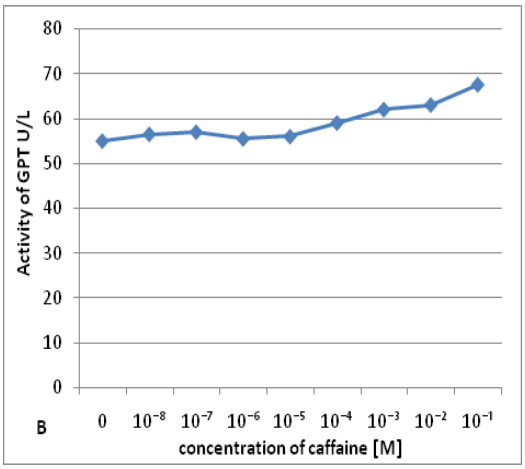


Figure (3A) : The relationship between caffeine and GOT activity



Figure(4B) : The relationship between caffeine and GPT% activity



Figure(3B) : The relationship between caffeine and GPT activity

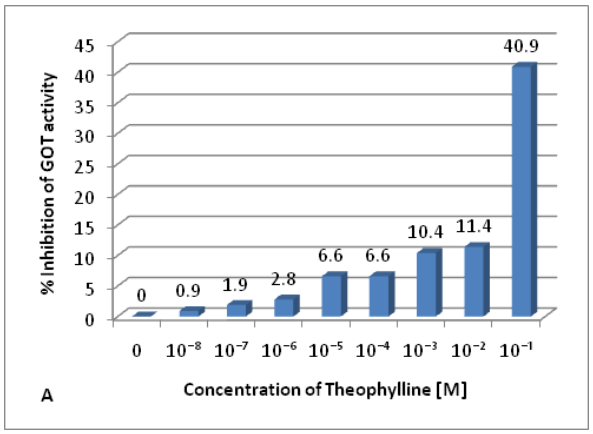


Figure (5A) : The relationship between theophylline and GOT % activity

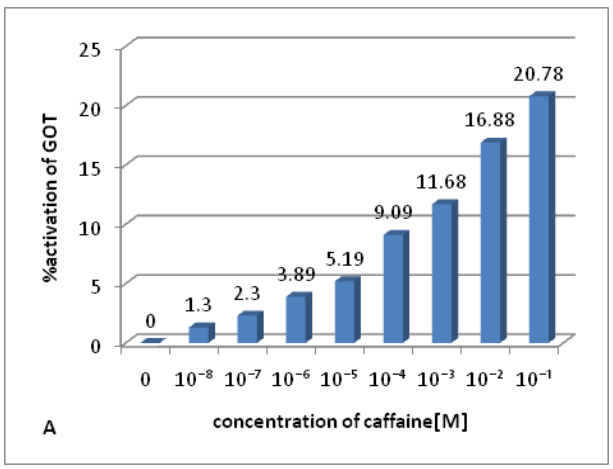
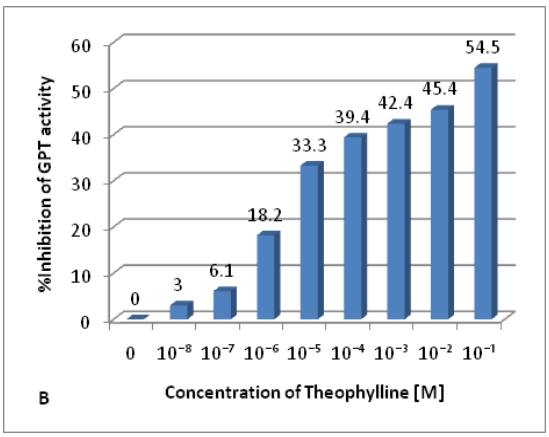


Figure (4A) : The relationship between caffeine and GOT % activity



Figure(5B) : The relationship between and theophylline GPT% activity

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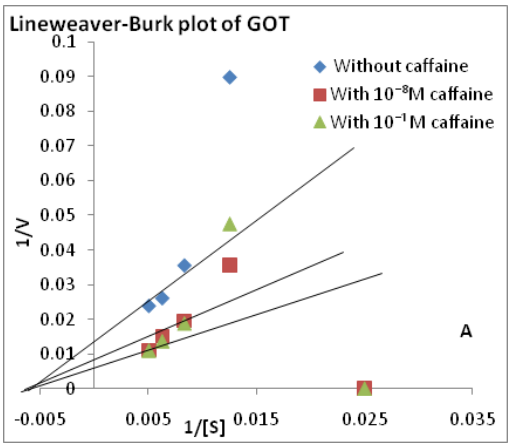

Figure (6A) : Lineweaver-Burk plots for caffeine effects on GOT

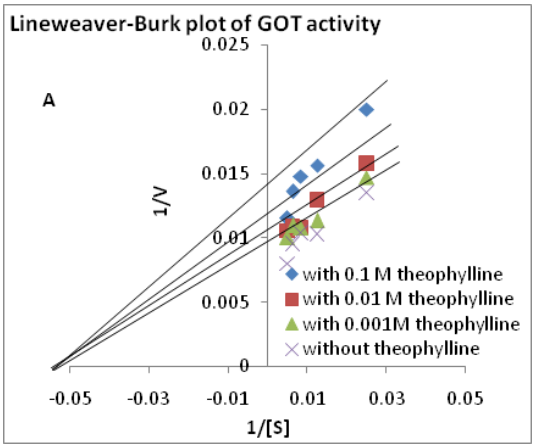

Figure (7A) : Lineweaver-Burk plots for theophylline effects on GOT

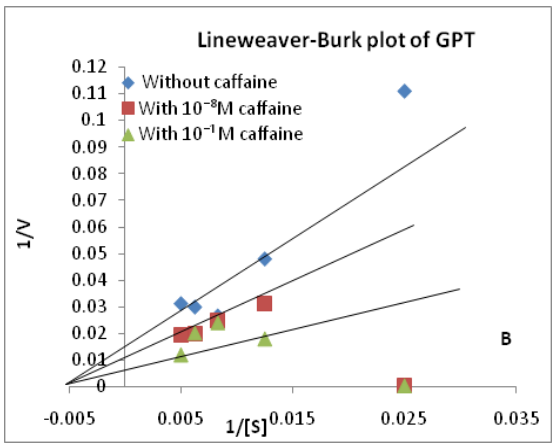

Figure (6B) : Lineweaver-Burk plots for effects on G caffeine GPT

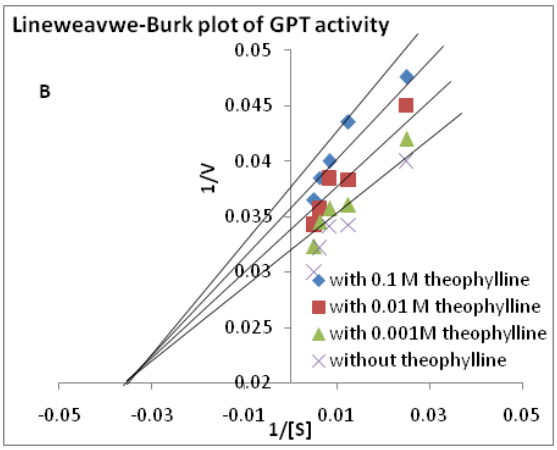

Figure (7B) : Lineweaver-Burk plots for theophylline effects on GPT

Table1 : The kinetic properties of GOT,GPT with caffeine

Enzymes	Con.of caffeine	K_{map} (M)	V map U/L	Ki (M)	Type of effect
GOT	10^{-1}	200	200	0.15	Non-competitive
	10^{-8}	200	100	3×10^{-8}	
GPT	10^{-1}	400	250	0.1666	Non-competitive
	10^{-8}	400	111.111	1×10^{-7}	

Table 2 : The kinetic properties of GOT and GPT, with theophylline.

Enzymes	Con.of theophylline	V map U/L	Ki (M)	Type of inhibition
GOT	10^{-1}	76.92	16×10^{-2}	Non- competitive
	10^{-2}	90.90	26×10^{-3}	
	10^{-3}	111.11	8×10^{-4}	

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GPT	10^{-1}	27.7	$62x10^{-2}$	Non-competitive
	10^{-2}	29.411	$10x10^{-2}$	
	10^{-3}	30.30	$15x10^{-3}$	

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