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Clinical and thermodynamicalproperties of catalase enzyme in sera of Iraqi diabetic patients

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ABSTRACT

Catalase is an antioxidantenzyme existing in most of the animalcells; it defends them from oxidative damage by catalyzing the rapid decay of hydrogen peroxide in two kinds of reactions. The aim of the present study was to assess the catalase activity in normal subjects and patients whom suffer from diabetes mellitus. Serum catalase activity was assessed by spectrophotometric method in 120 diabetic patients (type II) and 60 healthy subjects. Catalase activity increased in diabetic patients compared to control group. The increase in the serum catalase enzyme is belonged to the oxidative damage of human tissues by increament oxygen free radicals in the body. To correlate our results with the catalase thermodynamically behavior, the thermodynamic constants of activation ΔH, Ea and ΔS, were determined using Arrhenius plot and found to be 11.96 KJ/Mol, 22.44 KJ/male and 2.39 KJ/Mol/K, respectively.
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KEYWORDS

Diabetes mellitus;
Catalase;
Oxidative stress;
Entropy;
Rnthalpy.x

INTRODUCTION

Diabetes mellitus is a collection of metabolic disordersillustrated by hyperglycaemia that is produced from defectsin insulin secretion, insulin action or both[1]. It isclassified into two types. Type 1 diabetes,which is known as juvenile onset diabetes, and type 2 diabetes thatknown as non-insulin dependentdiabetes. Type 2 diabetes induces by two primary metabolic defects: insulin resistance and progressive pancreatic β-cell dysfunction[2]. β-Cell dysfunction appliedon insulin resistance results in hyperglycaemia and consequently to type 2 diabe-tes[3]. Treatmentis aimed todecrease blood glucose levels to normal or near normal values[4,5]. Exercise and diet are the first managements ofselection for patients with type 2 diabetes mellitus. When they do not completesufficient blood glucosecontrol, they start with oral antidiabetes treatment is then advocated[6]. First-line monotherapy usuallyinitiates with metformin or sulfonylurea(an insulin Secretagogue), which inhibits hepaticgluconeogenesis[7]. When monotherapy failure, these agentsare oftengiven in combination[8]. Through diabetes, constant hyperglycemia causes raised production of free radicals, particularly reactive oxygen species (ROS), for all

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tissues from glucose auto-oxidation and protein glycosylation[9, 10, 11]. Oxidative stress results from an imbalance between radical-scavenging systems and radical-generating, i.e. decreased activity of antioxidant defensesor raised free radical production or both. The oxidative stress may be implicatedin the pathogenesis of diabetes, not only by oxygen free-radical production, but also because of auto-oxidation of glucose, non-enzymatic protein glycosylation[12], impaired glutathione metabolism[13]. There are defense mechanisms from free radicals as the enzymes superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT),whose activities contribute to remove superoxide, hydrogen peroxide and hydroxyl radicals[14]. The catalase reaction isessentially a dismutation reaction resembling SOD; one$H_2O_2$is reduced to H_2O and the other is oxidized to O_2[15]. Red blood cells (RBCs) are the major source of antioxidants[16, 17] and the Catalase is asignificant antioxidant formed by RBC; hence, catalase levels can indicate ROS levels[18]. The present study evaluates the clinical and thermodynamic properties of catalase enzyme activity in sera of Iraqi diabetic patients.

MATERIALS AND METHODS

Patients

A clinical study was performed in Al-Najaf Center for Diabetes and Endocrinology (Al-Najaf City, Iraq) during 2015. The inclusion criteria were clinical T2DM with duration of diabetes of at least one year. Controls were selected from healthy adult vol-unteers with established levels of FBS<126 mg/dl. Patient characteristics and laboratory values are shown in TABLE 1. A total of 120 subjects with T2DM and 60 healthy persons were enrolled. The patients were under treatment with glibenclamide or metformin.

Thermodynamic studies

The relation between the rate of an enzymatic reaction and activation energy is obtained by the empirical formula of the Arrhenius equation (equation 1)

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

Where$V_1$and$V_2$are the enzyme activities at the temperatures T_1 and T_2; Ea is the energy of activation (kJ mol^{-1}) which can be determined from the slope of the Arrhenius plot of ln(V) against1/T.

The activation enthalpy (ΔH) can be calculated by equation 2.

$$\Delta H = E_a - RT \tag{2}$$

Finally the entropy (ΔS) was calculated by equation3 (Eyring-Polanyi),which correlates ΔH,Ea, and Arrhenius equation (equation 1);

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

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

Analytical methods

Preparation of catalase from human erythrocytes

Blood was drawn onto citrate phosphate-dex-

TABLE 1 : Characteristics of studied patients and controls; quantitative variables are expressed as mean ±SD

Parameter	Group					
	Healthy subjects		Patients treated with glibenclamide		Patients treated with metformin	
	Male	Female	Male	Female	Male	Female
Number	30	30	30	30	30	30
Age (yrs)	42.4±9.1	40.5±11.3	46.3±10.7	47.2±11.3	48.6±11.5	45.3±10.5
BMI (kg/m^2)	24.5 ± 1.7	24.2 ± 1.3	26.9 ± 1.9	25.5 ± 1.9	25.2 ± 1.7	25.3 ± 1.6
HbA1c	5.3 ± 0.4	5.4 ± 0.5	8.9 ± 3.2	8.8 ± 2.9	8.8 ± 3.3	8.7 ± 3.1
Catalase (Mean ± SD) k.U/liter	25.59±4.17	20.92±1.47	26.36±7.56 NS	25.38±6.76 *	28.13±13.34 **	24.31±7.69 *

NS: non-significance versus healthy donors (male); *: significance versus versus healthy donors (female); **: significance versus versus healthy donors (male); ***: significance versus versus healthy donors (female)

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trose tube. The erythrocytes were pelleted by cen-trifugation at 1800xgfor 2 min. The pellet wasresuspended in 5 volumes of 20 mM phosphate bufferin 0.9 % NaCl containing 1 mM EDTA andrepelleted as above. The washing process was repeated 3times. Finally, a 50 ml erythrocyte pellet was lysed in9950 ml of distilled water and the heamolysate wascentrifuged at 13000 xg for 5 min. Supernatant was kept in therefrigerator and used for themeasurement of catalase activity.

Determination of catalase activity

Theassessment of catalase activity is based on the reaction of un-decomposed hydrogen peroxide with ammonium molybdate to give a yellowish color, which has a maximum absorbance at 354 nm. 20 μl of sample was incubated with 1.5 ml of 60 mM phos-phate buffer solution (pH 7.4) at 37 °C. The reac-tion was started by the addition 0.5 ml of 30 mM hydrogen peroxide. After incubation for three min-utes, catalase activity was determined by mixing the reaction mixture with ammonium molybdate. Absor-bance was measured at 354 nm. Catalase activity was calculated from therate constant of a first-order reaction equation.

Effects of pHand temperature

Catalase activity was measured in a pH range of 3.0-11.0. Different buffer systems were used in ac-cordance with respective pH ranges: 50 mM cit-rate-phosphate buffer for pH 3.0-6.0, 50 mM potas-sium 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. To characterize the effects of temperature, standard reaction mixtures were as-sayed for catalase activity at different temperatures, in a range of 15°C-80°C.

Statistical analysis

Data analysis was performed using SPSS 17 for Windows (SPSS Inc., Chicago, IL, USA). Data were expressed as mean ± SD were evaluated by one-way analysis of variance (ANOVA). The statistical significance level was considered at $P < 0.05$.

RESULTS

Our study has shown that the level of CAT was elevated significantly in diabetic patients than con-trol.

Effect of temperature on enzyme activity

Figure 1 showed the optimum temperature curve for Catalase. The complete assays of enzyme were incubated at different temperatures from 25 to 45°C for 3 minutes. Results showed that catalase had an optimum temperature at 35°C and after 35°C the tem-perature is decreased because of denaturation of enzyme.

The enzyme activity, when studied at different pH values ranging from 3.0 to 10.0 is found to be maximum at pH 7.0 (Figure-2)

Thermodynamic parameters (Ea, ΔH, andΔS)

The activation energy (Ea) can be determined from the slope of the empirical formula of the Arrhenius plot of natural logarithm of the catalase activity versus the reciprocal value of temperature (Figure 3a). The activation energy was found to be 22.44 kJ/mol. Both enthalpy of activation (ΔH) and
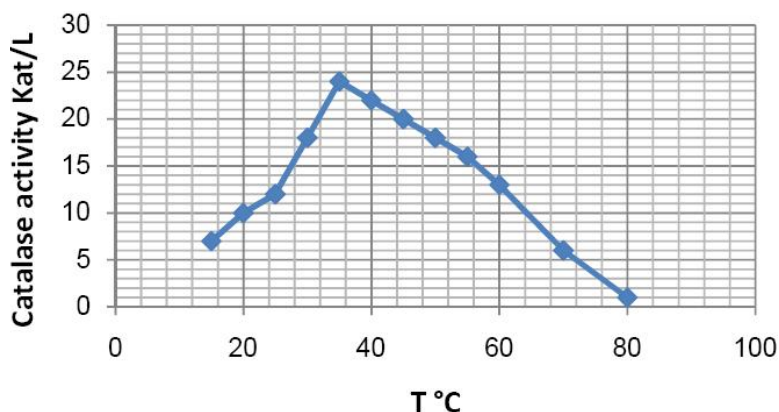

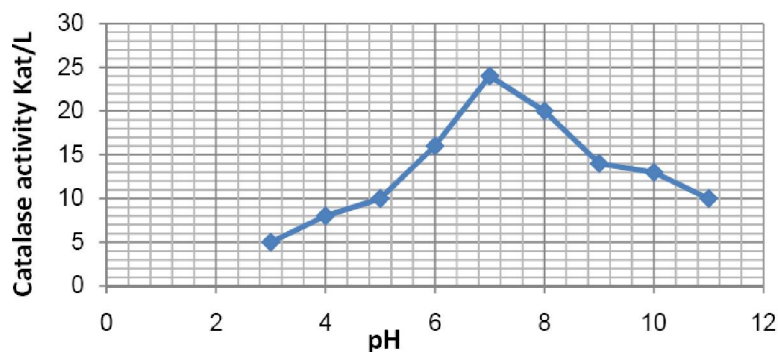**Figure 1 : The effect of temperature on enzyme activity.**

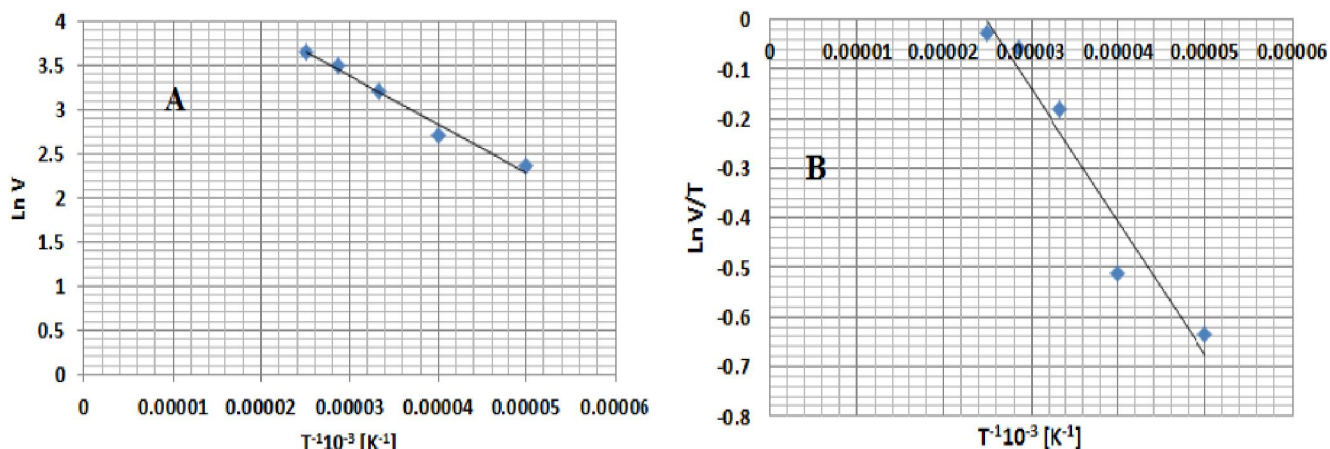Figure 2: the effect of pH on catalase activity

Figure 3 : Arrhenius plot for the activity of catalase, for the calculation of(a) activation energy, and (b) entropy of activation and enthalpy of activation.

entropy of activation (ΔS) were calculated using Arrhenius plot as shown in Figure 3b and were found to be 11.96 kJ/mol, 2.39 kJ/mol respectively.

DISCUSSION

Catalase is one of numerous antioxidant defense enzymes(Peroxidase,SOD,Glutathione peroxidase) that catalyses dismutation of hydrogen peroxide into oxygen and water. It is a tetrameric heme including enzyme present in all aerobic organisms is acting as a key role in protecting cells versus oxidative stress[19]. The increment of catalase activity in patients with diabetes as compared to the control group may be related to promotion ROS levels. Higher levels of ROS have been exposed to play a role in the progress of diabetic complications. An accumulation of ROS in diabetes cannot be accurately balanced by antioxidant systems. Consequently, when oxidative stress growths as a result of a pathologic state, a protection system stimulates the regulation

and expression of antioxidant enzymes. The results of the present study designates the presence of some change in oxidant antioxidant balance in diabetic patients. The increase in the serum catalase is associated with the oxidative stress.. The pH has an important role in the activity of the enzyme. The catalase wasfound to yield maximum activity at pH 7 (Figure 2). These results,perhapsclarifiedby the fact that acidic pH has an inhibitory effect on theenzyme leading todecreasing its activity. Also, the survival of the active sites in amino acids will be affectedby the change in pH, which may modify the ionization ofthese amino acids. The optimum temperature, where the maximumcatalase activity carries out, is equal to 35°C. The kinetic energy of molecules raises with an increase in temperature. When the temperaturewas additional increased, the molecules of enzyme surpassthe barrier of energy. This lead to the breakage of hydrophobic and hydrogen bonds that are dependable forpreserving the three dimensional structure of the enzyme[20, 21].

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