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ALDOSE REDUCTASE ENZYME'S CLINICAL AND THERMODYNAMIC CHARACTERISTICS IN THE SERUM OF IRAQI DIABETIC PATIENTS

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Abstract

A superfamily of aldo-keto reductases includes the monomeric NADPH-dependent cytosolic enzyme aldose reductase (AR). When hyperglycemia, increased glucose levels stimulate the AR, which activates the polyol pathway and results in glucose metabolism, the dangerous aldehydes created by reactive oxygen species (ROS) into innocuous alcohols. NADPH is a crucial cofactor in the formation of glutathione (GSH), a crucial antioxidant, and its consumption lowers GSH levels. The main causes problems of diabetes are osmotic stress brought on by the buildup of excess AR and oxidative stress brought on by a decline in the NADPH/NADP+ ratio and decreased NAD+. The aim of this work is to measure the AR enzyme's activity in healthy and diabetes patients.

A spectrophotometric was used to measure the serum (AR) activity in 120 type II diabetes patients and 60 healthy individuals. Increase in serum (AR) is related to the body's internalized oxygen free radicals, which cause oxidative damage to human tissues. The thermodynamic constants of activation, ΔH, Ea, and ΔS, were calculated using the Arrhenius plot in order to reconcile our findings with the thermodynamic behavior of (AR) enzyme's. These values were found to be (10.55, 19.93, and 2.85) KJ/mol, respectively. Due to its capacity to change NADPH into NADH, the active polyol pathway in diabetes mellitus significantly contributes to the NADH/NAD+ redox imbalance. Lack of NADPH can cause oxidative stress via limiting glutathione metabolism, the excess NADH's ability to cause it generating ROS through the mitochondrial electron transport chain.

Keywords: *Diabetes mellitus; Aldose reductase; Oxidative stress; Entropy; Enthalpy*.

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Introduction

Hyperglycemia, a metabolic disease caused by abnormalities in insulin secretion, action, or both, is the hallmark of diabetes mellitus [1]. It is separated into two groups. Type 2 diabetes, also known as non-insulin dependent diabetes, and type 1 diabetes, also known as juvenile onset diabetes. The two primary metabolic problems that produce type 2 diabetes are insulin resistance and advancing pancreatic cell failure [2]. Type 2 diabetes is ultimately brought on by hyperglycemia brought on by cell dysfunction in response to insulin resistance [3]. Reducing blood glucose levels to normal or nearly normal levels is the aim of treatment. [4, 5]. For patients with type 2 diabetes mellitus, exercise and diet are the initial therapeutic strategies of choice. When they are unable to control their blood sugar levels enough, oral diabetic medication is then advised [6]. Metformin or an insulin secretagogue called a sulfonylurea, which suppresses hepatic gluconeogenesis, are typically used as the first line of monotherapy [7]. These medications are frequently administered in combination when monotherapy fails [8]. Chronic hyperglycemia brought on by diabetes increases the generation of free radicals, notably reactive oxygen species (ROS), in all tissues as a result of protein glycosylation and glucose auto-oxidation[9- 11]. An imbalance between the systems that produce and remove free radicals—which can result from either decreased antioxidant defense activity or increased free radical production—causes oxidative stress. Auto-oxidation of glucose, non-enzymatic protein glycosylation, and impaired glutathione metabolism are other potential contributors to the involvement of oxidative stress in the development of diabetes. Oxidative stress is caused by an imbalance between the systems responsible for producing and scavenging free radicals, which can arise from either diminished antioxidant defense activity or increased free radical production. In addition to oxygen free radical formation, auto-oxidation of glucose, non-enzymatic protein glycosylation, and decreased glutathione metabolism may potentially contribute to oxidative stress' role in the development of diabetes [12, 13].

Typically, in a two-step metabolic process known as the polyol pathway, glucose is first converted to sorbitol and subsequently to fructose. Two enzymes make up the polyol pathway. The first enzyme, AR, catalyzes the conversion of glucose to sorbitol with the help of its cofactor NADPH, and the second enzyme, sorbitol dehydrogenase (SDH), with the help of its cofactor NAD+, converts sorbitol to fructose [14]. In order to create the cellular building blocks and energy, glucose is typically processed through the Krebs cycle and subsequently the glycolysis route. When there is hyperglycemia, the increased glucose levels stimulate the AR, which activates the polyol pathway and results in glucose metabolism. When cellular glucose levels are high, sorbitol is converted by AR to fructose in addition to the dangerous aldehydes created by reactive oxygen species (ROS) into innocuous alcohols. When AR converts high intracellular glucose to sorbitol, it uses the cofactor NADPH. [15]. NADPH is a crucial cofactor in the formation of glutathione (GSH), a crucial antioxidant, and its consumption lowers GSH levels. The main causes of the numerous problems of diabetes are osmotic stress brought on by the buildup of excess sorbitol and oxidative stress brought

on by a decline in the NADPH/NADP+ ratio and lower NAD^+. An excessive supply of electron donors for the mitochondrial electron transport chain is one of the main effects of the NADH/NAD+ redox imbalance [16]. Complex I, which transfers electrons from NADH to CoQ, would be overloaded by an excess of NADH. [17]. One characteristic of complex I electron transport is that it produces more superoxide the more electrons it moves. [18-21] This is due to the possibility that additional leaky electrons could partially reduce oxygen and generate an excess of superoxide, the precursor to all ROS. as shown by [22–24] in (Fig. 1). Hence, as numerous researchers have demonstrated [25–30], an excess of NADH in diabetes induced by prolonged hyperglycemia can kill cells by increasing oxidative stress, decreasing mitochondrial function, and accelerating cell death.

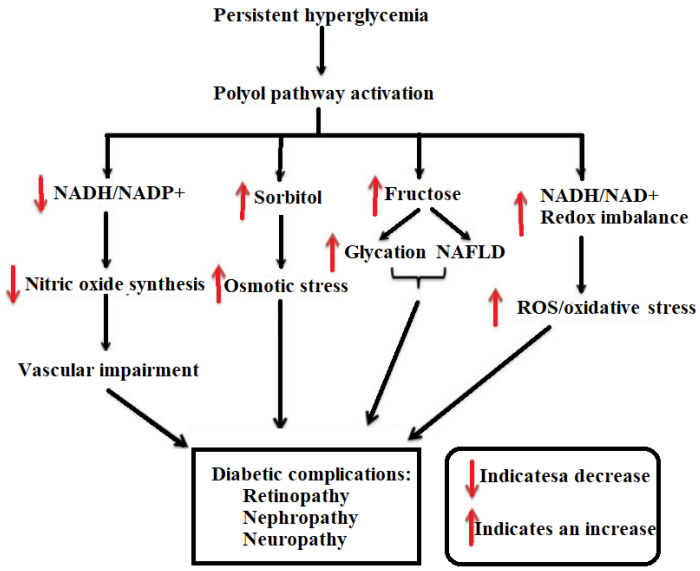

Figure 1: implications of persistent hyperglycemia's activation of the polyol pathway. [17]

Raising the fructose content, which leads to increased protein glycation and the beginning of non-alcoholic fatty liver disease (NFALD), reducing the NADPH/NADP ratio and nitric oxide production, and increasing the NADH/NAD ratio, which leads to ROS formation and oxidative stress. These events may lead to the development of diabetes problems as retinopathy, nephropathy, and neuropathy.

In the current study, the AR enzyme activity in the sera of diabetic patients from Iraq is evaluated for its clinical and thermodynamic characteristics.

Materials and Methods

Patients

Al-Imamain Alkadhimain Medical City (Bagdad City, Iraq) conducted a clinical trial in 2023. Clinical T2DM with a minimum one-year history of diabetes was required for participation. Adult volunteers who were in good health and had FBS levels that were known to be 126 mg/dl were chosen as controls. Table 1 displays patient characteristics and laboratory results. A total of 60 healthy individuals and 120 T2DM patients were included in the study. The patients were receiving glibenclamide or metformin therapy.

Table 1: The characteristics of the study population are expressed as mean SD for quantitative variables.

Variables	Patients receiving glibenclamide treatment (n=72)	Patients receiving metformin treatment (n=49)	Controls (n=56)	p- value
Age, years				
Mean±SD	54.13±10.77	54.02±9.90	51.13±8.71	0.185
Range	27-75	33-73	37-70	
Gender				
Male	44(61.11%)	22(44.9%)	30(53.57%)	0.212
Female	28(38.89%)	27(55.1%)	26(46.43%)	
BMI				
Mean±SD	30.47±5.71	30.70±3.78	31.51±7.19	0.586
Range	19.1-48.4	22.0-39.5	22.3-65.6	
HbA1c, %				
Mean±SD	8.10±1.87[a]	7.83±2.16[a]	5.69±0.93[b]	<0.001
Range	4.7-13.1	4.8-12.8	4.2-8.8	
AR, U/L				
Mean	22.26±9.91[a]	26.39±10.58[a]	48.39±24.43[b]	<0.001
Range	5.0-39	7-54	24-135	

Different small letters indicate significant differences.

Studies of Thermodynamics

An enzymatic process' rate and activation energy are related in Equation 1 of the empirical Arhenius equation.

$$E_a = R \cdot \ln\left(\frac{V_2}{V_1}\right).\left(\frac{1}{T_1} - \frac{1}{T_2}\right) \quad (1)$$

The energy of activation (E_a) is the product of the slope of the Arrhenius plot of the ln (V) against $1/T$ and the enzyme activity V_1 and V_2 at temperatures T_1 and T_2, respectively. Equation 2 can be used to compute the activation enthalpy (ΔH).

$$\Delta H = E_a - RT \quad (2)$$

Equation 3 (Eyring-Polanyi), which connects the Arrhenius equation (equation 1), was used to derive the entropy (ΔS).

$$\ln\left(\frac{V_{\max}}{T}\right) = \ln\left(\frac{K_B}{h}\right) + \frac{\Delta S}{R} - \frac{\Delta H}{R}.\frac{1}{T} \quad (3)$$

Where T, K_B, h, and R- are absolute temperature, Boltzmann constant, Planck constant and gas constant respectively.

Analytical techniques

Human erythrocytes are used to produce AR.

Onto a citrate phosphate-dextrose tube, blood was taken. After centrifuging the erythrocytes for two minutes at 1800 x g, they were pelleted. The pellet was re-pelletized as before after being re-suspended in 5 volumes of 20 mM phosphate buffer in 0.9% NaCl with 1 mM EDTA. Three times the washing process was carried out. The heamolysate was then centrifuged at 13000 x g for 5 min after being lysed in 9950 ml of distilled water with a 50 ml erythrocyte pellet. A refrigerator-stored supernatant was utilized to assess the AR activity.

Determination of AR activity

Undecomposed hydrogen peroxide reacts with ammonium molybdate to produce a yellowish tint with a maximum absorption at 354 nm, which is used to measure AR activity. At 37 °C, 20 µl of the sample was incubated with 1.5 ml of a pH 7.4 60 mM phosphate buffer solution. The addition of 0.5 ml of 30 mM hydrogen peroxide initiated the process. AR activity was assessed by combining the reaction mixture with ammonium molybdate

following a three-minute incubation. At 354 nm, absorbance was measured. A first-order reaction equation's rate constant was used to compute AR activity.

Effects of PH and temperature

A pH range of 3.0 to 11.0 was used to test the AR activity. In line with the corresponding pH ranges, different buffer systems were employed: 50 mM citrate-phosphate buffer for pH 3.0-6.0, 50 mM potassium phosphate buffer for pH 6.0-7.0, 50 mM tris-HCl buffer for pH 7.0-9.0, and 50 mM carbonate buffer for pH 9.0-10.0. Standard reaction mixtures were tested for AR activity across a variety of temperatures, from 15°C to 80°C, to describe the effects of temperature.

Analytical Statistics

The Statistical Package for Social Sciences (SPSS) software version 25.0 was used to conduct the statistical analyses (SPSS, Chicago). Data were analyzed using one-way analysis of variance and represented as mean standard deviation (ANOVA). The threshold for statistical significance was set at P 0.05.

RESULTS

According to the study, diabetic individuals had considerably higher levels of AR than the control group.

Temperature's and pH levels impact on the activity of enzymes

The AR optimal temperature curve was displayed in (Figure 2). The entire enzyme assays were incubated for three minutes at various temperatures ranging from 25 to 45°C. The findings indicated that the optimum temperature for AR was 40°C, and that the enzyme's denaturation caused the temperature to drop after that point.

When the enzyme activity is examined at several pH levels Between pH 3.0 and pH 10.0, pH 10.0 has the maximum activity (Figure3).

Parameters of thermodynamics (Ea, ΔH, and ΔS)

The activation energy (E_a) can be calculated using the empirical formula of the Arrhenius plot of the natural logarithm of AR activity versus the reciprocal value of temperature (Figure 4a). It was discovered that the activation energy was 19.93 kJ/mol. Figure 4b's Arrhenius plot was used to compute the enthalpy of activation (ΔH) and entropy of activation (ΔS), and the results showed that they were both 10.55 kJ/mol and 2.85 kJ/mol, respectively.

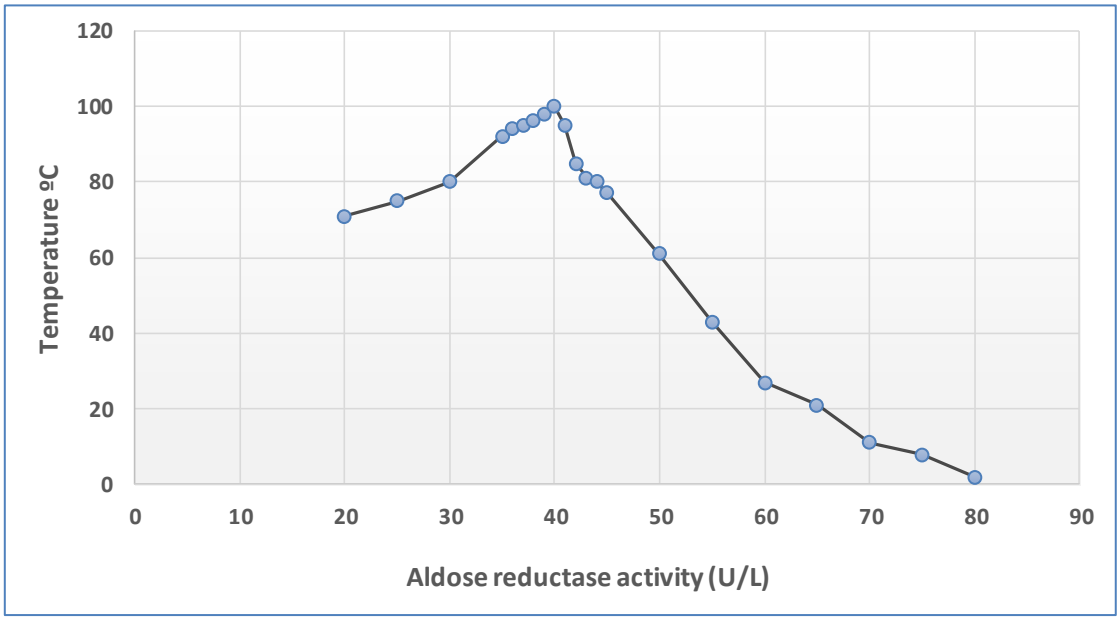

Figure 2: The effect of temperature on enzyme activity.

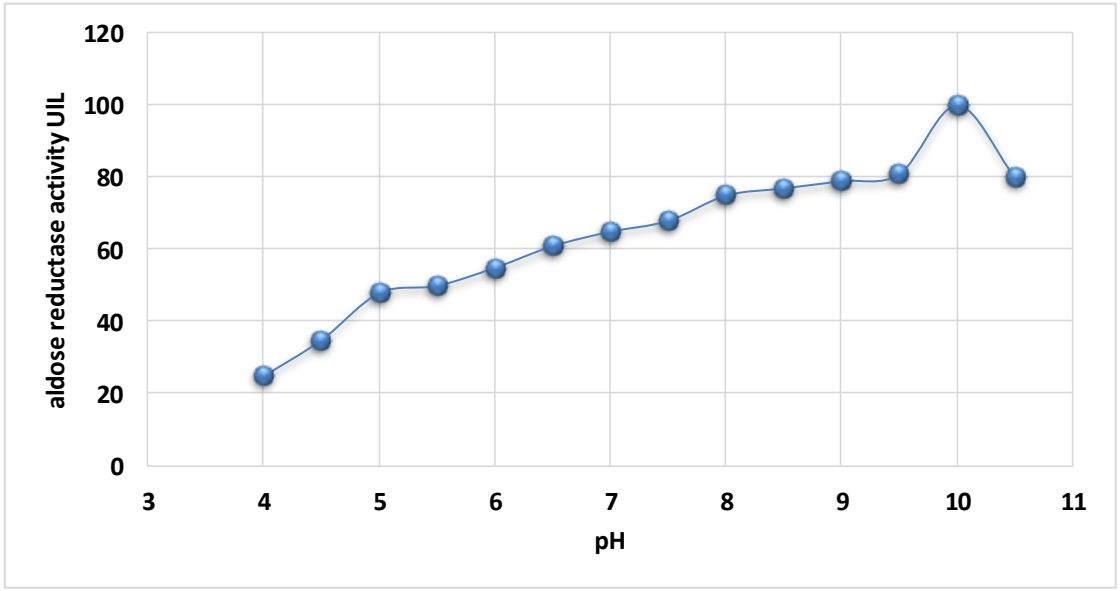

Figure 3: The effect of pH on catalase activity.

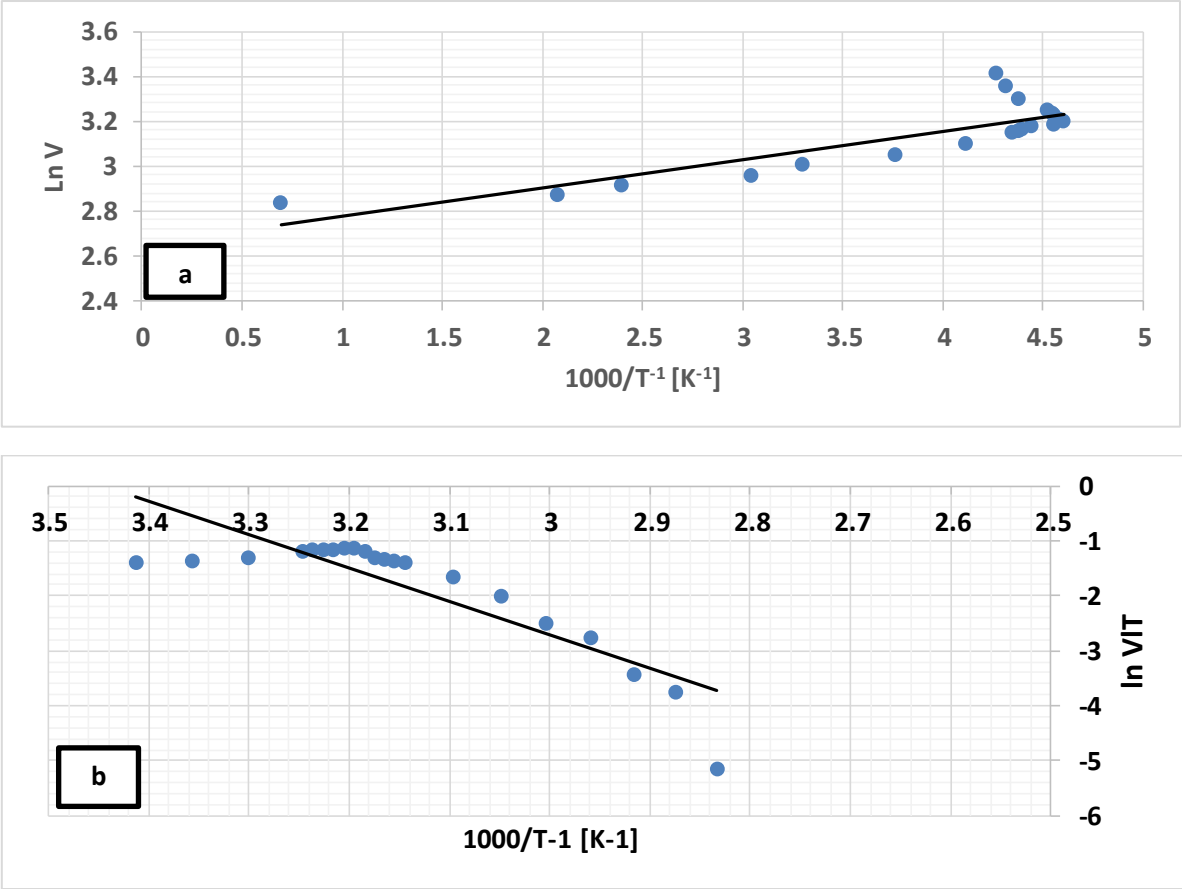

Figure 4: Arrhenius plot for AR activity, (a) activation energy, (b) activation entropy, and activation enthalpy calculations.

DISCUSSION

One of the many antioxidant defense enzymes, including glutathione peroxidase, peroxidase, and SOD, catalyzes the dismutation of hydrogen peroxide into oxygen and water. All aerobic species include this tetrameric heme-containing enzyme, which plays a critical role in defending cells against oxidative stress [31]. Diabetes patients' greater AR activity in comparison to the control group may be due to elevated ROS levels. Higher levels of ROS have been associated with the emergence of diabetes complications. In diabetes, an accumulation of ROS cannot be effectively fought off by antioxidant systems. Hence, a defense mechanism promotes the regulation and expression of antioxidant enzymes when oxidative stress increases as a result of a pathologic state. The findings of the present study indicate that oxidant-antioxidant equilibrium has changed in diabetes patients, and that ROS have been implicated in the development of diabetic problems. Increased serum AR levels are related to oxidative stress [32]. The pH plays a significant part in the enzyme's action. At pH 10, the AR showed its highest level of activity (Figure 3). These findings may be made clearer by the fact that an enzyme is inhibited by an alkaline pH, which lowers its activity. Moreover, the pH change will have an impact on how long amino acids' active sites survive, which could alter how quickly diabetes problems related to ionization of these

amino acids develop. The ideal temperature at which AR activity is at its highest is 40 °C. As the temperature rises, so does the kinetic energy of molecules. The enzyme molecules overcome the energy barrier when the temperature is raised further. As a result, the hydrophobic and hydrogen connections that are essential for maintaining the enzyme's three-dimensional structure were broken [33].

CONCLUDING

In the polyol pathway, which is linked to abnormal glucose metabolism and diabetes consequences, AR is a key enzyme. Due to its capacity to change NADPH into NADH, the active polyol pathway in diabetes mellitus significantly contributes to the NADH/NAD+ redox imbalance. Lack of NADPH can cause oxidative stress via limiting glutathione metabolism, in addition to excess NADH's ability to cause it through generating ROS through the mitochondrial electron transport chain and other mechanisms. In order to reconcile our results with the thermodynamic behavior of (AR) enzyme's, the thermodynamic constants of activation, H, E_a, and S, were computed. These were determined to be, respectively, 10.55 kJ/mol, 19.93 kJ/mol, and 2.85 KJ/mol.

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