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# Effects of Some Drugs on Enzyme Activity of Catalase from Bovine Liver

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The effects of gentamicin sulphate, acetyl salicylic acid, ampicillin sodium, paracetamol, potassium penicillin and augmentin were investigated on the *in-vitro* enzyme activity of catalase. Catalase (CAT:EC1.11.1.6) was purified from bovine liver by a simple and rapid method. The purification process was done by 2′,5′-ADP sepharose 4B affinity chromatography. Although the purified enzyme showed a tetrameric band on sodium dodecyl sulphate polyacryilamide gel electrophrosis but bovine liver showed a one band. The enzyme activity was measured spectrophotometrically at 240 nm, according to the method of Aebi. From these six drugs, paracetamol, potassium penicillin and augmentin inhibited the activity of the purified enzyme; gentamicin sulphate, acetyl salicylic acid and ampicillin sodium showed little effect on the enzyme activity. The $I_{50}$ values for these three drugs were as 4.6, 0.35 and 0.49 mM, respectively. The $K_i$ constants were 20, 25 and 25 mM, respectively and they were competitive inhibitors.

**Key Words: Catalase, Bovine liver, Gentamicin sulphate, Acetyl salicylic acid, Ampicillin sodium, Paracetamol, Potassium penicillin, Augmentin**

## INTRODUCTION

Catalase (EC 1.11.16) is a tetrameric enzyme with identical each containing a haem prosthetic group[1–3]. It is known as an antioxidant agent[4] because of its role in protecting cells from the toxic effects of $H_2O_2$. Catalase is a characteristic enzyme of peroxysomes group, in which catalase is present as the most abundant protein. This enzyme is widely distributed in a variety of life forms, including animals, plants and microbes. Catalase plays an important role in removing toxic hydrogen peroxide in the cell[5]. Although a large amount of information about catalase is now available, certain features of this enzyme have been poorly understood for several decades[6, 7]. Some evidence points to the existence of multiple forms of this enzyme in mammalian organisms, particularly in the mouse and rat[8]. Catalase is characterized by an electron pair transition[9] in which $H_2O_2$ is decomposed to $O_2$ and $H_2O$. Numerous purification procedures have been developed for the isolation of catalase from a variety of species and organs[5].

Many drugs are being used for therapies and there are few literature reports related with changing of enzyme activities. A few reports have indicated that some increases and decreases were found in human liver enzyme activity levels such as aspartate aminotransferase, ascorbate, alanine aminotransferase and alkaline phosphatase[10–14]. Effects of ionic and zwitter ionic surfactants on the stabilization of bovine catalase have also been studied[15]. Determination of the kinetic parameters connected with inactivation of bovine liver catalase have been investigated[16].

Drugs are used to deal with various disorders but there are few reports of their effects on enzyme activities. Some studies found either increases or decreases in mammalian enzyme activities and the inhibitor or activator effects of some drugs have been investigated[17–19]. The present study, therefore, investigated the *in-vitro* effects of gentamicin sulphate, acetyl salicylic acid, ampicillin sodium, paracetamol, potassium penicillin and augmentin on catalase purified from bovine liver.

## EXPERIMENTAL

2′,5′-ADP-sepharose 4B, sephadex G-25, sephadex G-50, protein assay reagents were from Sigma Chem. Co. Medical drugs were provided by Adeka Co. All other chemicals used were of analytic grade and obtained either from Sigma-Aldrich or Merck.

**Preparation of the homogenate:** Liver from adult bovine was washed in isotonic saline containing 1 mM EDTA and stored at –78°C before use. 20 g of liver was cut into small pieces and homogenized in a blender with 50 mL of 50 mM potassium phosphate buffer, pH 7, containing 1 mM EDTA and 458 mM saccharose. The homogenate was centrifuged at 15,000 rpm for 60 min and precipitate removed. This process was repeated twice and the supernatant was used as a crude extract. The crude extract was brought to 30–70% ammonium sulphate precipitation with solid $(NH_4)_2SO_4$ and the precipitate gathered. The precipitate was dissolved in a small amount of 50 mM K-phosphate buffer at pH 7 and then dialyzed at 4′C in the same buffer.

**2′,5′-ADP sepharose 4B affinity chromatography:** 2 g dried 2′,5′- ADP sepharose 4B gel was used for a 10 mL column size. The gel was washed with 300 ml distilled water and suspended in 0.1 M K-acetate/0.1 M K-phosphate buffer. After precipitation of the gel, it was equilibrated with 50 mM K-phosphate buffer. The flow rates for washing and equilibration were adjusted to 20 mL/h. The dialyzed sample obtained previously was loaded on to the 2′,5′-ADP sepharose 4B affinity column and washed successively with 25 mL 0.1 M K-acetate + 0.1 M K-phosphate, pH 6.0 and 25 mL 0.1 M K-acetate + 0.1 M K-phosphate, pH 7.85. The latter washing was continued with 50 mM buffer until the final absorbance difference was 0.05 at 280 nm. The enzyme was eluted successively with a gradient of 0–0.5 mM CAT in 50 mM K-phosphate buffer. Active fractions were collected and dialyzed with equilibration buffer. All procedures were performed at 4°C[20–22].

**Activity determination:** Enzyme activity was determined spectrophotometrically at 25°C by direct measurement of the decrease of light absorption at 240 nm caused by the decomposition of hydrogen peroxide by the enzyme[23]. The activity of catalase was given as mM $H_2O_2$/mg protein min.

**Protein determination:** Quantitative protein determination was done spectrophotometrically at 595 nm according to method of Bradford[24], with bovine serum albumin as a standard.

**SDS polyacrylamide gel electrophoresis (SDS-PAGE):** SDS polyacrylamide gel electrophoresis was performed by Laemmli's method to control enzyme purity[25]. SDS-PAGE was carried out in 4 and 10% acrylamide concentrations for stacking and running gel, respectively. To standard and sample, 20 µg bovine serum albumin was applied to the electrophoresis medium. Gels were stained overnight in 0.1% coomassie brilliant blue R-250, 50% methanol and 10% acetic acid. The gel was washed with several changes of the same solvent without the dye until the protein bands were clear.

***In-vitro* studies for drugs:** To determine the effects of drugs on catalase, enzyme activities were measured for gentamicin sulphate (1–5 mM), acetyl salicylic acid (1–5 mM), ampicillin sodium (0.5–2.5 mM), paracetamol (1–5 mM), potassium penicillin (0.1–0.5 mM) and augmentin (0.1–0.5 mM) were investigated for the *in-vitro* enzyme activity. Control cuvette activity in the absence of drug was taken as 100%. For each antibiotic an activity-drug graph was drawn. For three of these drugs (paracetamol, potassium penicillin and augmentin) which had an inhibitory effect on the enzyme, drug concentrations that produced 50% inhibition ($I_{50}$) were calculated from these graphs (Fig. 3).

For determining $K_i$ constants, fixed inhibitor concentrations (2.5 mM for paracetamol, 0.2 mM for potassium penicillin and 0.2 mM for augmentin) were used. In these studies, hydrogen peroxide was used as substrate at five different concentrations (5–40 mM).The Lineweaver-Burk graphs (1/V *vs.* 1/[S]) were obtained for each inhibitor. $K_i$ constants and inhibition types were estimated from the graphs (Fig. 4.).

## RESULTS AND DISCUSSION

In this study, catalase was purified from bovine liver. Fig. 1 shows the SDS-PAGE obtained for determining the purity of the enzyme. Effects of gentamicin sulphate, acetyl salicylic acid, ampicillin sodium, paracetamol, potassium penicillin and augmentin were investigated on the purified enzyme. Three drugs (gentamicin sulphate, acetyl salicylic acid and ampicillin sodium) have insignificant effects on the enzymes activity (Fig. 2). The other drugs (paracetamol, potassium penicillin and augmentin) inhibited the enzyme with increased concentration of the drugs. $I_{50}$ values were estimated as 4.6, 0.35 and 0.49 mM, respectively (Fig. 3); $K_i$ constants were 20, 25 and 25 mM, respectively (Fig. 4, Table-1). The inhibition type for these three drugs was competitive.

Many chemicals and drugs at relatively low dosages affect the metabolism of biota by altering normal enzyme activity, particularly inhibition of a specific enzyme[26]. For example, catalase enzymes have been inhibited by ascorbate alone

as well as $Cu^{2+}$.[27] 3,3′-diaminobenzidine[28], thiol reagents and N-bromosuccinimide, tryptophan, indole acetic acid, cysteine and formaldehyde[29], cyanide and azide ions, sodium-*n*-dodecyl sulphate, 2-mercaptoethanol and aminotriazole[30].

TABLE-1
$I_{50}$ VALUES, $K_i$ CONSTANTS AND INHIBITION TYPES OF PARACETAMOL, POTASSIUM PENICILLIN AND AUGMENTIN

| Inhibitors | $I_{50}$ values | [I] (mM) | $K_i$ constants (mM) | Inhibition type |
|---|---|---|---|---|
| Paracetamol | 4.6 | 2.5 | 20 | Competitive |
| K-penicillin | 0.35 | 0.2 | 25 | Competitive |
| Augmentin | 0.49 | 0.2 | 25 | Competitive |

In this study, catalase has been purified from bovine liver by 2′,5′-ADP sepharose 4B affinity chromatography. Before affinity chromatography so as to remove the impurities and to obtain a concentrated enzyme, ammonium sulphate precipitation was conducted. Using the procedure described in this study, an enzyme with specific activity of 310 EU/mg protein was purified from 40 g bovine liver. The purified enzyme showed a tetrameric band on SDS-PAGE (Fig. 1).

Gentamicin sulphate, an aminoglycoside, is used for treatment of many aerobic gram-negative infections, such as *Escherichia coli*, *Klebsiella*, *Enterobacter*, *Proteus* and *Serratia*, and infection by methicilin-resistant *Staphylococci*. Sodium ampicillin is derived from the penicillin nucleus, 6-aminopenicillanin acid. This drug reduces the development of drug-resistant bacteria. In this study, we found that gentamicin sulphate, acetyl salicylic acid and ampicillin sodium had an insignificant effect on catalase activity. Nevertheless, sheep red blood cell glucose

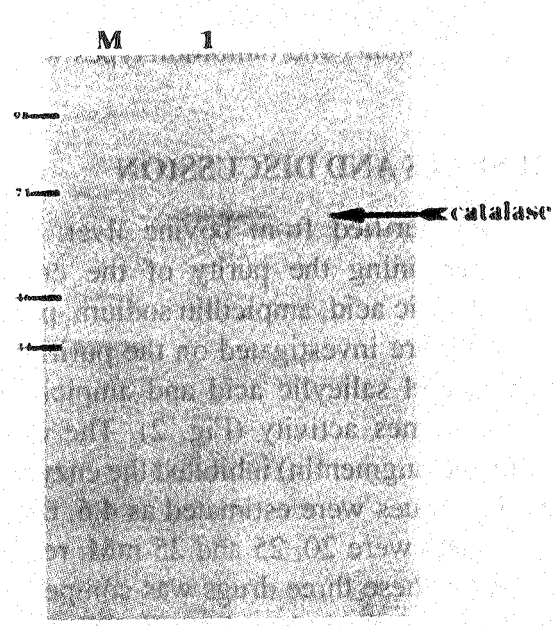


Fig. 1. SDS- polyacrylamide gel electrophoresis of purified CAT Lane 1: bovine liver catalase; Lane M: proteins from low molecular weight marker kit.

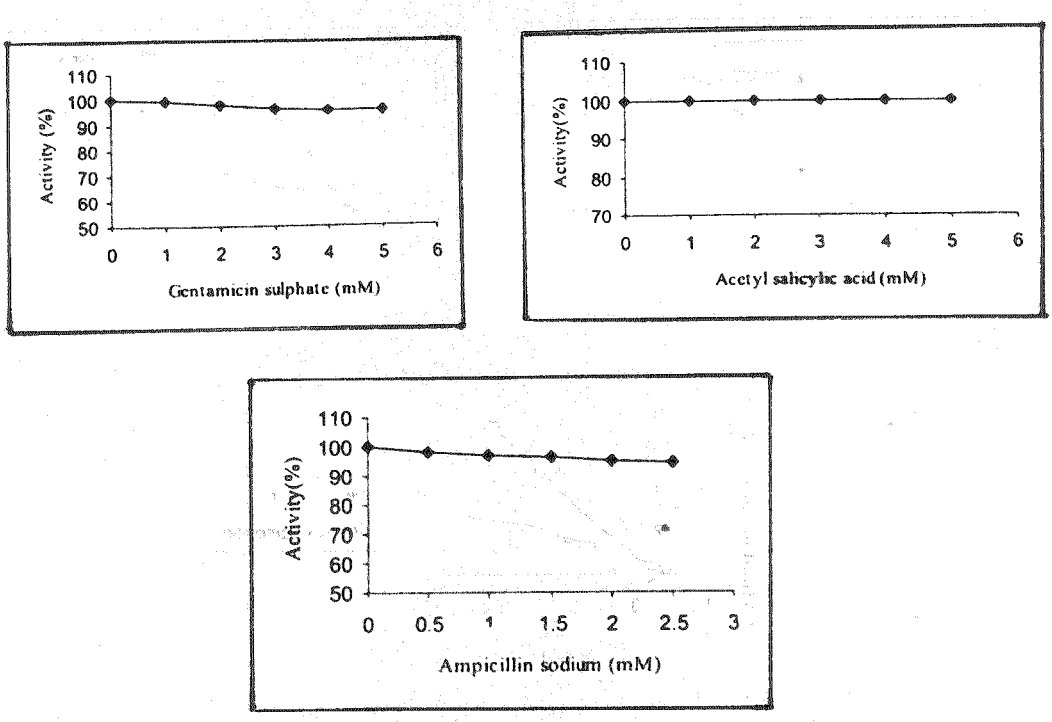


Fig. 2. % activity-[drug] graphs for catalase in the presence of gentamicin sulphate, acetyl salicylic acid and ampicillin sodium

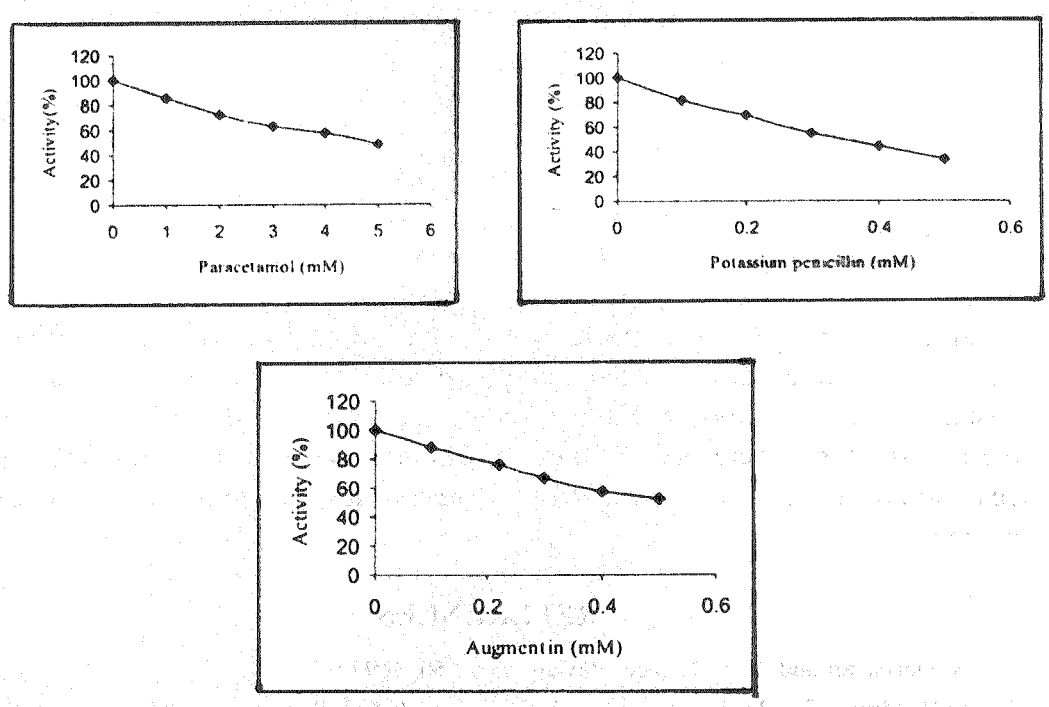


Fig. 3. % activity-[drug] graphs for catalase in the presence of paracetamol, potassium penicillin, and augmentin

6-phosphate dehydrogenase (G6PD) and human erythrocyte (G6PD) have been inhibited by gentamicin sulphate[19, 31]. Potassium penicillin is an antibiotic and kills certain bacteria that cause infection, or stops their growth. It treats many kinds of infections including those of the skin, brain, heart, respiratory tract, sinuses and ears. Paracetamol has been in use as an analgesic for home medication

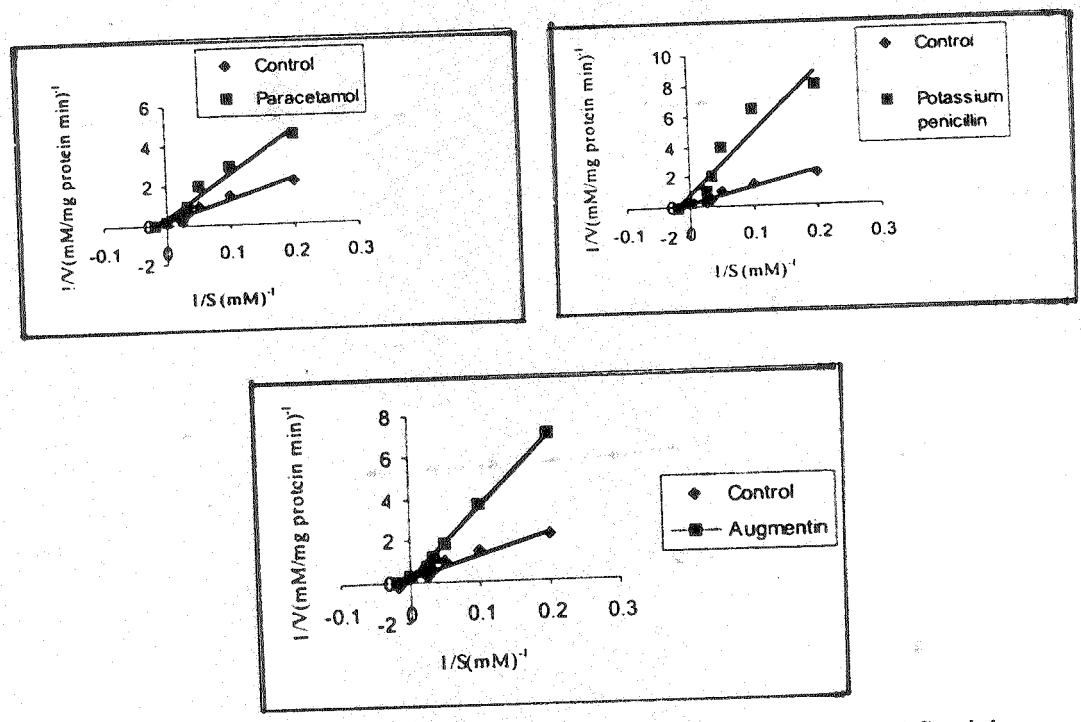


Fig. 4. Lineweaver-Burk graphs for five substrate ($H_2O_2$) concentrations and fixed drugs concentration of $K_i$ constants; (A) paracetamol; (B) potassium penicillin; (C) augmentin

for over 30 years and is accepted as a very effective treatment for the relief of pain and fever in adults and children. Augmentin is an oral antibacterial combination consisting of the semisynthetic antibiotic amoxicillin (present as amoxicillin trihydrate and amoxicillin sodium). This drug is used to treat or prevent infections that are proven or strongly suspected to be caused by bacteria.

This study showed that paracetamol, potassium penicillin and augmentin have strong inhibitory effect on catalase activity. Since the effects of these drugs on enzyme activity have not been previously reported, these results are of interest for further researches. Both the $K_i$ constants and $I_{50}$ values were determined for three of the studied drugs that inhibited the activity of bovine liver catalase. $K_i$ constants and $I_{50}$ values in Table-1 show that potassium penicillin is the most potent inhibitor. Therefore, if these drugs are given to bovines, their dosages should be very well controlled to prevent adverse effects on the catalase enzyme.

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