**Investigation of in Vitro Effects of Some Antibiotics on Chicken Heart Glutathione S-Transferase Enzyme Activity**

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**Abstract**

Glutathione S-transferase enzyme (GST; EC 2.5.1.18) is a very important antioxidant enzyme that plays important functions in living metabolism. Glutathione S-transferases have been identified as a Phase-II detoxification enzyme family. Xenobiotics, which originate from exogenous or endogenous sources, transform into metabolites with lower toxicity that are easily excreted from the living organism as a result of Phase II reactions. During this event, GST takes part in reactions that enable the conjugation of glutathione with many metabolites that may cause toxicity.

This study was carried out in two stages. First of all, the GST enzyme was purified and then the in vitro effects of some antibiotics on the purified GST enzyme activity were examined. GST enzyme was purified from chicken heart tissue by preparation of homogenate, ammonium sulfate precipitation and glutathione-agarose affinity chromatography. In the kinetic studies conducted with the purified GST enzyme, it was determined that the antibiotics amoxicillin, cefuroxime sodium and cefazolin sodium had an inhibitory effect on the enzyme. Activity%-[I] graphs were drawn with the data obtained as a result of the kinetic studies, and the IC50 values for the antibiotics in question were calculated as 0.66, 2.07 and 2.09 mM, respectively.

**Key Words:** Glutathione S-transferase, Antibiotic, Inhibition

1. **Introduction**

Living organisms encounter endogenous or exogenous substances containing toxicity throughout their life processes. They carry out their defense mechanisms against such harmful substances through their detoxification systems. Thus, xenobiotics that form in the body of the living thing or enter the body from outside are detoxified. To get rid of harmful substances, there are reactions called Phase I, Phase II and Phase III. The GST enzyme is involved in Phase II reactions. GST can bind with many organic anions to carry out detoxification reactions with compounds arising from endogenous and exogenous sources [1]. As a result of phase II reactions, the living organism is protected from the attacks of electrophilic substances with very high toxicity [2]. GST binds glutathione (GSH) and its substrate by bringing them closer together in its active site [3]. In this case, the sulfhydryl group on GSH becomes active and the aggressive electrotrophic substrate reacts with GSH [4].

GSH is endogenously synthesized in the liver through anabolic pathways using glutamic acid, cysteine and glycine [5]. Reduced glutathione (GSH) is a thiol-containing tripeptide of low molecular weight that is found in almost all living cells [6]. Glutathione (GSH) is considered an antioxidant because it eliminates free radicals such as H2O2, which cause serious damage to tissues, and eliminates the effects of reactive oxygen species (ROS) that cause damage to tissues [7]. The GSH/GSSG ratio is approximately 500 in erythrocytes. If a decrease in GSH level is observed, the onset and progression of many degenerative diseases are observed [8]. For example, aging causes symptoms of many diseases, including diabetes, renal failure, pneumonia, malignancy, amyotrophic lateral sclerosis, Parkinson's, Alzheimer's, and cataracts [9].

The aim of this study is to examine the effects of antibiotics such as amoxicillin, cefazolin sodium and cefuroxime sodium on GST enzyme activity.

1. **Materials and Methods**

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

The chicken hearts used in the experiments were obtained from Bingöl Meat and Milk Institution in accordance with the cold chain rules and kept in the deep freezer at -20°C. To prepare the homogenate solution, the frozen heart was cut into small pieces, 5 g of chicken heart was taken and 3 times the amount of homogenate buffer was added. The homogenate, which was made homogeneous using a homogenizer, was placed in Ependorf tubes and centrifuged at 13,000 x g for one hour. The precipitate was separated and homogenate was obtained. These processes were carried out at +4 °C.

**2.2. Purification of the Enzyme and Determination of Activity**

The enzyme sample was applied to the glutathione agarose affinity column equilibrated with 10 mM KH2PO4 and 0.1M KCl, pH: 8.0 buffer. Gradient elution was performed to obtain the enzyme pure. The solution for the prepared elution was created from a solution containing 50 mM Tris-HCl and (1.25-10 mM, pH: 9.5) GSH. The activities of the obtained eluates were measured on a spectrophotometer set to 340 nm wavelength. Enzyme activity was performed according to the method used by Habig et al. [10].

**2.3. Kinetic Studies**

To prepare the IC50 chart, the activity value of the purified enzyme was measured as a control in the inhibitor-free environment. This measured value was accepted as 100%. Afterwards, IC50 values were calculated by measuring the values of antibiotics at different concentrations. These concentration values; for cefuroxime sodium; 0.896, 1.792, 4.48 and 8.96 mM, 0.419, 0.838, 1.676, 4.19 and 8.38 mM for cefazolin sodium, 0.228, 0.456, 0.912, 1.14, 1.368 and 1,596 mM for amoxicillin. The graphic equation was obtained by creating % Activity-[I] concentration graphs with these concentration values obtained. IC50 values were calculated using this equation.

1. **Results and Discussion**

In the study, GST enzyme was purified from chicken heart tissue according to the processes mentioned above. In the kinetic study, % Activity - inhibitor concentration graphs were drawn for the antibiotics amoxicillin, cefuroxime sodium and cefazolin sodium, which inhibit enzyme activity, and Figure 1, Figure 2 and Figure 3 were created. With the help of these graphs, IC50 values for the antibiotics amoxicillin, cefuroxime sodium and cefazolin sodium were calculated as 0.66, 2.07 and 2.09 mM, respectively. These values are shown in Table 1.

**Figure 1**. Effect graph of amoxicillin antibiotic on GST enzyme activity

**Figure 2**. Effect graph of cefuroxime sodium antibiotic on GST enzyme activity.

**Figure 3**. Effect graph of cefazolin sodium antibiotic on GST enzyme activity

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**Table 1.** Effects of antibiotics on GST enzyme activities

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| --- | --- | --- |
| **Antibiotic** |  | **IC50 (mM)** |
| Amoxicillin  Cefuroxime sodium |  | 0.66  2.07 |
| Cefazolin sodium |  | 2.09 |
|  |  |  |

Today, many different substances have been identified that activate and inhibit the GST enzyme. Scientific studies have been conducted showing that while some of these are cations, most of them are drugs. In a study conducted by Casalino et al., the effects of Cd+2 and Mn+2 heavy metal cations on the activity of the GST enzyme isolated from rat liver tissue were examined. The rat was given 2.5 mg/kg CdCl2 or 2.0 mg/kg MnCl2 salts as a single dose. As a result, it was measured that the enzyme activity increased by approximately 36% one day after the experiment [11]. Türkanoğlu examined the GST enzyme activity purified from human blood serum with some metal cations. As a result of the research, it was reported that Cd+2, Hg+2 and Ni+2 cations reduced the activity of the GST enzyme [12]. Again, Chun-hua Zhang and his colleagues examined the effect of Cd+2 cation on the activity of GST purified from rice. As a result of the study, it was revealed that Cd+2 cation inhibits the enzyme [13]. In their study, Türkan and his colleagues isolated GST enzymes from the liver, heart muscle and kidney tissues of the albino rat species and examined the in vivo effects of the antibiotics cefuroxime sodium and cefoperazone sodium on this enzyme. They found that enzyme activity decreased seven hours after drug use [14]. In their study, Bayindir et al. examined the effects of gentamicin and clindamycin on GST enzyme activity isolated from rat erythrocyte tissue in vitro and calculated IC50 values. As a result of the experiment, it was reported that both antibiotics inhibited the enzyme. As a result of the experiment, IC50 values were found to be 1.69 and 6.9 mM, respectively [15].

1. **Conclusion**

In this study, the in vitro effects of antibiotics such as cefuroxime sodium, cefazolin sodium and amoxicillin on the GST enzyme isolated from chicken heart tissue were examined and IC50 values were calculated. Experimental results showed that these antibiotics inhibited enzyme activity. IC50 values of amoxicillin, cefuroxime sodium and cefazolin sodium were calculated as 0.66, 2.07 and 2.09 mM, respectively. Today, the therapeutic properties of antibiotics against bacterial infections have been demonstrated by many studies. However, the dose factor is very important in drug use. It is recommended that IC50 values be taken into consideration when using antibiotics whose kinetic effects on GST are examined.

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