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Effect of 3-Amino-1,2,4-Triazole-5-Carboxylic Acid on Human Blood Erythrocyte Catalase

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Abstract: Catalase is an antioxidant enzyme with great therapeutic potential that scavenges hydrogen peroxide, a reactive oxygen species produced during cellular metabolism. Substances containing 1,2,4-triazole structures are biologically important heterocyclic compounds found in the structure of many pharmaceutical drugs used in drug discovery studies against various types of diseases in the human body. In this study, the effect of phosphate buffer prepared at different pHs and 3-amino-1,2,4-triazole-5-carboxylic acid (ATZc) on catalase enzyme activity in human blood erythrocytes was determined. It was determined that the catalase enzyme was inhibited by ATZc at different pH levels. The weakest inhibition was observed at pH 5.5 (IC_{50}:49.01 µM), whereas the strongest inhibition was observed at pH 7.5 (IC_{50}:23.21 µM).

Keywords: 3-amino-1,2,4-triazole-5-carboxylic acid, Catalase, enzyme inhibition

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1. INTRODUCTION

Heterocyclic compounds are commonly found in nature and are the composition of various biologically active molecules, effective modern drugs, and plant protection products (1). Nitrogen-containing heterocycles have been found to have a significant impact on the discovery of new structures for pharmaceutical applications. These compounds are commonly seen in metabolic systems that are vital for living organisms. 1,2,4 triazoles, which have particularly unique structures and properties among various nitrogen-containing heterocycles, are used in various fields such as pharmaceutical chemistry, agricultural chemistry, materials science, and organic catalysts (2). Triazoles are five-membered rings containing three heteroatoms, a nitrogen atom, and two carbon atoms that can interact with various proteins, enzymes, and receptors in organisms through weak interaction bonding. Therefore, they can be viewed as bioisosteres of amides, esters, and carboxylic acids. Triazoles have various biological activities such as anticancer, antibacterial, antifungal, anti-inflammatory, antiviral, antioxidative, and anti-HIV. These triazole compounds show their biological activities through the inhibition of some other enzymes such as dihydrofolate reductase, DNA polymerase, DNA gyrase, and catalase (3).

Catalase is one of the most fundamental oxidoreductases in the human body and is a vital antioxidant enzyme involved in the catalytic removal of reactive oxygen species (ROS), prevention of cell damage, and inhibition of tumor cell growth (4). This enzyme catalyzes the breakdown of H_2O_2, which is highly toxic, into water and oxygen, thus protecting cells against toxic oxidants. Inactivation of the catalase enzyme has some side effects and can lead to various diseases such as cancer, hypertension, Parkinson's disease, and Alzheimer's disease. Human blood erythrocyte catalase is a large (240 kDa) enzyme with a tetrameric structure containing iron and four heme groups. This large structure facilitates its interaction with the substrate H_2O_2 (5). There are known inhibitors of catalase activity, such as azide, cyanide, and cyanogen bromide, but these inhibitors are not specific to the enzyme and can

inhibit different enzymes. 3-amino-1,2,4-triazole (ATZ) causes irreversible inhibition of the enzyme by covalent binding to the active center of the tetrameric form of catalase in the presence of H_2O_2, and this inhibitor is specific for catalase (6). Catalase is primarily localized in the liver. Given that the liver is the organ responsible for metabolizing and detoxifying drugs, this enzyme plays a crucial role in determining the potential side effects of medications (7). Erythrocytes are also a good model for investigating the toxicological effects of xenobiotics (drugs, pesticides, etc.) entering the human body (8). Because inhibition studies are important to investigate drug–zymes interactions, understand the mechanisms involved in the body, and determine the effective concentration and toxicity of drugs, we aimed to determine the effect of ATZc on catalase activity in human erythrocytes. In addition, the investigation of interactions at different pH levels can aid in understanding the effects of this compound in various cellular environments. Such studies can be valuable in biomedical research for the discovery and development of potential therapeutic agents.

2. MATERIALS AND METHODS

2.1. Materials

Hydrogen peroxide (H_2O_2), dipotassium phosphate (K_2HPO_4), dihydrogen phosphate (KH_2PO_4), 3-amino-1,2,4-triazole-5-carboxylic acid were obtained from Sigma-Aldrich. The study was approved by the local ethics committee (Balikesir University Faculty of Medicine Clinical Research Ethics Committee, Balikesir/Turkey, Decision No:2022/44 and Date:09.03.2022).

2.2. Preparation of Hemolysate

A 20-mL blood sample was collected from healthy volunteers, and the supernatant was separated by centrifugation at 5000 rpm for 20 min at +4 °C. Then, it was washed three times with 0.9% NaCl. The blood sample was then hemolyzed with three times the volume of cold water. The hemolysate was centrifuged again at 15000 rpm for 40 min at +4 °C.

2.3. Catalase Activity Determination

Enzyme activity was determined by the spectrophotometric method by measuring the absorbance decrease at 240 nm during the conversion of H_2O_2 to water and oxygen in the experimental environment (9). For activity determination, 30 mM H_2O_2 substrate solution was used and 50 mM phosphate activity buffer was prepared at different pH ranges (5-5.5-6-6.5-7-7.5-8-8.5).

2.4. Determination of the Inhibition Effect of 3-amino-1,2,4-triazole-5-carboxylic acid on Catalase

The inhibition study of ATZc (inhibitor) was performed at different pHs and constant substrate concentrations. A total reaction volume of 3 mL was created by taking 0.1 mL of hemolysate and different volumes of inhibitor (0.01 M) solution. First, enzyme activity was found in the inhibitor-free environment and this value was accepted as 100% activity. The change in absorbance at 240 nm in one minute was recorded. From the absorbance values obtained, % activity-inhibitor graphs were drawn, and IC_{50} values were calculated from their slopes.

3. RESULTS AND DISCUSSION

Triazoles are attracting increasing attention because of their broad biological activities. In particular, ATZ derivatives have received special attention because they demonstrate a broad range of bioactivity, including potential applications against thrombotic disorders, fibrotic, autoimmune diseases, central nervous system disorders, obesity, and diabetes (10). Today, there are triazole ring-containing drugs with different properties and in use for various therapeutic purposes. These drugs are used as antifungals, such as fluconazole, itraconazole, and voriconazole; as antidepressants, including triazolam, alprazolam, and estazolam; and as antivirals, such as ribavirin. The drugs letrozole, anastrozole, and vorozole, which are also aromatase enzyme inhibitors used in breast cancer treatment, contain a 1,2,4-triazole ring (11). At the same time, many compounds derived from triazole are effective in inhibiting various enzymes such as phosphatase, lactamase, butyrylcholinesterase, acetylcholinesterase, alpha-amylase, and alpha-glucosidase (12).

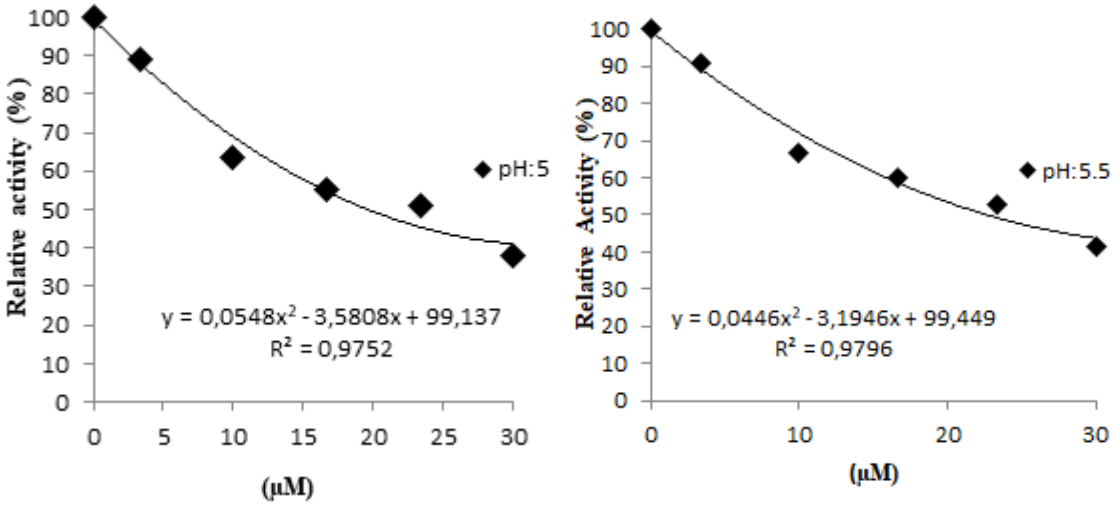
Catalase, an antioxidant enzyme, plays an important role in the defense system to eliminate the destructive effects of ROS. Therefore, any factor that can weaken the activity of this enzyme can cause ROS accumulation and oxidative damage to proteins, causing many diseases. ATZ is a specific inhibitor that covalently binds to the active site of catalase (13). Therefore, the catalase enzyme is the most suitable enzyme for studying the inhibition properties of triazole derivatives.

In this study, the inhibition effect of ATZc on catalase was investigated at different pH values. For this purpose, %activity-inhibitor graphs were plotted (Figure 1). IC_{50} values were calculated from the slopes of these graphs; the strongest inhibition was observed at pH:7.5 (IC_{50}: 23.21 μM) and the weakest inhibition was observed at pH:5.5 (IC_{50}: 49.01 μM). It was found to have stronger inhibition at high pHs, whereas it had weaker inhibition at low pHs (Table 1). The pH of the medium has a significant impact on enzyme performance (14). Catalase exhibits activity in a broad optimum pH:5-10 range (15).

Table 1: IC_{50} values of 3-amino-1,2,4-triazole-5-carboxylic acid determined at different pH ranges.

pH (Buffer Solution)	IC_{50} (µM)
5	45.74
5.5	49.01
6	26.16
6.5	27.92
7	24.56
7.5	23.21
8	27.88
8.5	26.52

In the present experiment, the inhibition effect of ATZc in two forms (ATZ-carboxylic acid and ATZ-carboxylate) on catalase under different pH conditions was investigated. Carboxylic acid converts to carboxylate as pH increases, and carboxylate converts to carboxylic acid as pH decreases. Therefore, inhibition of ATZc was observed between pH:5-8.5. This inhibitor showed weak inhibition at pH 5-5.5 but strong inhibition in the basic form at pH 7.0-8.5. Thus, it shows that the active part of this inhibitor interacts with the chemical group of the enzyme, which is approximately 7–7.5 pKa.



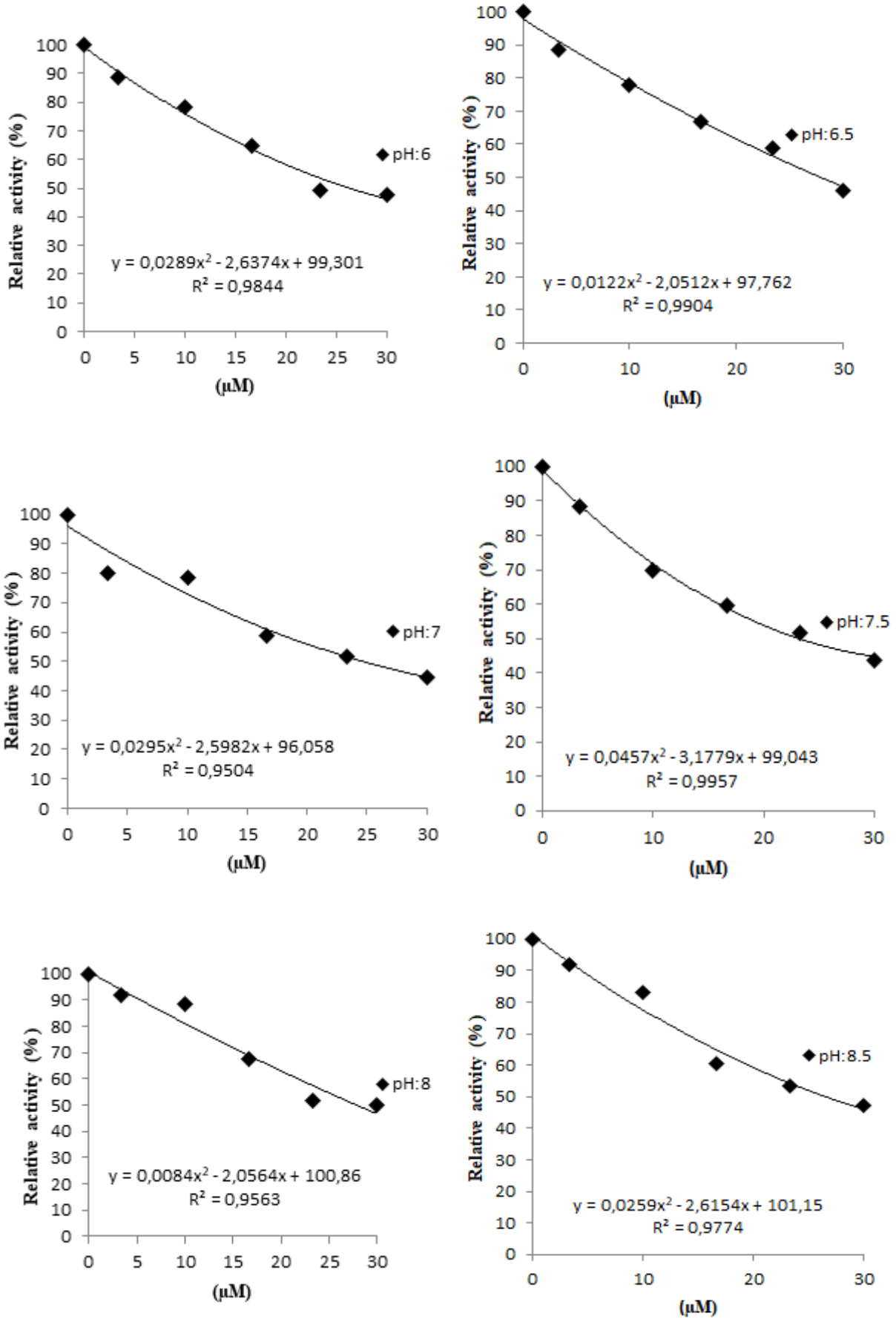


Figure 1: Inhibition graphs of 3-amino-1,2,4-triazole-5-carboxylic acid at different pHs.

In the literature, the inhibitory effect of ATZ on chicken liver catalase activity was reported as IC_{50}:3 mM at pH:7 (16), whereas in this study the strongest inhibition of human blood erythrocyte catalase activity was found at pH: 7.5 IC_{50}:23.21 µM. These pH values, on the other hand, are close together, but the inhibitor we used in our study contains a carboxyl (-COOH) group, unlike the ATZ compound (Figure 2c).

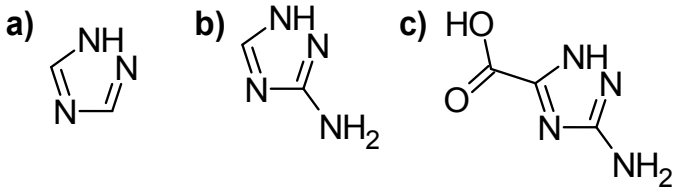


Figure 2: a) 1,2,4-triazole **b)** 3-amino-1,2,4-triazole (ATZ) **c)** 3-amino-1,2,4-triazole-5-carboxylic acid (ATZc).

In another study, it was found that ATZ infused peripherally in spontaneously hypertensive rats (SHR) disrupted sympathetic activity and also increased antiyosinergic activity. Additionally, it has been found to have a vascular relaxation effect and reduce arterial pressure in SHRs (17). Tada et al. reported that the ATZ is a competitive inhibitor of imidazole glycerol phosphate dehydratase (18).

4. CONCLUSION

In conclusion, in this study, the pH-dependent effect of ATZc on human erythrocyte catalase enzyme activity was determined. There have been no inhibition studies on catalase and 3-amino-1,2,4-triazole-5-carboxylic acid in the literature. An important part of the active substances used for therapeutic purposes are drugs that act as enzyme inhibitors. Therefore, determining the effect of ATZc, which has a 1,2,4-triazole structure, on the catalase enzyme is crucial for drug development research, but it should be supported by *in vivo* studies for its use in treatment.

5. CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

6. ACKNOWLEDGMENTS

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