**In Vitro Effects of Some Cations on Glutathione S-Transferase Enzyme Purified from Chicken Heart**

*Suat ZOR1,0000-0001-5424-0542, Mehmet ÇİFTCİ2,\*,0000-0002-1748-3729*

*1Batman University,* [*Social Sciences Vocational School*](https://batman.edu.tr/Birimler/1472)*, Batman,, Türkiye*

*2Bingol University,* [*Faculty*](http://sshmyo.bingol.edu.tr/en/) *of Veterinary Science,Bingol, Türkiye*

|  |
| --- |
| **Abstract**  Glutathione S-transferase enzyme (GST; EC 2.5.1.18) is an important antioxidant enzyme in metabolism and takes part in reactions that enable the conjugation of glutathione with many metabolites that may cause toxicity. In this study, firstly, the GST enzyme was purified from chicken heart by homogenate preparation, ammonium sulfate precipitation and glutathione-agarose affinity chromatography. Then, the inhibition effects of Ag+, Pb2+ and Na+ ions on enzyme activity were examined in vitro. Enzyme activity was determined spectrophotometrically at 340 nm by the method of Habig et al. (1974). This method was applied in all kinetic studies. In the kinetic studies, it was found that Ag+ (in the range of 0.1-0.7 mM), Pb2+ (in the range of 0.1-0.7 mM) and Na+ (in the range of 1-5 mM) cations caused inhibition on the enzyme activity. IC50 values were found by drawing % Activity-[I] graphs for these cations showing inhibition effects. IC50 values for Ag+, Pb2+ and Na+ were found to be 0.239, 0.283 and 1.725 mM, respectively. |
| ***Key Words:*** *Glutathione S-transferase, Ag+, Pb2+,Na+, Inhibiton* |

1. **Introduction**

Since many products in today's world, from food and beverages to the items we wear, are produced synthetically, highly harmful chemicals are used in this production process. Many scientific studies have shown that these chemicals cause serious harm to humans and the environment. It has been reported that organic substances such as carbohydrates and proteins, especially those processed at high temperatures, have a carcinogenic effect [1]. In parallel with the development of technology, the amount of many toxic substances that threaten humans and the environment has inevitably increased in this process. Examples include substances such as heavy metals, plastic materials, pharmaceutical residues, pesticides and herbal medicines [2]. One of the sources that cause this toxicity is free radicals. Xenobiotics from exogenous sources are also involved in the formation of free radicals from endogenous and exogenous sources [3]. One of the main functions of the GST enzyme, which is the main subject of this study, is to render harmless these toxic substances entering the body through detoxification reactions [4]. As a result of the phase II reaction, the protection of the living organism from reactive electrophilic attacks occurs thanks to the GST enzyme [5]. The reason for the high GST rate in organs such as lung, liver, kidney and small intestine is the xenobiotics entering the body [6]. The GST enzyme has been accepted as a natural protective antioxidant because it eliminates these harmful substances as a result of a number of chemical reactions.

1. **Materials and Methods** 
   1. **Quantitative Protein Determination**

Quantitative protein determination was determined spectrophotometrically by the Bradford method at 595 nm [6].

* 1. **Activity Determination**

The activity of glutathione S-transferase (GST) enzyme was determined spectrophotometrically at a wavelength of 340 nm according to the method used by Habig et al. [7].

* 1. **Procuring Chicken Heart and Preparing the Homogenate**

The heart used in the experiments was obtained from Bingöl Meat and Milk Institution in accordance with the cold chain rules and was kept in the deep freezer at -20°C. The frozen heart was cut into small pieces to prepare the homogenate solution. Then, 5 g of chicken heart was taken. Three times the amount of homogenate buffer was added to the amount of heart taken and it was made homogeneous using a homogenizer in an ice tray. Afterwards, the resulting homogenate was centrifuged at 13,000 x g for one hour. After this process, the precipitate was separated and homogenate was obtained.

* 1. **Ammonium Sulfate Precipitation and Dialysis Procedures**

The resulting homogenate was precipitated with ammonium sulphate between 20-80% to ensure that most of the chicken GST enzyme precipitated. At each step, the homogenate was centrifuged at 13500xg for 15 minutes. During these processes, the activities of the precipitate and supernatant were measured at each stage. Thus, tubes with activity were identified and merged. The mixture resulting from the precipitation process was dialyzed against dialysis buffer (10 mM K-phosphate, 1 mM EDTA pH = 7.5) for approximately two hours in the dialysis bag.

* 1. **Purification of Enzyme by Affinity Chromatography**

The enzyme sample obtained after dialysis was applied to the glutathione agarose affinity column, whose flow rate was 20 mL/hour with a peristaltic pump and balanced with 10 mM KH2PO4 and 0.1M KCl, pH: 8.0 buffer. The column was then subjected to washing. This process was carried out with a buffer solution of 10 mM KH2PO4 and 0.1M KCl, pH: 8.0. The washing process was completed after the absorbance values measured in the fractions during the spectrophotometric measurement were approximately equal to the blank. Gradient elution was performed to obtain the enzyme pure. Then, the enzyme was eluted from the column using the elution solution containing 50 mM Tris-HCl and (1.25-10 mM, pH: 9.5) GSH [8].

* 1. **Kinetic Studies**

Ag+, Pb2+ and Na+ cations were used in inhibition studies. First of all, appropriate solutions were prepared for each cation and activity measurements were made. Using the activity values obtained as a result of activity measurements and the applied concentrations, % Activity-[I] graphs were drawn and with the help of these graphs, IC50 values were found for each cation.

1. **Results**

Activity%-[I] graphs drawn for drugs showing inhibitory effects on chicken heart GST enzyme are shown in Figures 1, 2, and 3. The IC50 values obtained for the Ag+, Pb2+ and Na+ which have an inhibitory effect on the enzyme, were 0.239, 0.283 and 1.725 mM, respectively, and are shown in Table 1.

Figure 1. Activity%-[Ag+] graph

Figure 2. Activity%-[Pb2+] graph

Figure 3. Activity%-[Na+] graph

Table 1. Obtained IC50 values

|  |  |
| --- | --- |
| Cation | IC50 (mM) |
| Ag+ | 0,239 |
| Pb2+ | 0,283 |
| Na+ | 1,725 |

1. **Conclusion**

Glutathione S-transferase enzyme (GST; EC 2.5.1.18) is an important antioxidant enzyme in metabolism and takes part in reactions that provide conjugation of glutathione with many metabolites that may cause toxicity. In this study, the effects of cations such as Ag+, Pb2+ and Na+ on the enzyme were investigated. As a result of the research, it was determined that Ag+, Pb2+ and Na+ cations significantly inhibited the enzyme. Therefore, IC50 values were found by drawing Activity%-[I] graphs for these cations (Figures 1, 2, 3 and Table 1). According to these IC50 values (inhibitor concentration that reduces the enzyme activity by half), the decreasing inhibitory powers of the drugs can be listed as Ag+, Pb2+ and Na+. Because while Ag+ with a concentration of 0.239 mM causes 50% inhibition, this value is 0.283 mM for Pb2+ and 1.725 mM for Na+. According to the IC50 values obtained in this study, it is understood that the strongest inhibitor is Ag+, followed by Pb2+ and Na+. As a result, it will be important to conduct more detailed inhibition studies for these three cations in future studies, determine Ki constants and conduct *in vivo* studies. As a result, people exposed to heavy metals with low IC50 values such as Ag+ and Pb2+ need to be very careful and take the necessary precautions.

**References**

1. Senyuva, H.Z., and Gokmen, V. Surname N., (2005). *Food Additives and Contaminatio,* 22 (3), 204-209.
2. Senyuva, H.Z., and Gökmen, V. (2005). *Food Additives and Contaminatio,* 22 (3), 204-209.
3. Rebbeck, T.R. (1997). Molecıılar Epidemiology of the Human Glutathione S-Transferase Genotypes GSTM1 and GSTT1 in Cancer Susceptibility, *Cancer Epidemiology, Biomarkers &Prevention*, 6(9),733-743.
4. Awasthi, Y. C., Dao, D. D., and Saneto, R. P. (1980). Interrelationship between anionic and cationicforms of glutathione S-transferases of human liver. *Biochem J.,* 191(1), 1-10
5. Gyamfi, M. A., Ohtani. II., Shinno, E., and Aniya, Y. (2004). Inhibition of glutathionestransferases by thonningianin A, isolated from the African medicinal herb, Thonningia sanguinea, in vitro. Food and *Chemical Toxicology,* 42(9): 1401-1418.
6. Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72: 248-251.
7. Habig WH, Pabst MJ, Jakoby WB (1974). Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. *Journal of Biololgical Chemistry*, 249: 7130-7139.
8. Toribio F., Martinet L.E., Pascual P., Lopez B.J., 1996. Methods for purification of glutathione peroxidase and related enzymes, *J. Chromatog. B.* 684: 77-97.