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Purification of Glutathione S−Transferase Enzyme from Human Erythrocytes and Detection of the Effect of Separated and Mixture of Metformin and Daonil Drugs on its Activity

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Abstract : Glutathione S-transferases (GSTs) are a super family of enzymes involved in the detoxification of a various xenobiotics including diabetes mellitus drugs.The present studyexplained the effects of metformin and daonil drugs on the enzyme activity of glutathione−S−transferase (EC 2.5.1.18) obtained from human erythrocytes.For this purpose, erythrocyte glutathione S−transferase enzyme was purified **15.37** fold by DEAE-cellulose chromatography with a yield of **12.88**%.During thepurification, the temperature was kept under control (4°C). Enzyme purification was checked by performing SDS−PAGE. Twobandswere obtained approximately at 26 and 34 kDa. GST enzyme activity was determined by spectroscopic monitoring of the formation of 1−chloro−2,4−dinitrobenzene-glutathione (CDNB−GSH) conjugate. The activity of GSTenzyme after treated with diabetes mellitus drugs was **3.62**, **3.57** and **3.66** μmol/min/ml for metformin, daonil and mixture of them respectively while it was **3.24**μmol/min/ml before treated with drugs.
Key words: Glutathione−S−transferase, metformin, daonil, diabetes mellitus.

Introduction:

The first detection of glutathione S−transferases (GSTs) were in animalsat 1960because of their important functions in the metabolism and detoxification of xenobiotics[1]. GSTs enzymes are found in eukaryotes and prokaryotes[2-4]. (GSTs) (EC 2.5.1.18)are isoenzymes found inmammals, insects, plants, and microbes[5]. GSTs are found in the cytosols and membranes of manytissues such as in the liver, small intestine, large intestine, kidney, lung, breast, muscle, spleen, testis and placenta[6]. GSTs can be devided according to their subcellular location and function into 4 subgroups: cytosolic, mitochondrial, microsomal and bacterial antibiotic resistance proteins[7]. GST enzyme plays an important role in detoxification of xenobiotics by a conversion of a lipophilic, non-polar xenobiotic into amore water-soluble less toxic metabolite, to becomemore easily to be excreted from thecell by conjugating there with reduced GSH where GSH neutralizes medicationsthroughthe sulfhydryl (−SH) group in its molecules[8-11].

GST catalyze the general reaction:

$$GSH + R\text{-}X \longrightarrow GSR + HX$$

GSTs catalyze the reaction between a reduced glutathione (GSH; glutamyl–cysteinyl–glycine) and a reactive electrophilic center for various substrates,as a result a polar S glutathionylated product formed to reducing oxidative stress[3]. Theaim of this study to explain the effects of some drugs on the activity of GST enzyme

Material and Methods:

Bovine serum albumin, acetone, chloroform, ammonium sulphate, ethanol, methanol, DEAE cellulose, β−mercaptoethanol, 1−chloro−2, 4−dinitrobenzene (CDNB), TEMED, bromophenol blue, glutathione (GSH), molecular weight standard for SDS/PAGE.

Assay the activity of GST enzyme:

The activity of GST enzyme was determined for hemolysate and various enzyme preparations by using 1−chloro−2, 4−dinitrobenzene (CDNB) as substrate according to Habig method[6], where absorbance measured at 340 nm:

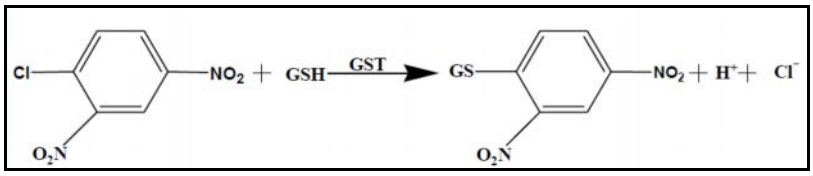

Figure1: Reaction between 1−chloro−2, 4−dinitrobenzene (CDNB) and glutathione in the presence of glutathione S−transferase enzyme.

Protein determination:

Determination of protein content of extract was determined during each purification steps by Bradford's method[12]that is based on the binding of Coomassie Brilliant Blue G−250 to protein. The absorbance of this complex shows at 595 nm. Bovine serum albumin (BSA) used as a standard.

Purification Procedures of GST enzyme:

All purification's steps were conducted at 4℃.

Hemolysis of Blood:

Fresh venous human blood sample was taken into EDTA tube. Next, the venous blood centrifuged at 4000 ×g for 10 min to remove the layers of plasma and leukocyte. The erythrocytes were washed with cold 0.9% saline for three times by centrifugation. After that the cells hemolysed by three times of its volume from ice-cold distilled water and stirring for 20 min and freeze-thaw[13].

Acetone Precipitation:

The protein was precipitated by adding chilled acetone (30%) to the hemolysate and centrifuged at 14000 ×g for 20 min. Using potassium phosphate buffer (0.05 M, pH 7.5) to suspended the precipitate. Next the sample was stirred for 15 min and the insoluble material removed by centrifugation at 14000 ×g for 15 min.

Ethanol-Chloroform Treatment:

Mixture of ethanol−chloroform was prepared by 3:1of chloroform to ethanol and added that mixture to the resultant solution by one volume for each them and stirring vigorously for about 1 min. The solution was centrifuged to remove the denaturated hemoglobin. The ethanol-chloroform is then evaporated in vacuum.

DEAE-Cellulose Chromatography:

Before running the ethanol−chloroform fractions, the DEAE−cellulose column (1.5× 40 cm) was equilibrated with 10 mM Tris−chloride, pH 7.5, containing 0.1 mM EDTA and 0.1 mM GSH. After running the enzyme was eluted with 10 mM Tris−chloride, pH 7.5, containing 0.1 mM EDTA, 1mM GSH and (0.1 – 1 M) NaCl. Fractions of 1.5 ml each were collected at a flow rate of 0.4 ml/min. The active fractions were concentrated by using a freeze-dryer (lyophilization) technique.

Polyacrylamide Gel Electrophoresis:

After the purification steps SDS/PAGE electrophoresis was performed using the buffer system described by[14] to verify the enzyme purity. The stacking and resolving gels containing 4.5 % (w/v) and 12.5 % (w/v) of acrylamide, respectively, and the amount of sample was running to the electrophoresis medium is 20 µl.

Result and Discussion:

The glutathione S−transferase GST enzyme was purified from human erythrocytes by using acetone fractionation, ethanol-chloroform treatment, DEAE cellulose chromatography, the purification steps for GST enzyme from human erythrocytes are explained in table 1. The first step of purification for GST enzyme from initial extract was fractional precipitation of protein by using $(NH_4)_2SO_4$ and acetone methods. The precipitation by acetone 30% recovered highest activity 1.09 µmol/min/mgwhile the ammonium sulfate precipitation was not performed because of partial inactivation of the enzyme. The next step after acetone precipitation was ethanol−chloroform treatment resulted in 7.64−fold purification of GST. From table 1.The specific activity of GST was3.86µmol/min/mg after EDAE−cellulosechromatography where notes by progress the purification process the specific activity raised while the activity and total protein were less which mean increasing in the purification of enzyme for each step[15-32]. Figure 2. and figure 3. showed the major single peak that purified GST give after EDAE−cellulose chromatography.

Table 1. Purification steps for human erythrocytes GST:

Yield %	Purification fold	Total protein mg	Specific activity µmol/min/mg	Activity µmol/min/ml	Purification steps
100	1.00	446.13	0.25	111.53	Crude extract
71.74	4.34	73.46	1.09	80.02	Acetone precipitation %30
37.28	7.64	21.66	1.92	41.58	Ethanol−chloroform treatment
12.88	12.37	3.72	3.86	12.37	DEAE−cellulose chromatography

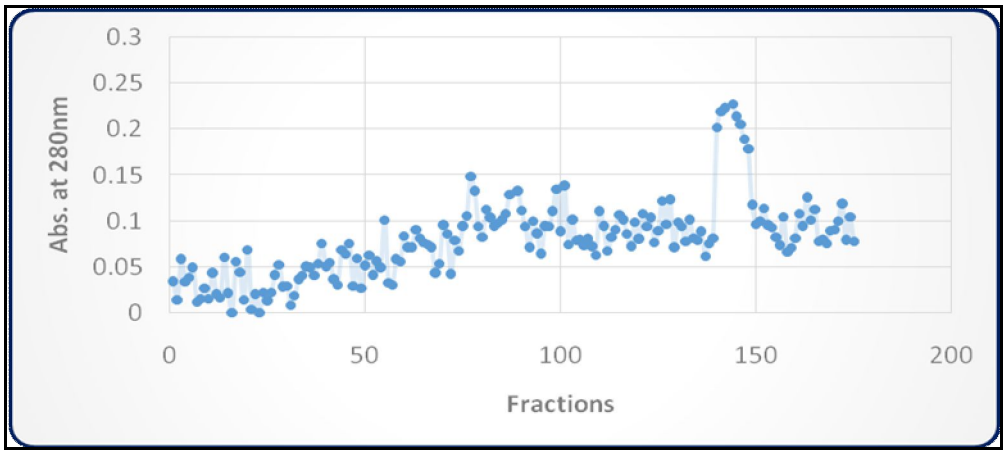

Figure 2. DEAE cellulose chromatography of human erythrocytes GST.

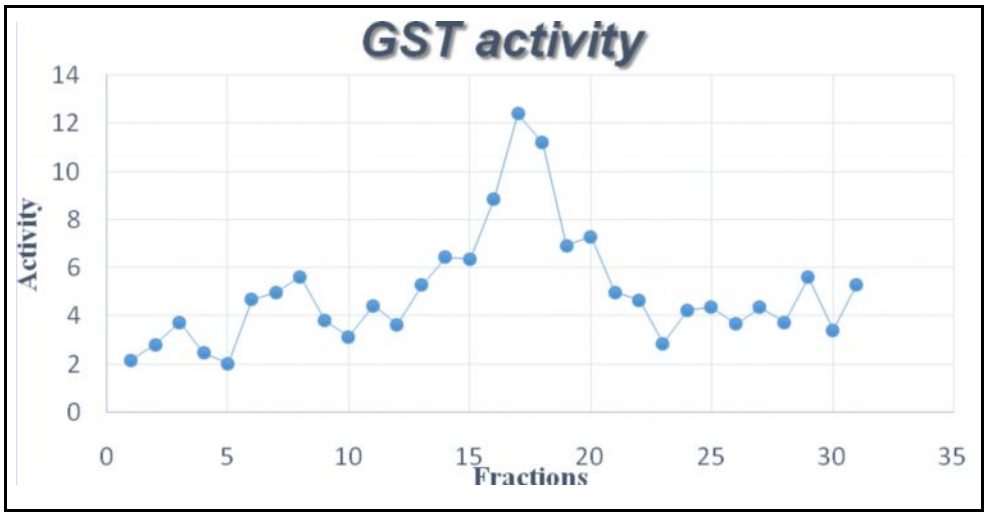

Figure 3. GST activity, absorbance at 280 nm.

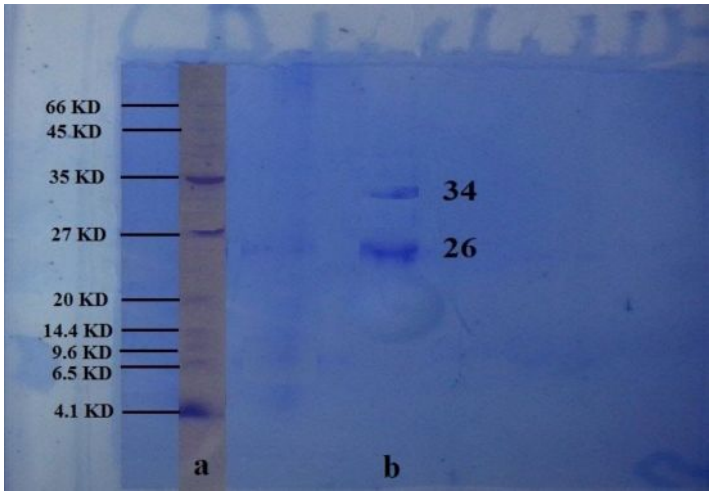

Figure 4. SDS−polyacrylamide gel electrophoresis of human erythrocyte GST (a) standard protein (b) purified human erythrocyte GST.

The electrophoresis technique for purified GST from human erythrocytes by DEAE−cellulose chromatography was performed by using Sodium Dodecyl Sulfate Poly Acryl Gel Electrophoresis (SDS-PAGE), to determined the molecular weight of GST enzyme where it was notes appeared two bands one of them approximately at 26 kD and the other appeared at 34 KD and this agreed with references of found two isomers for GST enzyme.

Table 2.Comparison between GST enzyme activity before purified and after purified with treatment drugs

GST purified activity with drugs µmol/min/ml	GST activity without drugs µmol/min/ml	Types of drugs
3.24	3.62	Metformin
3.24	3.57	Daonil
3.24	3.66	Daonil and Metformin

Table 2 showed the activity of GST enzyme before purification and the activity after purification with treatment diabetes mellitus drugs (metformin and daonil). The results showed a change in the activity of GST enzyme where it raised after purified and treatment with drugs, which explained the effect of this drugs on the activity of GST for transfer this xenobiotics out of the body. Also showed from table 2. The activity before

purification less than after purification due to found other effects on enzyme while there is no other protein effects after purification.

References:

1. Wilce MCJ and Parker MW., (1994), "Structure and function of glutathione S−transferases, a comprehensive review of mammalian GSTs", Biochim Biophys Acta; 1205:1–18.
2. Sheehan D., Meade G., Foley V. M. and Dowd C. A., (2001), "Structure, function and evolution of glutathione transferases: implications for classification of non−mammalian members of an ancient enzyme superfamily", Biochem. J.; 360: 1–16.
3. Dixon D., Lapthorn A and Edwards R., (2002), "Plant glutathione transferases", Genome Biology . 3; REVIEWS3004.
4. Allocati N, Federici L, Masulli M. and Di I. C.,(2009), "Glutathione transferases in bacteria", FEBS J; 276: 58–75.
5. Mannervik B. and Danielson U. H., "Glutathione transferases-structure and catalytic activity", CRC Crit. Rev. Biochem; (1988),23: 283–337.
6. Habig W., Pabst M. and Jakoby W., (1974), "Glutathione S−transferases. The first enzymatic step in mercapturic acid formation", J Biol Chem, 249; 7130–7139.
7. Öztetik E., "A tale of plant glutathione S−transferases: since 1970",(2008), Bot. Rev.; 74: 419–437.
8. Cedeberg J., "Oxidative stress, antioxidative defence and outcome of gestation in experimental diabetic pregnancy", Thesis, Ph. D. Sweden, Psala Unversity.(2001),
9. Chauhan G., "Glutathione S−transferases: family of enzymes", Free radicals in biology and medicine; (2003),77: 222, spring.
10. Salinas A. E. and Wong M. G., "Glutathione S−transferases – a review"(1999), Curr. Med. Chem; 6: 279–309.
11. Hayes J. D., Flanagan J. U. and Jowsey I. R., (2005), "Glutathione transferases", Annu. Rev. Pharmacol. Toxicol; 45: 51–88.
12. Bradford M., "A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of proteindye binding", (1976),Anal Biochem; 72:248–254.
13. Aydemer T. and Kavrayan D., (2009),"Purification of glutathione S−Transferase from chicken Erythrocyte",Artificial Cells, Blood Substitutes, and Biotechnology; 37: 92-100.
14. Laemmli U. K., "Cleavage of structural proteins during the assembly of the head of bacteriophage T4",(1970), Nature; 227: 680–685.
15. Al-Gubury, H. Y., , Almaamory, E. S., Alsaady, H. H., and Almurshidy, G. S., 2015., Research Journal of Pharmaceutical, Biological and Chemical Sciences, 6: 929-939.
16. Al-Gazally, M. E.; Al-Awad, A. S.; Kzar, H. H., 2016.Evaluating the superoxide dismutase-1 status in wild type and mutant at codons 12 and 13 of KRAS gene spectrum for the patients with sporadic colorectal cancer, International Journal of PharmTech Research, 9: 272-279.
17. Aljeboree, A. M.,2016.Adsorption of crystal violet dye by Fugas Sawdust from aqueous solution , International Journal of ChemTech Research,. 9(3): p. 412-423.
18. Omran, A. R.; Baiee, M. A.; Juda, S. A.; Salman, J. M.; Alkaim, A. F., 2016.Removal of congo red dye from aqueous solution using a new adsorbent surface developed from aquatic plant (Phragmites australis), International journal of ChemTech Research, 9: 334-342.
19. Salman, J. M.; Al-Mamoori, A. M. J.; Al-Saadi, A. H.; al Terehi, M. N., 2012.Dna damage in common carp fish (cyprenus carpio l.) induced by acute exposure to copper & cadmium, World Applied Sciences Journal, 20: 679-682.
20. Jafar, N. N.; Al-Dahmoshi, H. O. M.; Jeburalmamoori, A. M.; Al-Khafajii, N. S. K.; Al-Masoudi, N., 2013.Synthesis and biological activity of new derivatives of 6-chloro-5-((4-chlorophenyl) diazenyl)pyrimidine-2,4-diamine and 4-chloro-6-methoxy-N,N-dimethylpyrimidin-2-amine, Biomedical and Pharmacology Journal, 6: 453-465.
21. Al-Gubury H. Y., and Al-Murshidy G. S., 2015. Photocatalytic decolorization of brilliant cresyl blue using zinc oxide, International Journal of PharmTech Research, 8: 289-297.
22. Al-Terehi, M., Al-Saadi, A. H., Al-Sherefi, A. N., and Zaidan, H. K., 2016.Optimization polyplexe stability in different glucose concentrations, International Journal of ChemTech Research, 9: 396-401.

23. Al-Khafaji, N. S. K.; Shareef, H. K.; Al-Dahmoshi, H. O. M., 2015.Analysis of β-lactamases among multi drug resistant Klebsiella pneumoniae in Hilla city-Iraq, Research Journal of Pharmaceutical, Biological and Chemical Sciences, 6: 903-907.
24. Al-Dahmoshi, H. O. M.; Al-Saffar, A. K. H.; Al-Khafaji, N. S. K.; Ali, J. A.; Muttaleb, W. H., 2016.Non invasive markers to predict liver damage among patients with chronic HBV, Hilla City-Iraq, International Journal of PharmTech Research, 9: 388-393.
25. Alsayigh, H.; Ali, S. M.; Al Mahbob, T.; Al Salihy, S., 2016.Molecular detection of human papillomavirus genotype-31 in tissues from patients with prostate cancer and benign prostatic hyperplasia, International Journal of PharmTech Research, 9: 277-283.
26. Hadi, A. G.; Humedy, E. H.; Saddam, N. S.; Abd-Alameer, F. S., 2016.Spectrophotometric study of complex formation between hematoxylin and Al3+ and Fe3+ ions, International Journal of PharmTech Research, 9: 292-298.
27. Al-Terehi, M., Al-kilabi, I. H. AL–Mamoori, A. M. J. Al-Jboori, M. J., Al-Saadi, A. H., and Zaidan, H. K., 2016.Some heavy metals concentrations in tumor tissue, International Journal of ChemTech Research, 9: 407-411.
28. Smaism, M. F., 2016.Assessment of leptin levels in the different genotypes and leptin receptor genes in the women with polycystic ovary syndrome and diabetes mellitus type 2 in Iraq population, International Journal of PharmTech Research, 9: 269-276.
29. Al-Gazally, M. E.; Al-Awad, A. S.; Kzar, H. H., 2016.Assessment of antioxidant status in different genotypes/phenotypes at codon 72 of TP53 gene for patients with sporadic colorectal cancer in Babylon province, International Journal of PharmTech Research, 9: 280-286.
30. Karam, F. F.; Hussein, F. H.; Baqir, S. J.; Alkaim, A. F., 2016. Optimal conditions for treatment of contaminated waters with anthracene by Fenton processes in close system reactor, Journal of Chemical and Pharmaceutical Science, 9: 1111-1115.
31. Kamil, A. M.; Mohammed, H. T.; Alkaim, A. F.; Hussein, F. H., 2016. Adsorption of Congo red on multiwall carbon nanotubes: Effect of operational parameters, Journal of Chemical and Pharmaceutical Sciences, 9: 1128-1133.
32. Raheem, R. A.; Al-gubury, H. Y.; Aljeboree, A. M.; Alkaim, A. F., 2016. Photocatalytic degradation of reactive green dye by using Zinc oxide, journal of Chemical and Pharmaceutical Science, 9: 1134-1138.

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