

## Elevation of Serum Gamma-Glutamyltransferase Enzyme and the Role of Some Divalent Metals in Hepatitis Sera



Chemistry

KEYWORDS : GGTL;inhibition;Meta ions ;hepatitis

**Namama S. Hamad** Department of chemistry,College of science,University of Sulaimania

**Diary I.Tofiq** Department of chemistry,College of science,University of Sulaimania

**Avan A.Ahmad** Department of chemistry,College of science,University of Sulaimania

### ABSTRACT

*The effect of different divalent ions as potential and activator of serum gamma-glutamyltransferase GGT activities in human (male and female) with hepatitis was assessed using biochemical assays. Serum fluid was obtained from 50 cases (30 normal). Serum GGT activity were elevated in hepatitis ( $88.41 \pm 9.31$  IU/L)in comparison with control object ( $23 \pm 6.7$ ) by about four fold. The study was carried out in optimum pH value 8.2 and 37 C0 by which the enzyme possess highest activity. Effects of potassium ,sodium, copper, calcium ,and zinc on serum GGT activities were tested after addition of these metal (2mM)in the presence of the salts, they shows the inhibitory effect ( $63.41 \pm 2.43$  IU/L),( $69.56 \pm 4.75$  IU/L),( $69.90 \pm 8.31$  IU/L),( $64.26 \pm 3.81$  IU/L)and ( $60.87 \pm 6.08$ ) respectively, while magnesium and cobalt reflects a stimulatory effect on enzyme activity in hepatitis sera ( $164.32 \pm 0.81$  IU/L) and ( $101.5 \pm 0.55$  IU/L),from these results it can be concluded that the metals studied may have a role in initiating and controlling cellular proliferation, through their effects on modulating the activities of the possible marker for GGT enzyme activity.*

### Introduction:

Gamma-glutamyltransferase(E.2.3.2.2.GGT),which is also known asGTP,GPT, $\gamma$ -GTP, $\gamma$ -GT, $\gamma$ -GPT,or $\gamma$ -glutamyltranspeptidase,is a cell membrane-bound enzyme that catalyzes the transfer of the  $\gamma$ -glutamyl residue of  $\gamma$ -glutamyl peptides to appropriate acceptors, is distributed in a wide range of living cell. It is well-known that GGT catalyzes the first step of GSH catabolism in mammals and composes the  $\gamma$ -glutamyl cycle, Glutathion play an important role in protecting cells against oxidants that are produced during normal metabolism (1,2).

Orlowski and Meister proposed that GGTs play a physiological role in the  $\gamma$ -glutamyl cycle which is involved in the amino acid uptake through the cell membrane in to mammalian cells(3). Various alternative functions of GGT have also been postulated, such as the sequential hydrolysis of glutathione and it is conjugates by GGT and membrane-bound amino peptide(4,5),detoxification through the synthesis of mercapturic acid (6,7).

Serum GGT activity used as a sensitive marker of hepatobiliary disorders , exhibits tissue specific expression under various physiological and pathological conditions. The activity of GGT is the highest in embryo liver and decreases rapidly to the lowest level after birth,and it is a widely distributed enzyme that has been extensively studied in relation to hepatocarcinogenesis (8,9).

The way in which liver diseases lead to an increase in serum GGT are not well understood, in the early 1970s a number of authors recognized that as a liver function test, GGT was particularly sensitive to alcoholic liver disease (10). An extension of this idea led to the discovery that GGT was elevated in a high proportion of alcoholics not currently showing the symptoms of liver disease (11,12 ). Many papers have dealt with the study of the effectiveness of various inhibitors on the activity of GGT activity(13,14).

Most metals are an essential cofactor in multiple enzymatic reaction, therefore, it is important to study the activity role of these metals in other cases of acute liver injury due not to viral but to toxic agents and compare it with serum  $\gamma$ -glutamyltranspeptidase activity. For example, Zinc is involved in the activation of approximately 300 different metallo-enzymes and metal-activated enzyme in vivo and is regarded as essential for the metabolism of nucleic acids and proteins (15,16). Sodium and potassium are also the major positive ion of body fluids and affected by some diseases, and chromium is a mineral the body needs in very small amounts, but it plays a significant role in human nutrition. It has been determined that

humans need nearly 72 trace elements, including very low concentration of heavy metals, such Cu, Sn, V, Cr, Mo, Mn and Co. Most metals are toxic at high concentration (18).

The determination of blood component values using laboratory exams is an important procedure to aid the diagnosis of several diseases and dysfunction, as they provide reliable results, and may also give inputs for research studies on nutrition, physiological, and pathology(18).

Inhibition of GGT enzyme activity by these metals can have multiple applications including but not limited to: reduction of renal toxicity ,cancer therapeutic ,treatment for asthma and other vascular diseases,thus an inhibitor of GGT that can be used clinically has been long sought. Overall , these inhibitors are potentially useful in the treatment of cancer ,liver disease ,and even cardiovascular disease.

The aim of our study was to determine the effect of some important physiological mono and divalent metal ions on GGT enzyme activity in hepatitis patients.

### Materials and Methods:

A-chemical: all chemicals used in this project were of high Analytical grade.

B-blood collection and serum separation:-

These were of 30 normal healthy adults in addition to 50 samples representing hepatitis individuals ,aged between 21-67 years,from where blood samples were collected,these samples were diagnosed by consultants and proved by GGT test (hepatitis),they are not accompanied diseases, the blood left at room temperature until it has clotted. After clot formation the serum isolated by centrifugation,the separated serum was used on the same day of enzyme activity.

### C-Determination of GGT activity:

The GGT activity was determined by the hydrolysis of  $\gamma$ -glutamyl p-nitroanilide in the presence of the reagent (Szasz,1969 ;1974). Standard assay included final reagent concentration 2mM of gamma-glutamyl p-nitroanilide, 62 mM of glycylglycine and 95 mM of Tris-HCl, pH 8.1. The rate of p-nitroanilide formation was measured at 405 nm by using spectrophotometer. The results were expressed as U/L ,one unit of enzyme represents the amount of enzyme that catalyzes the release of 1mmol of nitroaniline/min.

GGT activity was calculated using the equation:  
GGT activity (IU/L) = ( $\Delta$  Abs /min)  $\times$  2121

Where the absorbance read at 405 nm .

**D- Inhibition and stimulation studies:**

These experiments were done using 2mM of each the (K+, Na+, Cu+2, Mg+2 ,Co+2 ,Zn+2 and Ca+2) for kinetic analysis. The same protocol (C) was applied ,and the only addition made is the addition of 2mM of each metals separately , the protocol was applied by using a series of different concentration of metals, to obtain maximum inhibition and maximum stimulation, we used following concentration ( 0.5,1.0,1.5,2.0,2.5,3.0) mM of each metals.

E- Biostatistical analysis:

A T-test analysis was applied for investigating data obtained.

**Results:**

The GGT activities in hepatitis serum (50 sample) was determined quantitatively and compared with that of normal (30 sample), it was seen that there was an elevation occurred in hepatitis (88.41±9.31 IU/L) in comparison with that of normal individuals (23±6.7 IU/L) fig

Fig (1) show using of different concentration of K+, Na+,Cu+2,Ca+2 and Zn+2 ions it was found that a concentration of (2mM) these ions can results in highest degree in inhibition for GGT activity.

**Fig(1): Inhibitory effect of (Na+,K+,Cu+2,Ca+2 and Zn+2 ) ions using different concentration**

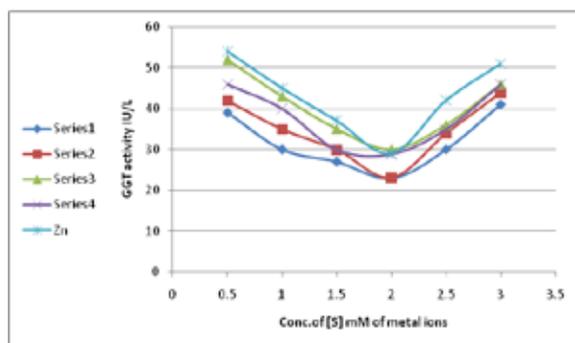


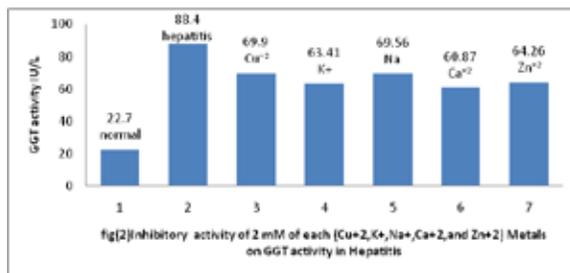
Table (1) figure (2) show the inhibitory effect of Na+,K+,Cu+2,Ca+2 and Zn+2 ions on the activity of GGT enzyme activity in hepatitis serum, , copper , ,potassium, sodium ,calcium, and zinc significantly decreased activity of GGT (69.90±8.31 IU/L),(63.41±2.43 IU/L),(69.56±4.75 IU/L),(60.87±6.08 IU/L) and (64.46±3.81 IU/L),p<0.001 respectively.

Serum	GGT activity IU/L Inhibition of	Presence of
	Absence of Cu <sup>2+</sup>	Cu+2
normal	23±6.7	-
hepatitis	88.41±9.31	± 8.31 69.90
	Absence of K <sup>+</sup>	Presence of K <sup>+</sup>
hepatitis	± 9.31 88.41	± 2.43 63.41
	Absence of Na <sup>+</sup>	Presence of Na <sup>+</sup>
hepatitis	± 9.31 88.41	± 4.75 69.56
	Absence of Ca <sup>2+</sup>	Presence of Ca <sup>2+</sup>

hepatitis	± 9.31 88.41	± 6.08 60.87
	Absence of Zn <sup>2+</sup>	Presence of Zn <sup>2+</sup>
hepatitis	± 9.31 88.41	± 3.81 64.26

**Table (1): GGT-enzyme activity inhibition using (2mM) of different metals**

Table (2) shows the inhibition degree of the Na+,K+,Cu+2,Ca+2 and Zn+2 ions on GGT activity, compared with normal case. Calcium ion has higher inhibition degree (31.5) more than other metal ions.



components	% degree inhibition	% recovery
normal	--	100
Na <sup>+</sup>	21.32	78.68
K <sup>+</sup>	28.27	71.73
Cu <sup>2+</sup>	20.93	79.07
Ca <sup>2+</sup>	31.5	68.85
Zn <sup>2+</sup>	27.31	72.69

**Table(2):inhibition degree of Na+,K+,Cu+2,Ca+2 and Zn+2 ions (2mM)**

Fig (3) show using of different concentration of magnesium and cobalt ions, it was found that a concentration of (2mM) these ions can results in highest degree in stimulation for GGT activity.

**Figure (3): stimulatory effect of (Mg+2 and Co+2 ) ions using different concentration**

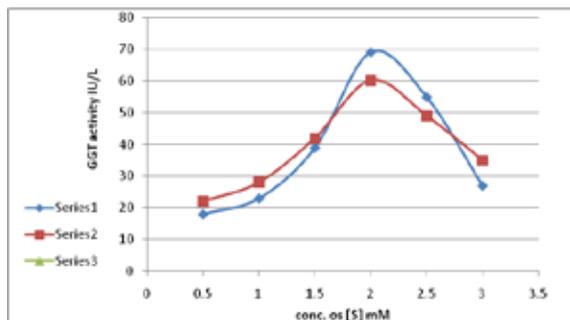
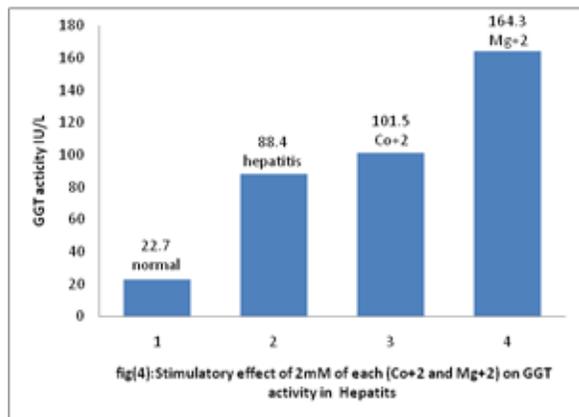


Table (3) and figure ( 2)show the stimulatory effect of magnesium and cobalt ions on the activity of GGT enzyme,(64.32± 0.81IU/L) and (101.5± 0.55IU/L) respectively .

**Table (3): GGT-enzyme activity stimulation using (2mM) of Mg+2 and Co+2**

Serum	GGT activity IU/L Stimulation of
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	Absence of Co <sup>+2</sup>	Presence of Co <sup>+2</sup>
normal	23±6.7	-
hepatitis	± 9.31 88.41	± 0.55 101.5
	Absence of Mg+2	Presence of Mg+2
hepatitis	± 9.31 88.41	± 0.81 164.32



**Table (4) shows the stimulation degree of the magnesium and cobalt ions on GGT activity, compared with normal case. Magnesium ion has higher stimulation degree (- 85) more than cobalt ion.**

components	% degree stimulation	% recovery
normal	---	100
Co+2	89	11.48
Mg+2	85 -	185.85

**Table (4):Stimulation degree of cobalt and magnesium on GGT activity at concentration equal to 2mM**

**Discussion:**

Our results showed that metal ions could affect activity of GGT. Data from our study indicate that magnesium stimulated the activity of GGT, Mg ion is a cofactor in various reaction related to carbohydrates, lipid, and protein metabolism, data obtained consisted with that published previously (20,21,22).

There are many enzymes also affected by these metals, for example copper inhibit both urease and glutamate dehydrogenase activities (23), next we compared the change in serum GGT activity between different metal ions, magnesium show highest degree of stimulation whereas the calcium show maximum degree of inhibition.

It is known that serum zinc concentration decreased in patients with liver disease in parallel with the development of disease stage because zinc absorption from the intestine decrease and the zinc content of the liver reduces due to the decrease in the number of function hepatocytes (24,25,26,27).

However, our study suggests that the serum zinc concentration in the majority of patients if a sufficient quantity of zinc is administered, because GGT increased in the liver diseases and can be inhibited by zinc as shown in our results. The relationship between liver diseases and trace element contents are debated, a significant direct correlation was observed among zinc and GSH plasma (28).

Previously published works, concentrated on the inhibitory effects of metal ions to be of great important. This is due to clinically treatment which provides a chemotherapy of disorders. Therefore, we concentrated on these metal ions to investigate them on GGT activity. It has been demonstrated that circulating GGT activity is elevated in patients with metabolic syndromes (29).

The results of this experiment indicate that the metal ions Na<sup>+</sup>, K<sup>+</sup>, Cu<sup>+2</sup>, Ca<sup>+2</sup> and Zn<sup>+2</sup> ions affect the activity of GGT enzyme as inhibitors, these metal ions can compete the substrate in the active site located on the GGT surface, while magnesium and cobalt act as activator for GGT enzyme. we showed that GGT activity which is increased in hepatitis can be treated by these inhibitors factor, these metal found in most food or vegetable.

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