

Optimization of Glutathione S-Transferase in the Sera of Young Female Iraqi Patients with Diabetes Mellitus Type1

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Abstract:

This study was attempted to determine optimum conditions, for Glutathione s-Transferase enzyme, in sera of three groups diabetic patients type1 depending on duration of disease without complications compared with control group. The aim of this study was to find optimum conditions were determined such as (pH, Substrate Concentration, Temperature, Incubation time, Enzyme concentration, and effect of(0.15M)(0.25M) of mono divalent compounds). And to find the kinetics parameters in the three groups of diabetic patients when compared with control.

It was found optimum pH(8.5,4.5,2.5,6.5).Temperatures(20c°,40c°,50c°,30c°). Incubation times (7min, 4min, 4min, 5min) substrate concentrations (12µl, 10µl, 5µl, 10µl) enzyme concentrations by enzyme volumes (125µl, 100µl, 75µl, 100µl) for group one, two, three and control group respectively., The maximum activity was presence in mono valent compounds were found in NaCl while in divalent compounds the maximum activity was presence CuSO₄ more than the other compounds. By using line weaver –Burk plot we estimate the three values of K_m and three values V_{max} for the three groups of diabetic patients and control. which enhance our result that there are confirm three isoenzymes for Glutathione –S- transferase.

Key words: Glutathione s-Transferase, DMT1, optimum condition, K_M, V_{max}.

Introduction:

Diabetes is a metabolic disease characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both. The chronic hyperglycemia of diabetes is associated with long –term damage, dysfunction and failure of different organs, especially the eyes, kidneys, nerves, heart and blood vessels[1]. Several pathogenic processes are involved in the development of diabetes, these range from autoimmune destruction of the β cells of the pancreas with consequent insulin deficiency to abnormalities that result in resistance to insulin action[2] . The basis of the abnormalities in carbohydrate, fat, and protein metabolism in diabetes is deficient action of insulin on target tissues[3].

Type one formerly called (juvenile_ onset or insulin dependent (IDDM), this type has peak age at onset of 12 years old. It is unusual to begin after age 40years old. Type one DM is due to beta cell destruction so that no insulin is produced and must be replaced by insulin injection[4].This form of diabetes which accounts for only 5-10% of those with diabetes results from autoimmune destruction of insulin- producing beta cells of the pancreas[5]. Glutathione transferase has historically also been called glutathione s-transferase, and it is this latter name that gives rise to the widely used abbreviation GST, this enzyme catalyze nucleophilic attack by reduced glutathione. GST on no polar compounds that contain an electrophilic carbon nitrogen, or

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sulphur atom. their substrates include halogen nitro benzene, arene, oxides, quinines, and alpha, beta unsaturated carbonyl[6]. Mammalian cytosolic GSTs are all dimeric with subunits of 199-244 amino acids in length. Based on amino acid sequence similarities, seven of cytosolic GST are recognized in mammalian species, designated alpha, mu, pi, sigma, theta, omega, and zeta. Other classes of cytosolic GST, namely beta, delta, epsilon, lambda, phi, tau, and the "U" class, have been identified in non mammalian species. In rodents and humans, cytosolic GST isoenzymes within a class typically share > 40% and those between classes share < 25 % identity[7].

Glutathione S-transferase (GSTs) belong to a group of multi gene and multi functional detoxification enzymes, which defend cells against a wide variety of toxic insults from chemical, metabolites, and oxidative stress[8]. An important condition affecting GST expression is oxidative stress, usually observed in diabetes[9]. Saito-Yamanaka *et al.* found decreased GST activity in the liver of streptozotocin – induced diabetic rats as compared with normal rat livers. These data point to the fact that GSTs may offer protection against diabetes mellitus[10,11,12].

Materials and Methods:

Patient and design. The study group consisted of 66 young female (15-30) years with DM1 divided to three groups (5,10,15) years according to duration of disease and they had no complication, The number of the groups (24,22,20) respectively compared with 22 controls, The samples collected from the center of diabetes / Al-Mustansiriya University, in January, February 2013.

Materials. 1-Chloro-2,4-dinitrobenzene (Hopkins & Williams, U.K). Ethanol (Fluka-Germany). Phosphate buffer

saline (PBS) (BDH, England). Reduced Glutathione (GSH) (Sigma chemicals, USA).

Optimum Conditions of GST in the sera of three groups DM1 & controls:

I. The Effect of Different pH on the activity of GST :

Nine hundred micro liter of enzyme cocktail [mix of 980 μ l PBS of different PH (10.5, 8.5, 6.5, 4.5, 2.5) and (10 μ l of 0.1M CDNB) and 10 μ l of 0.1M GSH] was placed on the tube of each sample and blank.

II. The Effect of Different Concentration of substrate on the activity of GST :

Nine hundred micro liter of enzyme cocktail [mix of 980 μ l PBS pH (8.5, 4.5, 2.5, 6.5) in group one ,group two, group three respectively and different volume of 0.1M CDNB (2.5, 10, 12, 15 μ l) and 10 μ l of 0.1M GSH] was placed on the each tube sample and blank.

III. The Effect of Different temperature on the activity of GST:

1. Nine hundred micro liter of enzyme cocktail [mix of 980 μ l PBS pH (8.5, 4.5, 2.5) and (12, 10, 5 μ l of 0.1M CDNB) of group one ,two, three respectively and 10 μ l of 0.1M GSH] was placed on the tube of each sample and blank.

2. Incubated at different temperature (20, 30, 40, 50, 60 $^{\circ}$ C) for 5 minutes . of each sample and blank.

IV. The Effect of Different Incubation Time on the activity of GST

1. Nine hundred micro liter of enzyme cocktail [mix of 980 μ l PBS pH (8.5, 4.5, 2.5) and (12, 10, 5 μ l of 0.1M CDNB) of group one ,two ,three respectively and 10 μ l of 0.1M GSH] was placed on the tube of each sample and blank.

2. Incubated at (20, 40, 50 $^{\circ}$ C) of group one , two, three respectively in

spectrophotometer for (2,4,5,7,10) minutes .

V. The Effect of Different enzyme concentration on the activity of GST:

1. Nine hundred micro liter of enzyme cocktail [mix of 980 μ l PBS pH (8.5,4.5,2.5) and(12,10,5 μ l of 0.1M CDNB)of group one ,two ,three respectively and 10 μ l of 0.1M GSH] was placed on the tube of each sample and blank.

2. Incubated at (20,40,50 c°) for(7,4,5 minutes)of group one , two, three respectively.

3. To the sample tube was added increasing volume of serum (50,75,100,125,150 μ l).

VI. The Effect of Different mono and divalent compound on the activity of GST:

1. Nine hundred micro liter of enzyme cocktail [mix of 980 μ l PBS pH (8.5,4.5,2.5) and(12,10,5 μ l of 0.1M CDNB)of group one ,two, three, respectively and 10 μ l of 0.1M GSH and10 μ l of (0.15,0.25M)of NaCl,NaI,MgSO₄, CaSO₄, CuSO₄, ZnSo₄] was placed on the tube of each sample and blank.

2. Incubated at (20,40,50 c°) for (7,4,5minute) of group one, two, three, respectively.

3. To the sample tube was added (125,100,75 μ ls) of the sample of group one ,two, three respectively.

Results and Discussion:

The influence of pH upon the activity of GST was investigated by using 100 μ l of sera as a source for enzyme and 980 μ l of PBS The assay conditions were conducted in same manner as described earlier at range of pH (2.5-10.5).Optimum GST activity was obtained at pH (8.5,4.5,2.5,6.5) in one, two, three groups diabetic patients and control respectively. The shift in pH of the environment may include the

induction of the protonation-deprotonation process occurring within the charge polar groups on the amino acid residues of GST enzyme present in the binding domain. Extremes in pH can cause inhibition of binding or dissociation of already formed CDNB-enzyme complexes. Such extremes ultimately results in some degree of denaturation of the protein involve[13,14]. The result of current study is similar to the observation of Kai Zhang *et al*(2003) who observed the spontaneous and the enzymatic with GSH were affected markedly by a change in pH buffer from 6.0 to 8.0 [15] . as shown in figure (1).

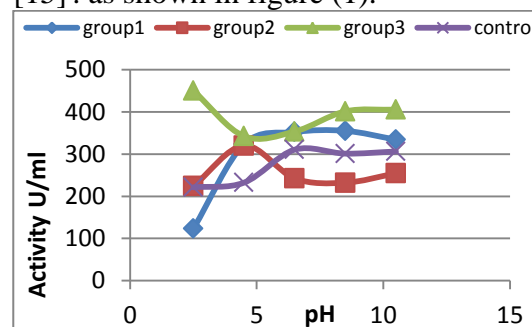


Fig. (1) The Effect of Different pH on GST activity

II. The Effect of different substrate concentration on GST activity:

To determine the effect of substrate concentration on the enzyme activity a series of experiments were performed where the concentration of the substrate was showed in Figure (2). Crystalline 1-chloro -2,4-dinitrobenzene (CDNB) was preferred for routine use as the substrate, because of its greater stability and commercial availability in satisfactory purity.

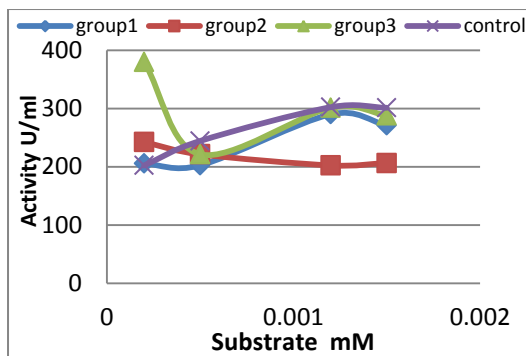


Fig.(2)The Effect of substrate concentration on GST activity

III. The Effect of different Temperature on GST activity:

When we increased temperature range (20-60c°) in three groups of diabetics and control, there was a concave up increase in the enzyme activity until it reached a maximum value at a temperature of (20,40,50,30 c°) for group one, two, three and control respectively then dropped gradually after that Figure (3).

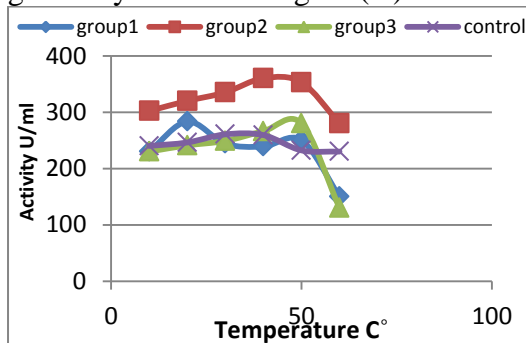


Fig. (3.17) The Effect of Different Temperature on GST activity

It seems that the loss of binding activity when temperature was raised may be due to the fact that dissociation rates show a greater increase with temperature than association rates, or it may be denaturation of enzyme. Then the increase in reaction velocity is due to an increase in a number of molecules that have sufficient energy to enter into the transition state, followed by a decrease in GST activity as the temperature continues upward with increasing break down of linkage

between (GST) enzyme & substrate (CDNB) include electrostatic attraction ,hydrogen bonding, Vander Waals forces and hydrophobic interaction , rise in temperature leads also to thermal denaturation of protein enzyme molecule[16,17].

IV. The Effect of different Incubation Time on GST activity :

To determine the stability of GST activity under assay conditions, a series of experiments were performed at different time intervals(2-10min) The results indicated that maximum enzyme activity was optianed (7,4,4,5min) at optimum temperature for group one ,two, three and control respectively. As shown in figure (4). The activity of GST increase by increasing the incubation time allowing to the enzyme to be completely saturated with the substrate CDNB until it reach to optimum time [18].

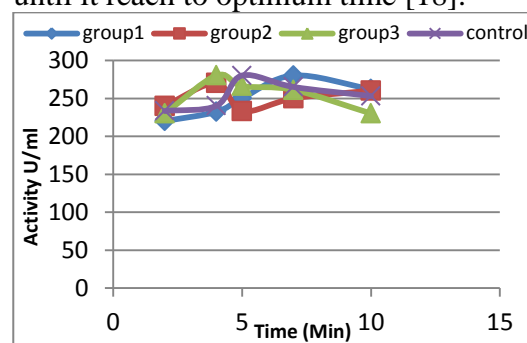


Fig. (4)The Effect of Different incubation time on GST activity

V. The Effect of Enzyme concentration on GST activity:

One of the conditions that affect rate of reaction is the amount of the enzyme: changed amount of the sera of female patients with type one DM (50, 75,100,125,150µls) of group one, two, three and control group were incubated with increasing volume of serum source of enzyme conjugated for optimum min of each group at optimum temperature of each group of three groups diabetics and control. The results are illustrated in Figure (5).

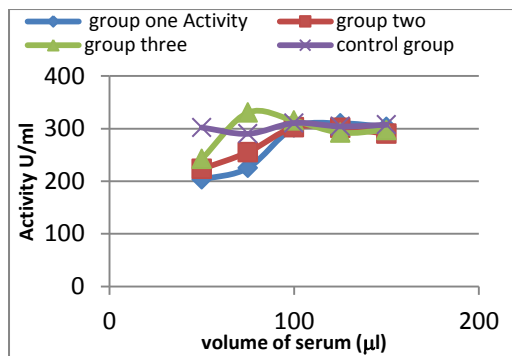


Fig. (5) The Effect of Different volume of serum on GST activity

It is obvious that the activity of GST enzyme in three groups diabetic patients and controls was risen by increasing the amount of enzyme concentration the optimum concentration of enzyme at (75,100,150 µls) for three group diabetic patients respectively when compared with control.

VI. The Effect of mono and divalent compounds on GST activity:

Many investigators observed that some chemical compounds like (0.15M) and (0.25M) of (NaCl, NaI, MgSO₄, CuSO₄, ZnSO₄) have an inhibitory effect or enhance GST activity . As showed in Figure (6) and Figure (7).

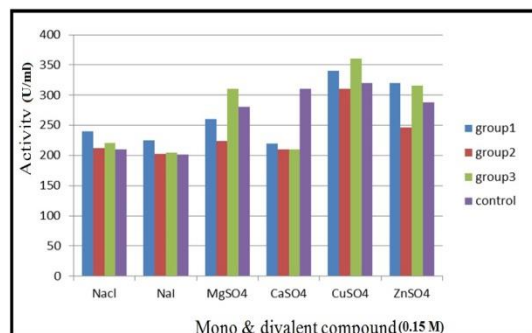


Fig. (6) The Effect of Mono, divalent compounds(0.15M) on GST activity.

In Mono valent compounds NaCl > NaI : group one more than other groups were in presence of NaCl . This could be due to the NaCl in low concentration or in physiological

concentration (0.15M) increase the binding between enzyme and substrate [19]. and chloride ion size less than iodine ion size which could lower inhibite the interaction between substrate and enzyme [20].

In divalent compounds CuSO₄> ZnSo₄ > MgSO₄> CaSO₄: group three more than other groups in the presence of CuSo₄. This could be due Some divalent cations appeared to enhance the binding reaction, this mean increase binding between substrate and enzyme therefore the activity will be increase[21].

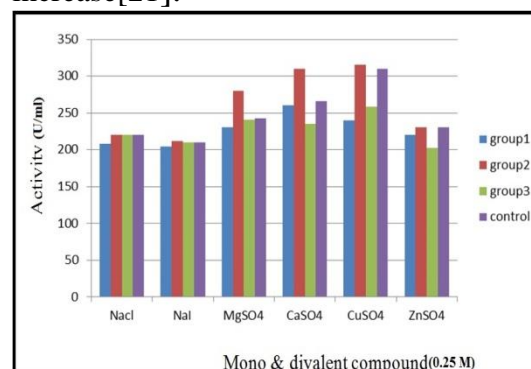


Fig. (7) The Effect of Mono, divalent compounds (0.25M) on GST activity

In mono valiant compounds (NaCl< NaI) : group one less than other groups,this could be due to NaCl in high salt concentration decrease the binding between enzyme and substrate[19]

In divalent compounds(ZnSo₄< CaSo₄< MgSO₄< CuSo₄) group three less than other groups in presence of ZnSo₄.This could be due to the large size of Zn ion when compared with other ions, which could inhibit the interaction between substrate and this compounds decreased activity of enzyme with increased concentration[20] .

Values of Km and Vmax on GST activity of three groups diabetics and control:

To determine the effect of substrate concentration on the enzyme activity we used Line weaver–Burk Plot to find

K_m and in three groups diabetics and control as show in figures(8)(9)(10)(11) :

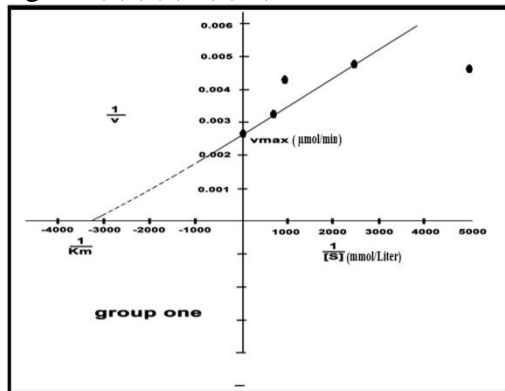


Fig. (8) Line weaver- Burk plot of substrate concentration in group one

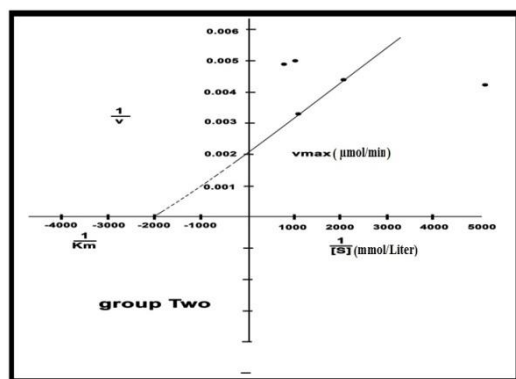


Fig.(9) Line weaver- Burk plot of substrate concentration in group two diabetic patients.

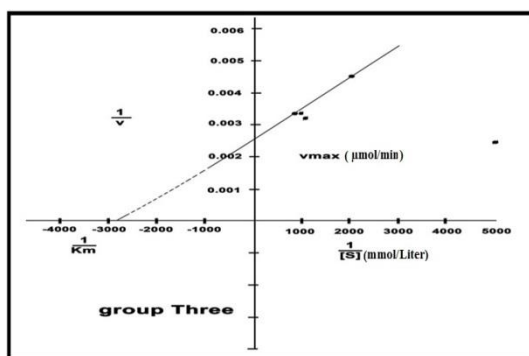


Fig. (10) Line weaver- Burk plot of substrate concentration in group three diabetic patients.

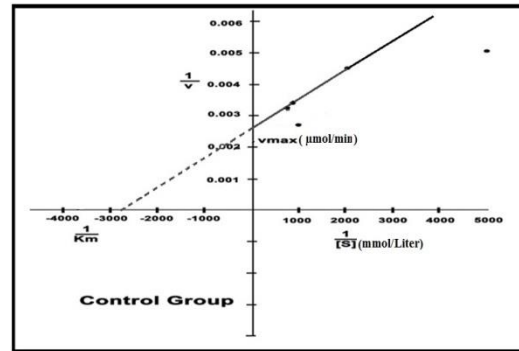


Fig.(11) Line weaver- Burk plot of substrate concentration in control group

Table(1) Show Kinetics Parameter (V_{max} , K_M) in three groups diabetics and control

Kinetic parameters	Group one	Group two	Group three	Control
V_{max}	$3.5 \times 10^2 \mu\text{mol/min}$	$4.5 \times 10^2 \mu\text{mol/min}$	$3.7 \times 10^2 \mu\text{mol/min}$	$3.8 \times 10^2 \mu\text{mol/min}$
K_M	$3.1 \times 10^{-4} \mu\text{mol/L}$	$5 \times 10^{-4} \mu\text{mol/L}$	$3.5 \times 10^{-4} \mu\text{mol/L}$	$3.2 \times 10^{-4} \mu\text{mol/L}$

Conclusion: We found three different values of K_m , V_{max} when compared each group of diabetic patients with control group. These results enhances optimum conditions were found and lead to confirm there are three isoenzymes depending on the same substrate of enzyme.

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الظروف المثلى لانزيم الكلوتاثايون س ترانسفيريز في امصال النساء العراقيات المصابات بداء السكري من النوع الاول

رواء مؤيد محمد ضياء

بري حبيب سيف الله

جامعة بغداد كلية العلوم للبنات-قسم الكيمياء.

الخلاصة:

حاولنا في هذه الدراسة ، تعيين الظروف المثلى لقياس فعالية انزيم الكلوتاثايون س ترانسفيريز ، في امصال ثلاث مجاميع من مرضى السكري من النوع الاول اعتمادا على فترة المرض بدون مضاعفات و المقارنة بمجموعة السيطرة .الهدف من الدراسة لايجاد الظروف المثلى مثل (دالة الحموضة ، تركيز المادة الاساس ، درجة الحرارة الزمنية للتفاعل ، تركيز الانزيم ، وتأثير (0.15 مولاري) (0.25 مولاري) لاملاح ايونات احادية وثنائية التكافؤ) ولايجاد المتغيرات الحركية في المجاميع الثلاث لمرضى السكري عند مقارنتها بمجموعة السيطرة.

حيث وجدنا دالة الحموضة (8.5 ، 4.5 ، 2.5 ، 6.5) درجات الحرارة (20 ، 40 ، 50 ، 30 م °) الفترة الزمنية للتفاعل (7 دقائق ، 4 دقائق ، 4 دقائق ، 5 دقائق) تراكيز المادة الاساس (12 ، 10 ، 5 ، 10 مايكرو لتر) تراكيز الانزيم بواسطة حجم الانزيم هي (125 ، 100 ، 75 ، 100 مايكرو لتر) للمجاميع الاولى والثانية والثالثة ومجموعة السيطرة على التوالي. ووجدنا قيم مختلفة لفعالية الانزيم عند قياس فعالية الانزيم بعد اضافة مركبات احادية وثنائية التكافؤ، وكانت اعلى فعالية للانزيم بوجود المركبات احادية التكافؤ في ملح كلوريد الصوديوم بينما في المركبات الثنائية التكافؤ كانت اعلى فعالية للانزيم بوجود ملح كبريتات النحاس عند مقارنتها مع بقية المركبات وتم استخدام رسم معادلة لينيفر-برك لتقدير قيم ثابت ميكالس والسرعة القصوى في المجاميع الثلاث لمرضى السكري ومجموعة السيطرة ووجدنا قيم مختلفة في المجاميع الثلاث لمرضى السكري مقارنة بمجموعة السيطرة التي عززت النتائج التي توصلنا اليها. والتي جعلتنا نؤكد بان هناك ثلاث ايزو انزيمات استنادا إلى ان المادة الاساس هي واحدة للمتناظرات الانزيمية ولفعاليات مختلفة ولظروف مثلى متباينة لها .

الكلمات المفتاحية: الكلوتاثايون- س- ترانسفيريز، داء السكري من النوع الثاني، الظروف المثلى، ثابت الالفة الانزيمية، السرعة القصوى.