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Research Article

In vitro Effects of Some Drugs and Metals on Aldose Reductase and Sorbitol Dehydrogenase in the Kidney of Goat (*Capra aegagrus hircus*)

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Abstract

The effects of some antibiotics and metals on goat (*Capra aegagrus hircus*) kidney aldose reductase (AR) and sorbitol dehydrogenase (SDH) activities were examined. For drugs and metals that exhibit inhibitory effect, K_i constants were calculated and inhibition types were determined by using Lineweaver-Burk curves. In our study, ceftriaxone antibiotic showed the highest inhibition effect on the AR enzyme with an IC_{50} value of 0.0274 mM. More potent AR inhibitors can be synthesized from the ceftriaxone compound. This compound is followed by amikacin sulfate and ciproflaksasin compounds with IC_{50} values of 0.061 and 0.25 mM, respectively. Co^{+2} metal ion showed the highest inhibition effect on the AR enzyme with an IC_{50} value of 0.000445 mM. This metal is followed by Fe^{+2} and Zn^{+2} with IC_{50} values of 0.0286 and 0.084 mM, respectively. In inhibition studies on SDH enzyme, rifamycin sodium antibiotic showed the highest inhibition with an IC_{50} value of 0.016 mM. More potent SDH inhibitors can be synthesized from the rifamycin sodium compound. This compound is followed by ceftriaxone and cefuroxime compounds with IC_{50} values of 0.025 and 0.16 mM, respectively. The highest inhibition of metals showed Co^{+2} ion with IC_{50} value 0.00044 mM. This metal is followed by Fe^{+2} and Zn^{+2} with IC_{50} values of 0.009 and 0.16 mM, respectively.

Key words: Aldose reductase, sorbitol dehydrogenase, goat kidney, drugs, enzymes inhibition

Keçi Böbreğinde (*Capra aegagrus hircus*) Bazı İlaç ve Metallerin Aldoz Redüktaz ve Sorbitol Dehidrogenaz Enzimleri Üzerine *in vitro* Etkileri

Öz

Bazı antibiyotik ve metallerin keçi (*Capra aegagrus hircus*) böbrek aldoz redüktaz (AR) ve sorbitol dehidrogenaz (SDH) aktiviteleri üzerine etkileri incelenmiştir. İnhibitör etkisi gösteren ilaç ve metaller için K_i sabitleri hesaplanmış ve Lineweaver-Burk eğrileri kullanılarak inhibisyon tipleri belirlenmiştir. Çalışmamızda AR enziminde en yüksek inhibisyon, 0.0274 mM IC_{50} değeri ile seftriakson antibiyotiği gösterdi. Seftriakson bileşiğinden daha güçlü AR inhibitörleri sentezlenebilir. Bu bileşiği IC_{50} değerleri sırasıyla 0.061 ve 0.25 mM olan amikasin sülfat ve siproflaksasin bileşikleri izlemektedir. Metallerin en yüksek inhibisyonu, 0.000445 mM IC_{50} değeri ile Co^{+2} iyonu göstermiştir. Bu metali sırasıyla 0.009 ve 1.43 mM IC_{50} değerleri ile Fe^{+2} ve Zn^{+2} iyonları takip etmektedir. SDH enziminde en yüksek inhibisyon, 0.016 mM IC_{50} değeri ile rifamisin sodyum antibiyotik gösterdi. Rifamisin sodyum bileşiğinden daha güçlü SDH inhibitörleri sentezlenebilir. Bu bileşiği sırasıyla 0.025 ve 0.16 mM IC_{50} değerlerine sahip seftriakson ve sefuroksim bileşikleri takip etmektedir. Metallerin en yüksek inhibisyonu 0.00044 mM IC_{50} değeri ile Co^{+2} iyonunu göstermiştir. Bu metali sırasıyla 0.009 ve 0.16 mM IC_{50} değerleri ile Fe^{+2} ve Zn^{+2} takip etmektedir.

Anahtar kelimeler: Aldoz redüktaz, sorbitol dehidrogenaz, keçi böbreği, ilaçlar, enzim inhibisyonu

Introduction

The polyol pathway consists of the enzymes aldose reductase and sorbitol dehydrogenase, the rate-limiting enzyme of this pathway. Hyperglycemia causes an increase in intracellular glucose in tissues such as insulin-independent lens, blood vessel, nerve, kidney for glucose transport. Increased intracellular glucose is reduced by aldose reductase enzyme and cofactor NADPH to sorbitol. Sorbitol, a polyol, is converted to fructose by oxidation with the second enzyme of the polyol pathway, sorbitol dehydrogenase, with the help of NAD^+ cofactor (SDH is found in low concentrations in these tissues). As a result, in tissues independent of insulin and glucose increase, sorbitol does not easily pass through the cell membrane, causing accumulation in tissues and subsequent conversion of fructose is slow (Obrosova et al. 2005; Tang et al. 2010) (Fig. 1).

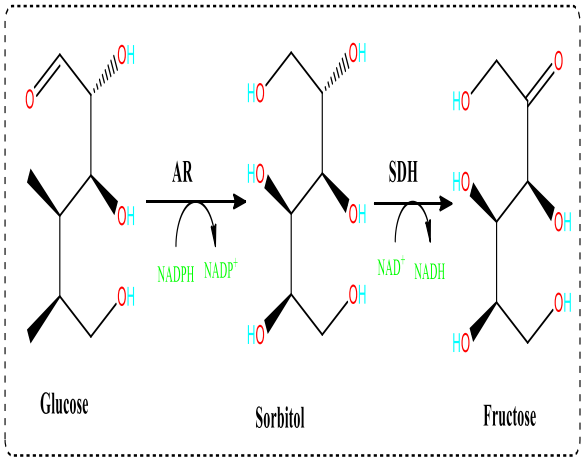

Figure 1. Polyol (sorbitol) pathway

The effects of the drugs on the activities of goat kidney aldose reductase and sorbitol dehydrogenase enzymes are as follows: Cefuroxime is a second-generation antibiotic from cephalosporin class. Cephalosporins are similar to penicillins in their antibacterial mechanism of action and chemical structure. The core of this class of antibiotics is the cephem derivative, 7-aminosephalosporanic acid. Cephazolin, the first-generation antibiotic, is the most common cephalosporin that binds to plasma proteins. Gentamicin, an aminoglycoside class, is obtained from Micromonospora purpurea (Manchanda et al. 2010). Among the antibiotics of the aminoglycoside class, gentamicin is the antibiotic which has the broadest spectrum and the highest antibacterial potency. It is much stronger than streptomycin, kanamycin, and amikacin. It is the first derivative in the class of aminoglycosides obtained by semisynthetic methods. Amikacin, which is semisynthetic, is derived from kanamycin A, a natural antibiotic. It is effective against ceftriaxone, pneumococcal, meningococcal, *Haemophilus influenzae*, and *Neisseria gonorrhoeae*, the third generation antibiotic of cephalosporins (Tomasz 1997). It is not indicated that it has no activity against B. fragilis, pseudomonas, staphylococci, and enterococci and it binds to plasma proteins in 90%. Penicillins are commonly used natural and semi-synthetic antibiotics with relatively low toxicity as well as strong bactericidal effects. Ampicillin is a broad-spectrum antibiotic of the penicillin class. Lincomycin is a natural antibiotic with narrow spectrum. The structure is unlike any other antibiotic. The mechanism of action is different from penicillins. Breaks the continuation of the peptidoglycan chain in bacteria. Vancomycin is a complex glycopeptide obtained from *Streptomyces orientalis*. Ciprofloxacin is the first generation antibiotic in the fluoroquinolone class (Davies 1994). Meropenem is a β-lactam subclass of carbapenem. It is effective against most bacterial species resistant to penicillin and cephalosporins. Cefoperazone, a cephalosporin class, is a third-generation antibiotic and has a wide spectrum. Cephalosporins are similar to penicillins in their antibacterial mechanism of action and chemical structure. Rifamycin is a semisynthetic antibiotic. Especially effective on *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococci viridans* and *Mycobacterium tuberculosis*, even in very small doses (Spratt 1994) (Fig. 2).

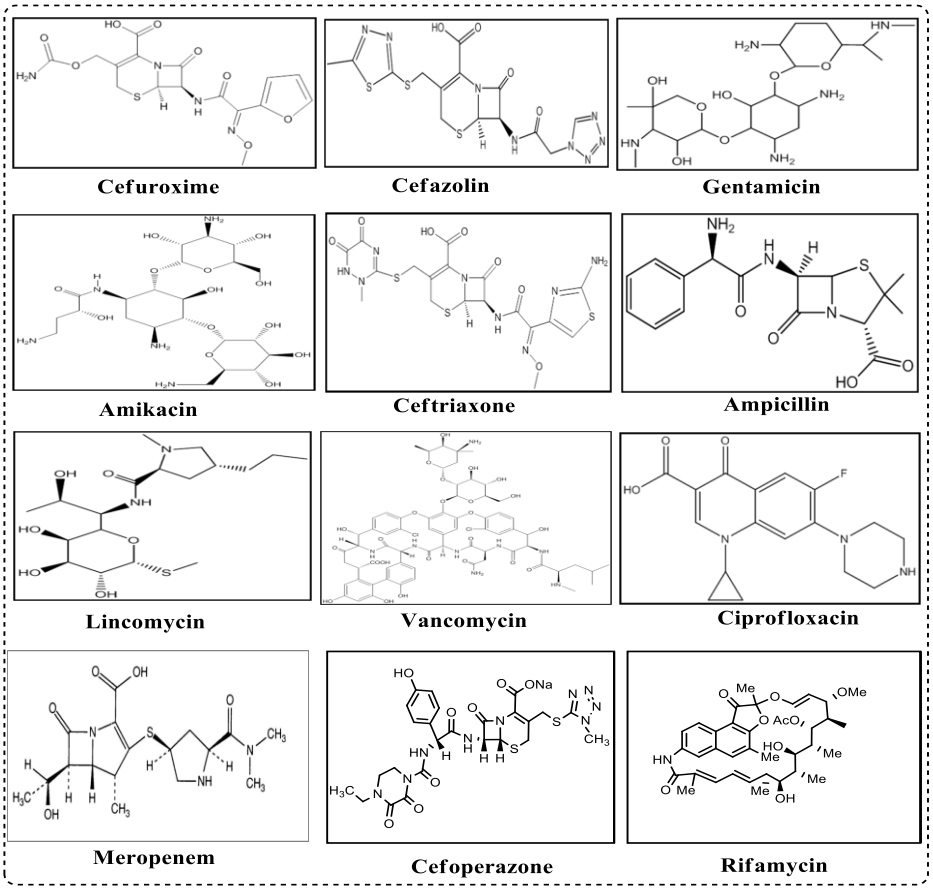

Figure 2. Sructurs of some drugs studied

In this study, the effects of some antibiotics and metals on goat (*Capra aegagrus hircus*) kidney aldose reductase and sorbitol dehydrogenase activities were examined. For drugs and metals that exhibit inhibitory effect, K_i constants were calculated and inhibition types were determined by using Lineweaver-Burk curves.

Materials and Methods

Aldose Reductase studies

Determination of the effects of some drugs and metals on the activity of goat kidney aldose reductase enzyme

To determine the effects of some drugs and metals on aldose reductase enzyme activity, different concentrations of drug and metal solutions were added to the cuvette medium and activity values were read. The stock solutions were diluted to form different concentrations of the drug and metal solutions used. When the stock solution volume used did not provide the required concentration, the buffer volume added to the cuvette was reduced, the concentration of the drug or metal solution was increased, so that the required concentration was adjusted (Kato et al. 2009).

Activity measurement of aldose reductase enzyme

In 1986, Cerelli et al. developed the procedure for measuring the activity of aldose reductase enzyme and modified the procedure. The reaction medium was prepared by adding 0.25 ml of Na-phosphate buffer, 0.1 ml of NADPH, 0.1 ml of isolated enzyme on 0.45 ml of purified water and 0.1 ml of DL-glyceraldehyde to a total volume of 1 ml. The reaction was initiated by the addition of DL-glyceraldehyde to the prepared cuvette. The decrease in NADPH concentration was monitored for 3 minutes at 340 nm using room temperature and spectrophotometer and then the range of linear absorbance values were determined and slope calculations were performed (Jung et al. 2010).

Sorbitol dehydrogenase studies

Determination of the effects of some drugs on the activity of goat kidney sorbitol dehydrogenase enzyme

In order to determine the effects of some drugs on SDH enzyme activity in goat kidney, activity values were read by adding different concentrations of drugs to the cuvette medium. The stock solutions were diluted to form different concentrations of the drug used. When the stock solution volume used did not provide the required

concentration, the buffer volume added to the cuvette was reduced to increase drug concentration. In this way, the required concentration was adjusted (Yamaki and Ishikawa 1986).

Measurement of activity of sorbitol dehydrogenase enzyme in goat kidney

The increase in absorbance caused by NADH reduction of NAD^+ during the oxidation reaction of sorbitol to fructose was measured at 340 nm for 3 min. 1 ml quartz cuvette content for this measurement; 50 mM Glycine / NaOH buffer (pH = 10.0), 10 mM sorbitol, 470 µM NAD+ and enzyme were prepared and absorbance values at 340 nm were measured. The millimolar extinction coefficient of NADH was used for activity calculation. SDH enzyme reduces nicotinamide adenine dinucleotide (NAD^+) in the presence of sorbitol. We can measure the rate of formation of NADH by the increase in absorbance at 340 nm, which depends on the activity of the SDH enzyme. The SDH enzyme unit gives the value in micromoles of sorbitol oxidized per minute. The following formula was used to calculate the enzyme unit (Yamaguchi et al. 1994).

$$\text{D-sorbitol} + NAD^+ \rightleftharpoons \text{D-fructose} + NADH + H^+$$

Studies for determination of IC_{50} and K_i values for drugs and metals with inhibiting effect

Drug and metals showing inhibition effects were determined by measuring activity at different inhibitor concentrations. % Activity- [I] graphs of these drugs and metals with high inhibitory effect were plotted and IC_{50} values were calculated from the equation of the curve. In order to determine the Ki values of some drugs and metals whose IC_{50} values were calculated, activity measurements were made with the concentration of drug or metal that halves the AR enzyme activity of the goat kidney and with the appropriate five substrate concentrations at two fixed drug or metal concentrations above and below this value. Five suitable substrate concentrations used in the studies were determined by preliminary experiment using stock solution. Lineweaver-Burk graph was drawn for each inhibitor with the obtained results. In the graph equation, $K_m / V_{max} (1+ [I] / K_i)$, which is equal to the slope for competitive inhibition, and K_i values were determined by using $V_{max} = VI_{max} (1+ [I] / K_i)$ formula for non-competitive and semi-competitive inhibition (Cornish-Bowden 1974; Liu et al. 2006).

Results

Aldose reductase results

SDS-PAGE was used to check the purity of the eluates obtained by the 2',5'-ADP Sepharose 4B affinity of goat kidney aldose reductase enzyme. For this reason, electrophoresis system was established as described in former studies and enzyme samples were loaded and run sequentially. The photograph showing the bands obtained for both enzymes is shown in Figure 3. Additionally, the pure enzyme carried out on SDS-PAGE was photographed and using the graph, the molar mass of the goat kidney aldose reductase and SDH enzymes were calculated about 42 kDa.

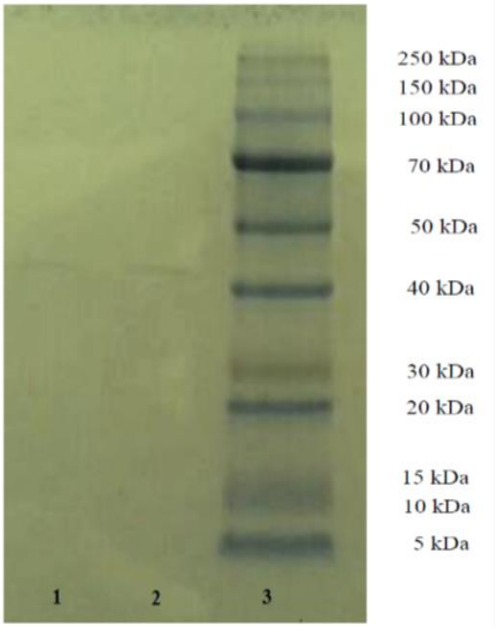

Figure 3. SDS-PAGE Photo (Lane 1:SDH; Lane 2: AR; Lane 3: Standard proteins)

As is known, the concentration of inhibitor that halves the enzyme activity is IC_{50}. In our study, AR enzyme inhibition was determined by calculating IC_{50} values. In our study, the highest inhibition showed ceftriaxone antibiotic with an IC_{50} value of 0.0274 mM. More potent AR inhibitors can be synthesized from the ceftriaxone compound. This compound is followed by amikacin sulfate and ciproflaksasin compounds with IC50 values of 0.061 and 0.25 mM, respectively. Inhibition magnitudes of the inhibitors studied on AR enzyme activity were ordered from large to small; ceftriaxone> amikacin sulfate> ciprofloxacin> vancomycin> cefoperazone> cefazolin> meropenem> cefuroxime> ampicillin (Table 1). The highest inhibition of metals showed Co^{+2} ion with IC_{50} value 0.000445 mM. This metal is followed by $FeSO_4$ with IC_{50} values of 0.0286, respectively (Table 2 and Fig. 4).

Table 1. IC_{50} and K_i values and inhibition types of some antibiotics on AR enzyme

Antibiotics Type	IC_{50} (mM)	K_i (mM)	Inhibition Type
Cefuroxime	6.54	7.52 ± 1.41	Noncompetition
Ceftriaxone	0.0274	0.018 ± 0.009	Competition
Amikacin Sulfate	0.061	0.017 ± 0.002	Noncompetition
Cefazolin	2.5	1.73 ± 0.18	Noncompetition
Gentamicin Sulfate	3.483	0.84 ± 0.13	Competition
Vancomycin	0.706	0.19 ± 0.054	Competition
Ampicillin	24.755	16.06 ± 7.59	Noncompetition
Siproflaks Press	0.25	0.103 ± 0.03	Competition
Meropenem	4.814	1.66 ± 0.066	Competition
Cefoperazone Sodium	0.858	0.46 ± 0.24	Competition
Lincomycin	Not enough inhibition	-	-

As a result of studies with aldose reductase inhibitors such as ponalrestat and tolrestat, it has been reported that it can not prevent retinopathy. Benfotiamine, a thiamine derivative, is a successful aldose reductase inhibitor in diabetic retinopathy. Activating the pentose phosphate pathway, benfotiamine inhibits the formation of free radicals. It is stated that it prevents the activation of the PKC pathway as a result of the decrease in mitochondrial ROS level. Studies with zenalrestat, an inhibitor of the same group, showed significant improvements in nerve conduction velocity.

Table 2. IC_{50} and K_i values and inhibition types of some metal ions on AR enzyme

Metal ions Types	IC_{50} (mM)	K_i (mM)	Inhibition Type
$CoCl_2$. $6H_2O$	0.000445	0.00019 ± 0.0004	Noncompetition
$FeSO_4$	0.0286	0.00264 ± 0.00081	Competition
$MgCl_2$	1.007	-	-
$Zn(CH_3COO)_2.H_2O$	0.084	0.017 ± 0.002	Competition
$CaCl_2$	Not enough inhibition	-	-
$CuSO_4.5H_2O$	Not enough inhibition	-	-
KCl	Not enough inhibition	-	-

The carboxylate functional group of the carbamoyl group of the ARI fidarestat and the other ARI and the minalrestat of cyclic imide class have been shown to increase the net binding energy of the enzyme-inhibitor complex as a result of its replacement with fluorine atom. However, aldose reductase inhibitors are not limited to their functions. In recent studies on ARIs, inhibitors have been shown to act as curative agents in the treatment of cancer types such as breast, liver, cervix, colon and ovarian cancers (Halder, Joshi, and Gupta, 2003).

Sorbitol dehydrogenase results

All inhibitors used on AR enzyme activity were also studied on SDH enzyme. In our study, rifamycin sodium antibiotic with the highest inhibition IC_{50} value was 0.016 mM. This compound is followed by ceftriaxone and cefuroxime sodium compounds with IC_{50} values of 0.025 and 0.15 mM, respectively. Among the compounds showing inhibitory effects on both enzymes of the polyol pathway, the best inhibitory effect was shown by the ceftriaxone compound (IC_{50} value for AR and 0.050 mM for SDH and 0.025 mM for SDH). Inhibitors which inhibit the entire polyol pathway can be synthesized with reference to the ceftriaxone compound and some modifications to this compound. The inhibition sizes of the inhibitors studied on the activity of SDH enzyme are from big to small; rifamycin sodium> ceftriaxone> cefuroxime> cefoperazone sodium> ciproflaksasin> amikacin sulfate> meropenem> cefazolin. It can be concluded that rifamycin sodium is a potent inhibitor of SDH (Table 3). The highest inhibition of metals showed Co^{+2} ion with IC_{50} value 0.000445 mM. This metal is followed by $FeSO_4$ and $MgCl_2$ with IC_{50} values of 0.009 and 1.43 mM, respectively (Table 4 and Fig. 4).

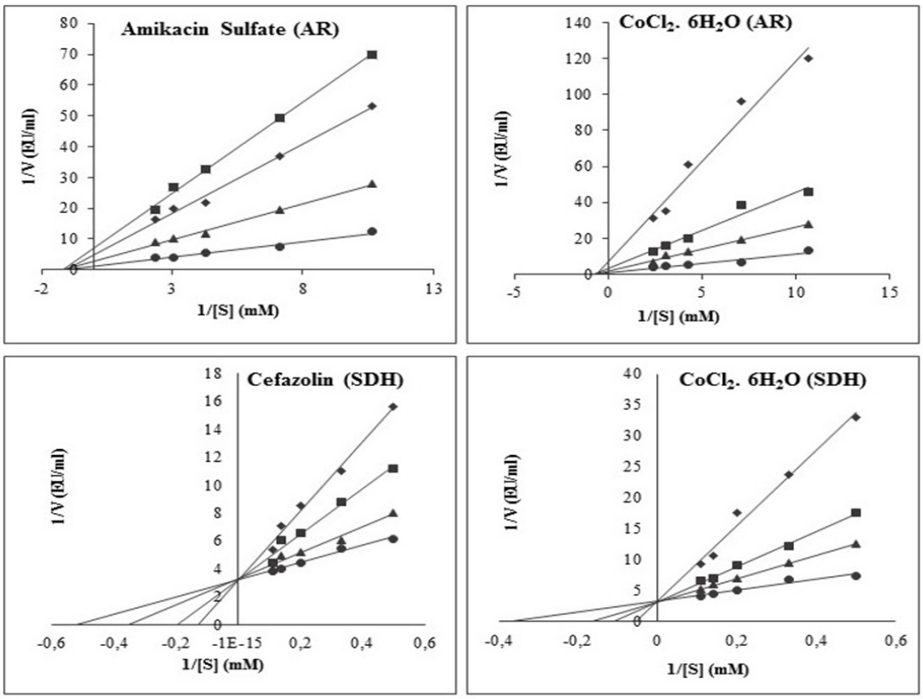

Figure 4. K_i graphs the best inhibitors of drugs and metal ions

Table 3. IC_{50} and K_i values and inhibition types of some antibiotics on SDH enzyme

Antibiotics Type	IC_{50} (mM)	K_i (mM)	Inhibition Type
Cefuroxime	0.15	7.52 ± 1.41	Noncompetitive
Ceftriaxone	0.025	0.075 ± 0.038	Competition
Amikacin Sulfate	0.7	0.62 ± 0.25	Noncompetitive
Cefazolin	13.86	0.013 ± 0.0075	Competition
Rifamycin Sodium	0.016	0.031 ± 0.027	Noncompetitive
Siproflaks Press	0.27	0.54 ± 0.39	Noncompetitive
Meropenem	4.88	3.5 ± 4.2	Competition
Cefoperazone Sodium	0.16	0.067 ± 0.074	Competition
Gentamicin	Not enough inhibition	-	-
Vancomycin	Activation	-	-
Ampicillin	Activation	-	-

In our study, goat kidney SDH enzyme was purified in four steps and ammonium sulfate precipitation (40-60%), DEAE Sephadex ion-exchange chromatography, CM-cellulose ion-exchange chromatography and gel filtration chromatography techniques were applied in the purification processes. With these methods, SDH enzyme was purified from goat kidney at 19.6 EU/mg specific activity, approximately 124.05 times in 0.05% yield. It has been reported that the inhibition of SDH enzyme by nucleosides and nucleotides inhibits the activity of Guanosine-2,3-di-P (K1=1.0 mM) and this inhibition is uncompetitive. Recent studies; It shows that inhibition of SDH may be effective in delaying the occurrence of complications caused by diabetes by correcting redox disorders related to the polyol mechanism activated in case of hyperglycemia. For this reason, many researchers are working to develop strong and effective SDH inhibitors. Quercetin, which is thought to be the specific inhibitor of aldose reductase, has been shown to inhibit the SDH enzyme significantly in the human brain and likewise quercetin in bovine brain. It has been reported to inhibit SDH (Schmidt et al. 1998).

Table 4. IC_{50} and K_i values and inhibition types of some metal ions on SDH enzyme

Metal Type	IC_{50} (mM)	Mean K_i (mM)	Inhibition Type
$CoCl_2$. $6H_2O$	0.00044	0.00018±0.0003	Competition
$FeSO_4$	0.009	-	-
$MgCl_2$	1.43	-	-
$Zn(CH_3COO)_2.H_2O$	0.16	0.14 ± 0.13	Competition
$CaCl_2$	Not enough inhibition	-	-
$CuSO_4.5H_2O$	Not enough inhibition	-	-
KCl	Not enough inhibition	-	-

Discussion

Diabetes is a multisystemic chronic metabolic syndrome characterized by hyperglycemia caused by the absence, deficiency or ineffectiveness of insulin hormone. The polyol pathway, which is assumed to play a major role in conditions such as hyperglycemia in diabetic patients, is reported to be a small pathway of glucose metabolism. Sorbitol dehydrogenase and aldose reductase enzymes are required for this pathway. Reduction of glucose to sorbitol and oxidation of sorbitol to fructose occurs by reactions catalyzed by AR and SDH enzymes in the polyol path (Lee and Chung 1999; Obrosova et al. 2005).

In healthy individuals, blood glucose levels are at a certain level, whereas aldose reductase enzyme has a low affinity for glucose and therefore does not use glucose as a substrate. In case of diabetic hyperglycemia, aldose reductase enzyme is stimulated; glucose is converted to sorbitol by the enzyme aldose reductase and converted to fructose by the sorbitol dehydrogenase enzyme and the resulting fructose is used as energy source. In 1956, Hers stated that sperm cells used fructose as an energy source. Yabe and Nishimura stated that aldose reductase enzyme plays an important role in the pathogenesis of diabetic complications (Poulsom et al. 1983).

Hers in 1956 as the energy source of sperm cells in the polyol pathway by reducing the reduction of glucose by aldose reductase sorbitol by sorbitol dehydrogenase catalyzed by a reaction catalyzed by sorbitol dehydrogenase enzyme showed in the seminal sac. In 1959, Van Heyningen reported the presence of a polyol pathway in the diabetic rat lens. Aldose reductase enzyme mRNA was found to be high in mouse testis. Aldose reductase is the first enzyme of the polyol pathway and uses aliphatic and aromatic aldehydes as substrates. The intracellular substrate of the enzyme is glucose and galactose. When AR uses glucose and galactose as the substrate, their sugar alcohols, sobitol and galactitol, are formed. However, since galactitol is not a good substrate for sorbitol dehydrogenase, it accumulates faster than sorbitol in the case of hyperglycemia. All aldose reductase inhibitors have a negative charge at physiological pH. Inhibitors are attached to these anionic binding sites of the enzyme by these negatively charged portions. Therefore, inhibitors prefer $NADP^+$, the oxidized form of NADPH. Aldose reductase inhibitors establish many van der Waals bonds with the hydrophobic region residues in the active site of the enzyme (Patel et al. 2012a, 2012b).

Sorbitol deposition has increased as a result of inhibition of sorbitol dehydrogenase enzyme. However, in this study, it was observed that the nerve conduction and the vascular complications in the nerves, eye and aortic tissues were decreased without the side effects of sorbitol accumulation. As a result, the pathogenesis in nerve, eye and aortic tissues has been shown to be more related to the oxidation of sorbitol to fructose by sorbitol dehydrogenase enzyme rather than reduction of sorbitol via aldose reductase enzyme. Therefore, increase in $NADH/NAD^+$ ratio in diabetics is seen as a very important determinant of pathogenesis in diabetic tissues. Studies on sorbitol dehydrogenase enzyme inhibition; inhibition may be useful in delaying the occurrence of diabetes-induced complications by correcting redox disorders associated with polyol pathway activity under hyperglycemia conditions. Therefore, studies are underway to develop strong and effective sorbitol dehydrogenase inhibitors (Tilton et al. 1995; Niculescu et al. 1997).

In this study, we purified AR and SDH enzymes from goat kidney and investigated their effects on some metal ions and some important antibiotic drugs. IC_{50} and K_i results were obtained at the mM level. inhibitors of these enzymes are important and are used in medicines.

Author contribution: MK and ŞB conceived and designed the research. MK conducted experiments. MK and ŞB analyzed the data. MK wrote the manuscript. All authors read and approved the manuscript.

Data availability: The authors affirm that all data necessary for confrming the conclusions of the article are present within the article and tables. Data will be provided on specific request.

Conflict of interest statement: The authors declare no competing interests.

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