Effect of Caffeine on Some Transferase Enzymes Activities

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Abstract

This study was designed to show the effects of caffeine on the activities of aspartate aminotransferase (AST), alanine amino transferase (ALT), gama glutamate transferase (γ-GT) enzymes in the sera. Caffeine demonstrated activation on (AST) and (ALT) activities, inhibitory effects on the (γ-GT) activity, and these effects increased with increasing the concentration of the compound. Kinetic properties of (AST) and (ALT) activities revealed (by caffeine) non-competitive type of activation, and of (γ-GT) activity competitive inhibitors

Keywords: GOT, GPT, GGT

1. Introduction

Caffeine is a natural compound that is made by plants. Caffeine is classified as a methyl xanthine alkaloid. Molecular structure of Caffeine has:

At present there are 63 different species of plants known to contain caffeine, the most common food sources of caffeine in the human diet have been coffee (from the coffee plant), tea (from the tea plant), and chocolate (from the cacao plant) [Koppelstaetter, 2005].

Caffeine from coffee or other beverages is easily absorbed by the stomach and small intestine within 45 minutes of ingestion, and it is rapidly distributed throughout all tissues of the body [Liguori A, 1997]. Caffeine is 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 [Fisone, 2004].

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 [Fredholm, 1999].

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 [Latini S, 2001; Addicot MA, 2009].

In view of the importance of transferase enzymes reactions like Glutamate Oxaloacetate Transaminase (GOT), Glutamate Pyruvate Transaminase (GPT) and Gama Glutamate Transferase (GGT) which form links between the metabolism of amino acids, carbohydrates and fats

1.1 Glutamate Oxaloacetate Transaminase (GOT) activity (EC 2.6.1.1)

It's called also aspartate aminotransferase (AST), is one of the most important of transaminases enzyme, and catalyzes the transfer of the amino group of aspartate to α-ketoglutrate. GOT is widely distributed in human tissues; heart, liver, skeletal muscle and kidney. The optimum conditions of maximum enzyme activity are pH=7.4, temp. = 37cº. The enzyme stability 3 days in 25 cº, 1 week in 5 cº and 1 month in -25 cº. The clinical usefulness of the enzyme is largely restricted to the diagnosis of heart and liver diseases. Large amount of GOT may be released into the blood. Very high levels are observed in acute liver disease while lesser elevation is seen in chronic liver disease [L.Stryer, 1988; Robert, 1997; Joan F., 1988].

1.2 Glutamate Pyruvate Transaminase (GPT) activity(EC 2.6.1.2)

It's also called alanine amino transferase (ALT) which is prevalent in mammalian tissue catalyzes the transfer of the amino group of alanine to α-ketoglutrate.GPT found in a highest concentration in liver in spite of its active occurrence in skeletal muscles, heart and kidney. The GPT activity in tissues is generally less than GOT. Significant elevation of S.GOT occurs in sever acute hepatitis where the enzyme is released in to the circulation. GPT level is found to increased in the following diseases; infection hepatitis, liver cirrhosis and biliary cirrhosis, obstructive jaundice, liver cancer [Joan F., 1988; Thommas M., 1982; Ottaway G.H., 1984; Harry R.Mathews, 1997].

1.3 Gama Glutamate Transferase (γ-GT) activity (EC 2.3.2.2)

It's also called Gama Glutamate Transpeptidase (γ-GT) or (GGT) is found in kidneys and liver and catalyzes the transfer of a gamma-glutamyl group from glutathione (GSH)to an amino acid. GGT levels are increased in most forms of liver disease, especially cholestasis, a plasma membrane-bound enzyme, provides the only activity capable to effect the hydrolysis of extracellular glutathione, thus favoring the cellular utilization of its constituent amino acids [Elsevier, 1997].
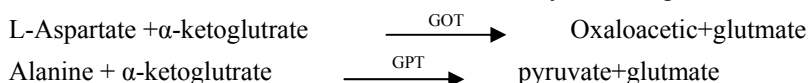
2. Aim of study

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

3. Materials and methods

3.1 Effect of caffeine on GOT and GPT activities

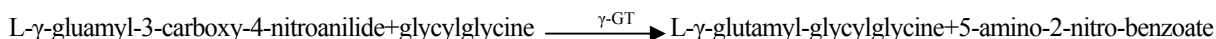Colorimetric determination of GOT or GPT activity according to the following reactions:

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

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

The pyruvate or oxaloacetat formed was measured in its derived form 2,4-dinitrophenylhydraone, which was absorbed at wave length 505 nm [Reitman, 1957; Cabaud, 1956; Karmen, 1955].

3.2 Effect of caffeine on γ-GT activity

Kinetic colorimetric method for the determination of γ-GT activity was assayed by Persijn and Van Der Slik [Persijn, 1976; Z.Klin, 1974]. The principle of the method was the measurement of the 5-amino-2-nitro-benzoate form from reaction, which was absorbed at wave length 405 nm.

L-γ-gluamyl-3-carboxy-4-nitroanilide+glycylglycine $\xrightarrow{\text{γ-GT}}$ L-γ-glutamyl-glycylglycine+5-amino-2-nitro-benzoate

3.2.1 A stock solution (0.1 M) of caffeine was prepared and the following concentration of $(1\times10^{-2}, 1\times10^{-3}, 1\times10^{-4}, 1\times10^{-5}, 1\times10^{-6}, 1\times10^{-7}, 1\times10^{-8})$M were prepared by diluting with distilled water.

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

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

The activity in the presence of inhibitor

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

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

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

3.2.2 A constant concentration of caffeine (1×10^{-1} and 1×10^{-8}) M were used with different substrate concentrations of (40,80,120,160,200)mmol/L for GOT,GPT and (0.4, 0.8, 1.2, 1.6 2)mmol/L for γ-GT, to study the type of inhibition or activation. Buffers were used to prepare different substrates concentrations of these enzymes, GOT, GPT (phosphate buffer pH 7.4, 100 mmol/L) and γ-GT (TRIS buffer pH 8.25, 100 mmol/L)

The enzymes activiteis were determined with and without caffeine, by using the Lineweaver-Burk equation and plotting 1/v against 1/[s] were evaluated values [Linweaver, 1934]:

a) ki, b) Apparent v_{max} (v_{mapp}), c) Apperent k_m (k_{mapp}), d) Type of inhibition or activation.

4. Results and discussion

This research addresses investigation of the effects of caffeine of GOT, GPT and γ-GT enzymes. The biochemical tests revealed that caffeine caused inhibitory effects on γ-GT enzyme activity, and activatory effects on GOT and GPT enzymes activities. Figure (1).

The normal value of the GOT enzyme activity was (77 U/L). The relationship between caffeine concentration versus and the activity of enzyme as shown in figure (2).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 (0.1M) (20.78 %) as shown in figure(3).

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 (4).these results observed that any increase in compound concentrations caused increase percentage of activation of enzyme. The greater activation of caffeine was demonstrated at concentration (0.1M)(22.72 %) as shown in figure(5).

The normal value of the γ-GT enzyme activity was (26.18 U/L). The relationship between caffeine concentration versus and the activity of enzyme as shown in figure (6).these results observed that any increase in compound concentrations caused increase in percentage of inhibition of enzyme. The greater inhibition of caffeine was demonstrated at concentration (0.1M) (55 %) as shown in figure(7).

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 [U.Satyanarayna, 2003].

Table (2) and figure (8) 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) U/L 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 (9) showed that the type of enzyme activation using Lineweaver-Burk plot for caffeine on serum 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 100 U/L, 400 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 (250,111,111) U/L 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 (0.166, 1 x 10^{-7}) M respectively.

The enzymes play important role in amino acid metabolism and in the urea and tricarboxylic acid cycles. We suggested that caffeine molecule has (N- and O=) groups by which, it activates the active sides of amino acids of GOT and GPT enzymes by increasing affinity of active sides of enzymes to react with the substrates. The results of our study is in agreement with before studies of same enzymes [Springer Berlin, 2004].

Table (2) and figure (10) showed that the type of enzyme inhibitor using Lineweaver-Burk plot for caffeine on serum γ-GT 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.

Molecular of caffeine inter action between the groups (N- and O=) with active sides of amino acids of γ-GT enzyme. The results of our study are in agreement with before studies of same enzyme [Burg, 2002].

The tripeptide glutathione (GSH) is used by cells to detoxify hydroperoxides, produced during oxidative stress, and is consumed in the process. Previous studies have indicated that cells can be protected against oxidative stress by extracellular GSH through its degradation catalyzed by the exoenzyme gamma-glutamyl transpeptidase (GGT) and its de novo synthesis within the cytosol. [A Kugelman, 1994] Because of this, induction of GSH depletion has been proposed as a good strategy for sensitizing tumor cells to anti tumor agents [Castro, 2002].

We hypothesized that (GGT) would be increased as part of the adaptation of cells to oxidative stress.

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Table 1. The effect of different concentrations of caffeine on the activity of GOT,GPT, γ-GT enzyme in human serum

Number	Conc.[M]	GOT activity U/L	% Activation	GPT activity U/L	% Activation	γ-GT activity U/L	% Inhibition
1	0	77	0	55	0	26.18	0
2	10^{-1}	78	20.78	56.5	22.72	11.78	55
3	10^{-2}	79	16.88	57	14.54	13.09	50
4	10^{-3}	80	11.68	55.5	12.72	20.94	20
5	10^{-4}	81	9.09	56	7.27	23.56	10
6	10^{-5}	84	5.19	59	5.45	24.87	5
7	10^{-6}	86	3.89	62	3.63	25.55	2.4
8	10^{-7}	90	2.3	63	2.73	26.18	0
9	10^{-8}	93	1.3	67.5	1.81	26.18	0

Table 2. The kinetic propereties of GOT,GPT, γ-GT with caffeine

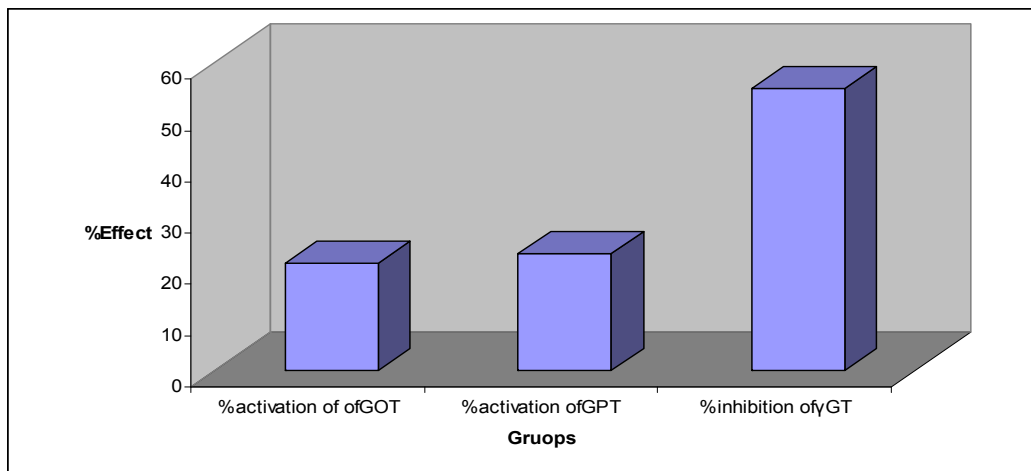| Enzymes	Con.of caffeine	K_{map} (M)	V map U/L	Ki (M)	Type of effection
GOT	10^{-1}	200	200	0.15	Non-competitive
	10^{-8}	200	100	3×10^{-8}	
GPT	10^{-1}	400	250	0.1666	Non-competitive
	10^{-8}	400	111.111	1×10^{-7}	
γ-GT	10^{-1}	1.429	37.037	0.0388	Competitive
	10^{-8}	0.714	37.037	1.27×10^{-8}	

Figure 1. % Effect of GOT, GPT, γ-GT enzymes activities with 0.1 M caffeine
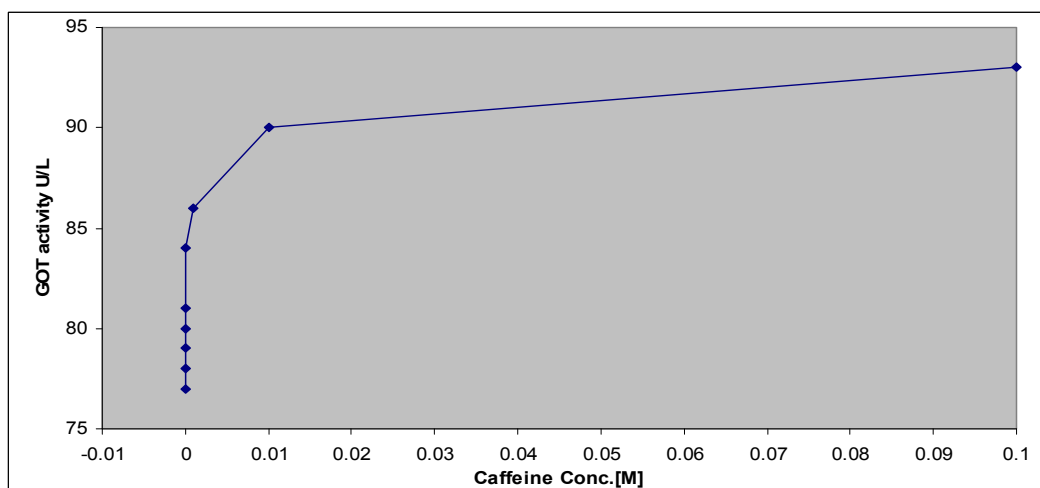

Figure 2. The relationship between concentration of caffeine and GOT enzyme activity
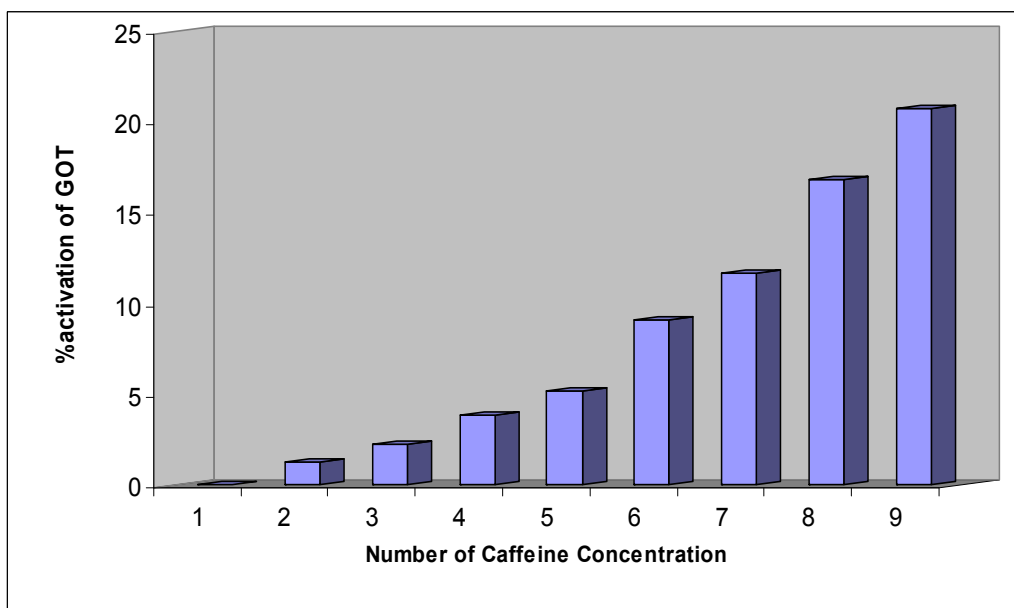

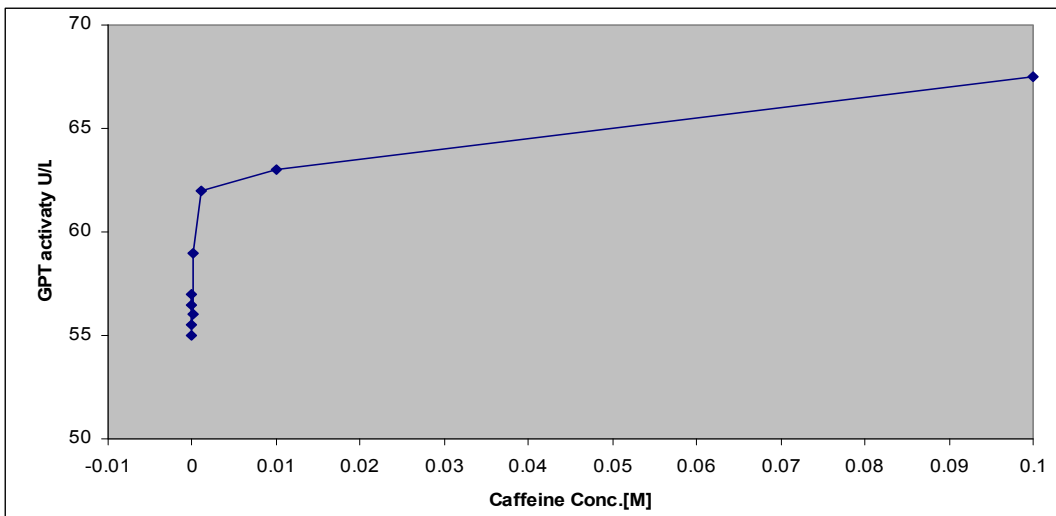Figure 3. The percentages of activation GOT enzyme and caffeine concentration

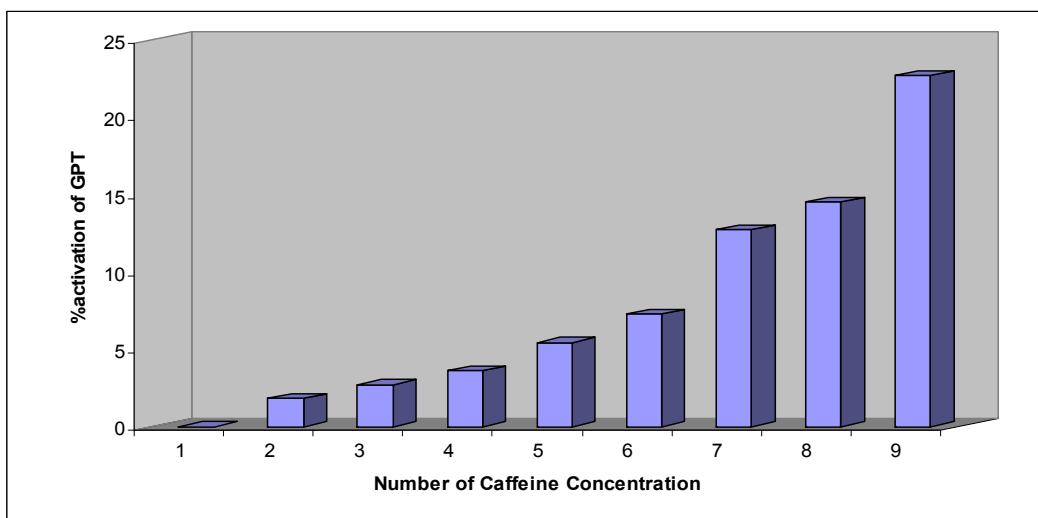Figure 4. The relationship between concentration of caffeine and GPT enzyme activity

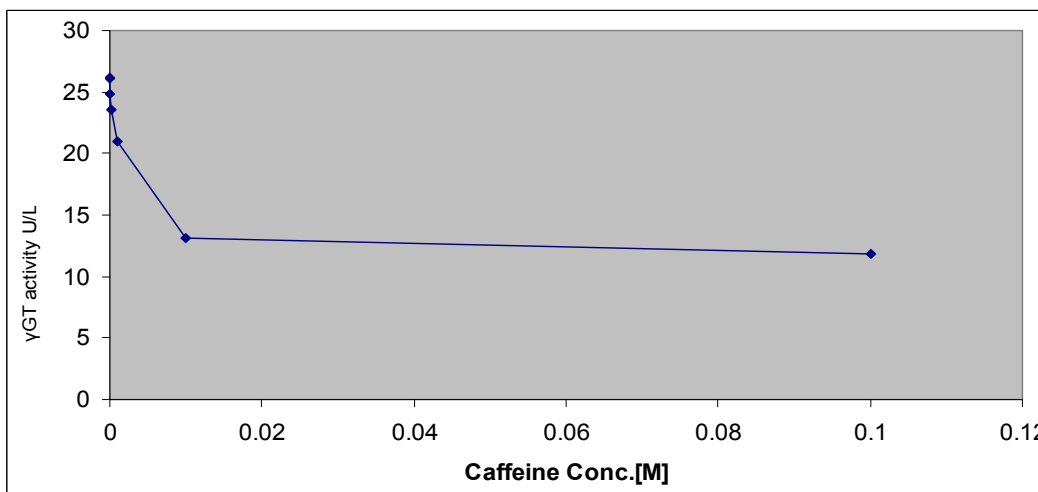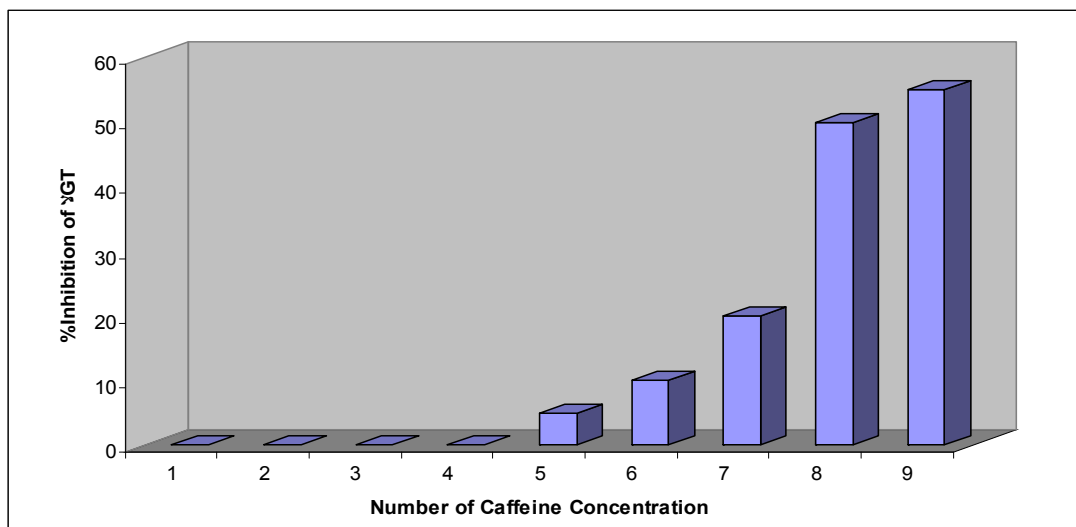Figure 5. The percentages of activation GPT enzyme and caffeine concentration

Figure 6. The relationship between concentration of caffeine and γ-GT enzyme activity

Figure 7. The percentages of inhibition γ-GT enzyme and caffeine concentration

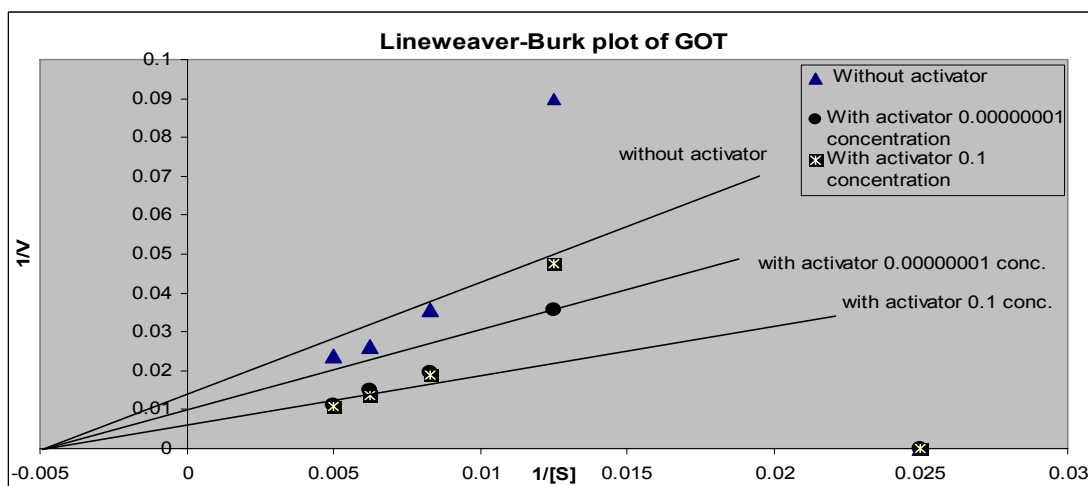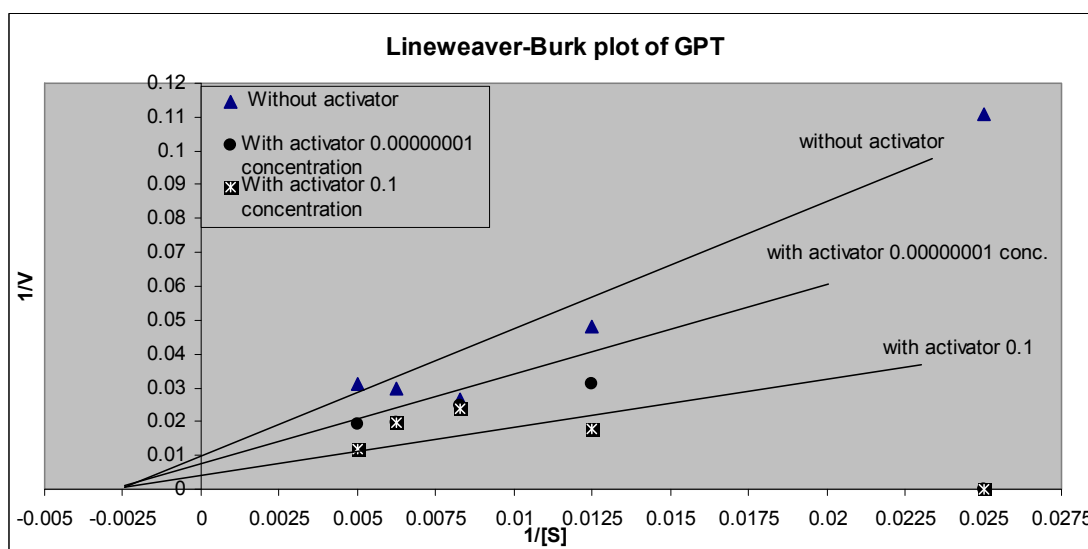Figure 8. Lineweaver-Burk plots for caffeine effects on GOT

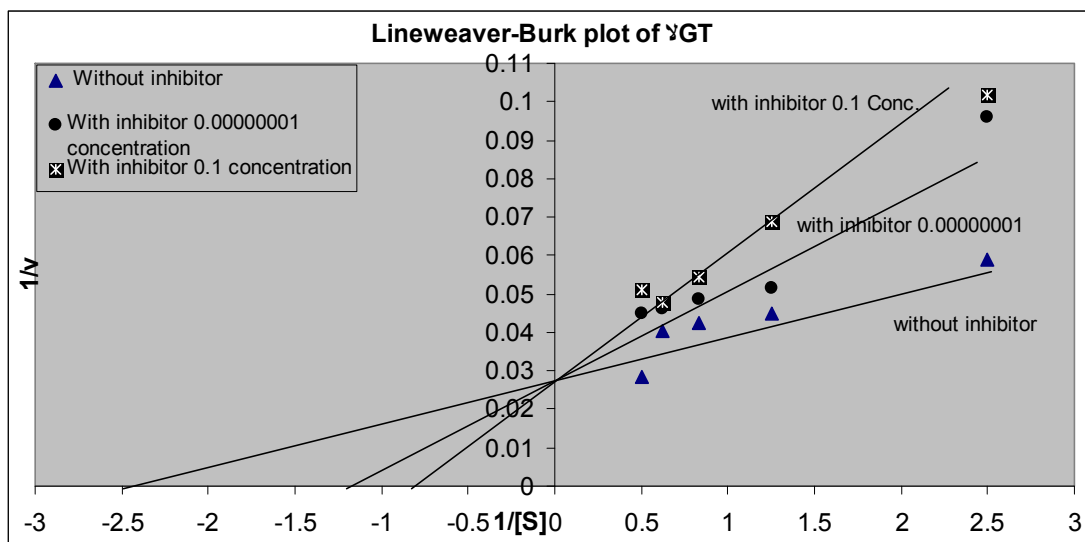Figure 9. Lineweaver-Burk plots for caffeine effects on GPT

Figure 10. Lineweaver-Burk plots for caffeine effects on γ-GT