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Investigation of the Inhibition Effects of Some Antidepressants on Lactoperoxidase Enzyme

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ABSTRACT: Lactoperoxidase (LPO) (E.C.1.11.1.7), which is a component of human and animal milk, is an oxidoreductase that is found in milk, saliva and tears, especially protecting the gut systems and mammary glands of newborns. In this study, Bovine LPO enzyme was purified 447.57 times with 35.24% yield and 31.33 EU / mg protein specific activity using Amberlite CG-50 H^+ resin and affinity chromatography. Enzyme activity was measured using ABTS as a chromogenic substrate (pH 6.0). After purification the *in vitro* effects of some antidepressants (Sodium valproate, mirtazapine, risperidone) were investigated on lactoperoxidase. K_i values for these antidepressants were found as 296.4±119.3 µM and 0.90±0.40 µM, respectively. Sodium valproate did not show an inhibition effect on LPO. Risperidone exhibited noncompetitive inhibition, mirtazapine showed competitive inhibition.

Keywords: Lactoperoxidase, bovine milk, antidepressant, inhibition

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INTRODUCTION

Among the enzymes, peroxidases included in the oxidoreductase enzyme class (POD: H_2O_2-Oxidoreductase E.C.1.11.1.7) that have the potential to be used in many fields today, which commonly found in prokaryotes, eukaryotes, and photosynthetic cells (Huystee, 1987).

By using various aromatic compounds as substrate, peroxidases eliminate the harmful effect of H_2O_2 that occurs during metabolism (Robert et al., 1993). It is required to remove H_2O_2, which has an oxidizing feature and formed as a result of biological systems, from the environment without losing time. This important task is performed by catalase and peroxidase enzymes, being the antioxidant enzymes in the cells (Halliwell, 1984).

Lactoperoxidase enzyme (LPO: Hydrogen Peroxide Oxidoreductase E.C. 1.11.1.7) is present in mammals' milk, tears, saliva, and airways surface fluid (Prince et al., 2000; Fontehet al., 2002). The enzyme is a glycoprotein containing hem group with a molecular weight of 78 kDa (Elagamy et al., 1992; Amornkul and Henning, 1997; Kumar and Bhatla, 1985). Due to the reason that lactoperoxidase enzyme contains 0.07% Fe^{2+} together with protein, it is included in the metalloprotein group. This iron atom in the structure, forms part of hem group in the catalytic center of the enzyme. Hem group enzyme in LPO, is constituted of protoporphyrin IX tightly connecting with disulfide bridges (Thanabal and LaMar,1989). The peptide chain in the enzyme contains 612 amino acid residues and 15 half cystine residues (Reiter and Harnulv, 1984). The isoelectric point of the LPO enzyme is 9.6 (Anonymous, 1955). Lactoperoxidase enzyme is a molecule containing 8-10% carbohydrate and having a glucose-binding region (Cals et al., 1991). In studies conducted in relation to secondary structure of LPO enzyme, it has been determined that 65% of molecules had β structure, 23% of molecules had α structure and that 12% of molecules had irregular structure (Sievers, 1980). The most commonly known substrates are simple phenols such as ABTS (2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid), guaiacol and catechol (Shindler and Bardsley, 1975).

Antidepressants are medicines that can help alleviate depression, anxiety disorders, seasonal mood disorder, and mild chronic depression symptoms and other conditions and they aim to correct the chemical imbalances of neurotransmitters (Beser -Gördeles and Öz, 2003).

Mirtazapine is used in the treatment of Major depressive disorder. It effects chemicals that can become unstable in brain and cause depression and anxiety (Brunton et al., 2005; Süzer, 2002).

Studies show that risperidone can be used as an antidepressant in bipolar disease (Sanger et al., 2001). Risperidone influences Dopamine (D2) and serotonin (5-HT2) receptor antagonists. Its antagonist effect on other receptors can explain other effects of risperidone. It also has antagonistic effects on alpha-1, alpha-2 and histamine-1 receptors **(**Grant et al., 1994).

It has been reported that sodium volproate is an antiepileptic substance that has its effect on the central nervous system (Emrich et al., 1985). Valproic acid is an antiepileptic drug, widely used as a mood stabilizer in the therapy of bipolar disorder. It also exhibits antidepressant effect (Pistovcakova et al., 2008)

In the present study we aimed to investigate the inhibitory effects of antidepressants (Sodium valproate, mirtazapine, risperidone) on bovine milk LPO enzyme activity. For this aim, LPO enzyme was purified from bovine milk then IC_{50}, K_i constants and inhibition types were firstly determined for these molecules. Since LPO has a crucial role for the immunity system, the inhibition of this enzyme means that the immune system is weakened, it is not desirable, especially for newborns. No previous study in the literature so far investigated the inhibitory effects of these molecules on bovine milk LPO.

MATERIALS AND METHODS

Activity Procedure

For checking the activity of LPO used procedure is based on the oxidation of ABTS as a substrate by H_2O_2, results in a product that absorbs at 412 nm **(**Shindler and Bardsley, 1975; Ozdemir and Uguz, 1995).

Purification procedure

Bovine milk was centrifuged to remove fat of milk. Amberlite CG 50 NH_4^+ resin was added in the milk for the bound proteins were elute after the eluate was applied to the Sepharose4B-L-tyrosine-sulphanilamide affinity column (Atasever et al. 2013).

SDS PAGE

The sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE) used to check the LPO purity. In this method the stacking and running gels contained 3% (w/v) and 10% (w/v) acrylamide, respectively, and 0.1% (w/v) SDS. After electrophoresis, proteins can be stained with Coomassie Blue R-250 (Laemmli, 1970).

Protein Determination

The protein concentration was determined according to the Bradford method (Bradford, 1976) and BSA (Bovine serum albümine) was used as a standard protein In this method, Coomassie Brilliant Blue G-250, binds to proteins, and the resulting coloured complex has a maximum absorbance at 595 nm (Gulcin et al. 2004).

In Vitro Inhibition Studies

Determined the IC_{50} concentrations for each molecules, constant ABTS concentration and five different inhibitor concentrations were used after an activity %-[Inhibitor] graphs were drawn. K_i values were determined with Lineweaver-Burk graphs at five different ABTS concentrations (0.083- 0.50 mM) and three different inhibitor concentrations. Analysis of data obtained was made by *t*-test and they are given as X±SD. (Atasever et al., 2013).

RESULTS AND DISCUSSION

The LPO system in milk has an antibacterial effect, it is based on the oxidation of SCN^- ions by the reaction catalyzed in the presence of H_2O_2. It is accepted that the active intermediary product that is formed during oxidation of thiocyanate to hypothiocyanate (OSCN) (Madureira, 2007). It has the ability to oxidize free sulfhydryl (-SH) groups in enzymes that are vital in bacterial metabolism (Madureira, 2007).

The biological importance of LPO involves its role in providing a natural defense system against the invasion of microorganisms (Koksal et al., 2016). LPO system has effect on a wide microorganism group. Apart from certain special cases, while it has a bacteriostatic effect against Gram (+) bacteria such as streptococlar and lactobaciller, it has a bactericidal effect against Gram (-) bacteria causing degradation in products such as Pseudomonas, E. Coli and enteropathogenic species of salmonella (Dufy, 1983).

Until now, many LPO inhibitors have been reported in the literature. For example, hydrasines, thiocarbamide compounds, sulfanilamides, propofol, some anesthetic drugs, some bacteria species, some phenolic acid compounds and phenolics, avermectins, adrenaline, melatonin, serotonin and norepinephrine, fungi and bacteria, hydrasines and some thiocarbamide compounds (Koksal et al., 2020; Koksal et al., 2016). But, there is not any research in the literature that investigated the inhibitory activity

of antidepressants on LPO. For his purpose, LPO was purified from bovine milk 447.57 times with 35.24% yield with an affinity technique and the purification table was prepared with the data obtained (Table 1).

Table 1. The purification of lactoperoxidase from bovine milk

Purification steps	Total Volume (ml)	Activity (EU/ml)	Protein (mg/ml)	Total Activity (EÜ)	Total Protein (mg)	Specific Activity (EU/mg)	Yield %	Purification Fold
Crude Homogenate	55.00	0.97	13.00	53.35	715.0	0.07	100	1.00
Affinity Chromatography	10.00	1.88	0.06	18.80	0.60	31.33	35.24	447.57

The purity of LPO was checked with the SDS-PAGE and the molecular weight was determined to be 78 kDa (Figure 1).

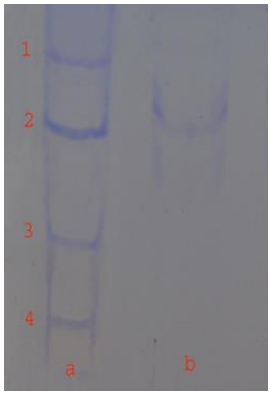

Figure 1. Sodium dodecylsulphate–polyacrylamide gel electrophoresis (SDS-PAGE) band of LPO. Column "b" is purified LPO. Column "a" is standard proteins (Line 1: 250 kDa, Line 2: 150 kDa, Line 3: 100 kDa, Line 4: 70 kDA, Line 5: 50 kDA, Line 6: 40 kDA, Line 7: 30 kDA, Line 8: 20 kDa, Line 9: 15 kDa from Thermo 26630, with a 250-20 kDa interval

LPO activity was measured for the different molecular structures of compounds used in this study (Figure 2) and the determined inhibition values and types were given in the (Table 2, Figure 3).

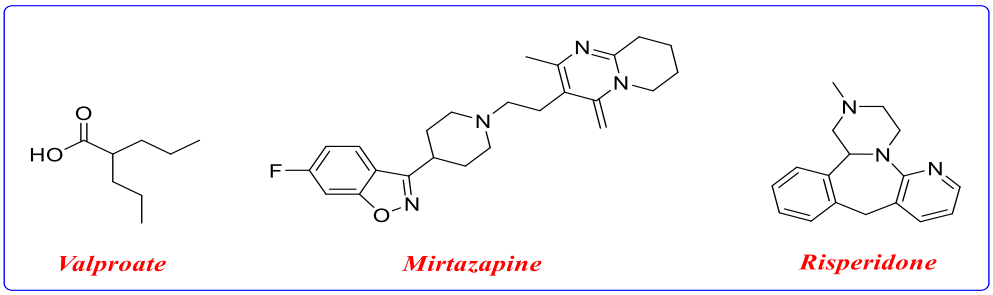

Figure 2. The molecular structures of antidepressants used in this study

Table 2. IC_{50} and K_i values and inhibition types for antidepressants used in this study

Antidepressant	IC_{50} (µM)	R^2	K_i (µM)	Inhibition Type
Sodium valproate	No inhibition			
Mirtazapine	263.35	0.96	296.4±119.3	Competitive
Risperidone	1.02	0.99	0.90±0.40	Noncompetitive

In this study, both K_i and IC_{50} parameters for these molecules on bovine LPO are first determined and summarized in Table 2. K_i and IC_{50} graphs of Mirtazapine and Risperidone are given in Figure 3.

As seen in Table 2, the obtained IC_{50} values of antidepressants against LPO are as follows: risperidone (1.02 µM, R^2: 0.99) > mirtazapine (263.35 µM, R^2: 0.96). On the other hand, K_i values of

these compounds decreased in the following order: risperidone (0.90±0.40 µM) > mirtazapine (296.4±119.3 µM).

Among these compounds, while sodium valproate does not show an inhibitory effect, risperidone has shown the strongest inhibition effect. Also, the inhibition effect of risperidone on LPO was found to be a noncompetitive inhibition. mirtazapine demonstrated competitive inhibition type. Risperidone caused to inhibition by binding to enzyme somewhere other than active site and mirtazapine caused to inhibition by only binding to enzyme–substrate complex.

Mirtazapine is a molecule with a steric hindrance relative to the risperidone molecule. Additionally, LPO inhibition by these molecules is dependent on the positioning of the inhibitor in the active site; the distance between the atoms in the molecules and active site amino acids **(**Koksal et al., 2017).

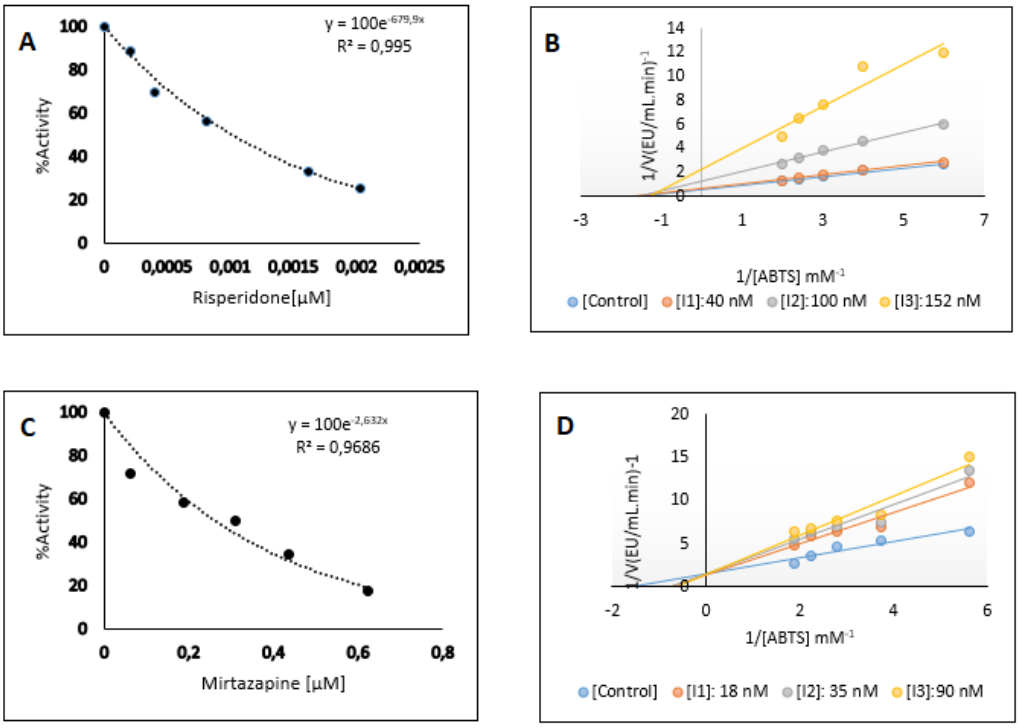

Figure 3. IC_{50} graphs (A,C) and Lineweaver–Burk graphs (B, D) of Risperidone and Mirtazapine for LPO, respectively

The i*n vitro* inhibition effects of mirtazapine, sodium valproate and risperidone antidepressants on the AChE enzyme was investigated and the following conclusions were reached. IC_{50} values were found to be 0.428 mM and 0.019 mM for Mirtazapin and Risperidone, respectively. The K_i values for each molecules were found to be 0.23±0.051 mM and 0.012± 0.0018 mM (Özcan, 2014).

In vitro inhibitory effects of mirtazapine was investigated against Dihydropyrimidine dehydrogenase enzyme. IC_{50} value was determined as 86.65 µM (Camadan, 2016).

In a study conducted to investigate *in vitro* drug-drug interactions, the effect of antidepressants on the Cytochrome P450 system was investigated. For CYP3A4, mirtazapine is also a very weak inhibition effect (K_i = 0.07 µM) (Owen et al., 1998).

Another study, *in vitro* metabolism of risperidone was investigated using the recombinant human cytochrome P450 enzymes. The contribution of CYP2D6 and CYP3A to the metabolism of risperidone may have significant value in predicting potential drug-drug interactions in the clinical use of risperidone (Fang et al., 1999).

Use of antidepressants in pregnancy is increasing to 2–8.7% over the recent years in Western countries (Ray and Stowe, 2014). Many *in vitro* inhibition studies in the literature provide guidance for clinical studies. When the results of *in vitro* studies are evaluated, it may be useful for drug design and dosage adjustment.

CONCLUSION

In this study being conducted, it has been reported that some antidepressants inhibit LPO enzyme activity. LPO activity is a very important enzyme during lactation. It has vital importance especially in protection of immune system. Reduction in LPO activity of milk during antidepressant usage, can be harmful for the newborn. Attention should be paid during medicine consumptions a dosage adjustment should be made. Care should be taken in the use of antidepressants specified in the study.

Conflict of Interest

The article authors declare that there is no conflict of interest between them.

Author's Contributions

The authors declare that they have contributed equally to the article.

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