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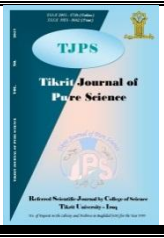

Study of some parameters and Partial purification of prolidase from serum women preeclampsia

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ABSTRACT

This study was carried out by partial purification of prolidase from blood serum patients with preeclampsia by ion exchange The degree of purification enzyme (1. 85) fold, enzyme yield (8.82) and specific activity is (8.88) IU/mg .The kinetic studies of partially purified The enzyme technology demonstrated the optimal substrate concentration which was (97) mmol/L and Vmax (71.42)(U/L),km(1.25) while optimal The optimum temperature was (37°C) and pH (9.2). molecular The weight of the partially purified enzyme was determined by a gel Electrophoresis method, in the presence of polyacrylamide gel and sodium Dodecyl Sulfate (SDS_PAGE) which showed that the approximate. The molecular weight was (54KD). We found a high level of Erythropoietin hormone in Pre-eclampsia patients which was a significant (P≤0.01) increase, (438.47) when she was in Control (251.70), the value of antidiuretic hormone in the patient was a significant (P≤0.05) increase (7.343) while in Control decrease value (2.155), the value of the ALD hormone in the patient was a significant decrease (P≤0.05) (288.7) and control was (143.6), Nitric Oxide in the patient was a significant (P≤0.05) (30.0) and control was increase (44.2) and vitamin D in the patient was a significant decrease (P≤0.05) (11.04) and control was(15.78) We conclude from the above results that there are some variables that rise with preeclampsia, such as the hormone ADH and the hormone EPO, but other variables such as ALD, vitamin D3 and nitric oxide decrease with increased pregnancy pressure, that is, with preeclampsia, in addition to that the main objective of the research is to purify the prolidease enzyme in the serum of women with preeclampsia and to know the duration of its effect This enzyme on pregnant women and linked it in another study with materials to find a new treatment..

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دراسة بعض المتغيرات والتنقية الجزئية لأنزيم البرولايديز لدى مصل النساء المصابات بتسمم الحمل

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الملخص

أجريت الدراسة لتنقية انزيم البرولايديز من مصل النساء المصابات بتسمم الحمل باستخدام تقنية التبادل الايوني حيث بلغت درجة التنقية في تقنية التبادل الايوني (1.85)والفعالية النوعية IU/L (8.88) وحصيلة انزيمية (8.82) . وأجريت الدراسة الحركية للأنزيم المنقى جزيئيا حيث كان تركيز المادة الأساس الأمثل 97 mmol وبلغت قيمة ثابت ميكالس منتن mM (1.25) والسرعة القصوى(71.42mM) بينما درجة الحرارة المثلى لعمل الانزيم هي (37) والاس الهيدروجيني الأمثل هو pH(9) .تم تقدير الوزن الجزيئي للأنزيم المنقى من المرضى بطريقة الترحيل الكهربائي على هلام متعدد الأكريل أمايد بوجود كبريتات دوديكايل الصوديوم (PAGE_SDS) حيث بلغ الوزن الجزيئي للأنزيم KD54 اما بالنسبة لدراسة عدة متغيرات حيث وجد ارتفاع معنوي لهرمون اريثروبويتين (P≤0.01) في المرضى(438.47) مقارنة مع السيطرة(251.70) اما الهرمون المضاد لأدرار البول حيث كانت النتيجة مستويات عالية من هذا الهرمون (P≤0.05) في المرضى (7.343 ±1.063) مقارنة مع السيطرة (2.155 ±0.571) اما مستوى هرمون الالدستيرون مستوى واطئ (P≤0.05) في المرضى حيث بلغت (20.82 ±288.7) مقارنة مع مجموعة السيطرة (33.8 ±143.6) اما بالنسبة لمستويات النتريك أوكسيد وفيتامين D3 كانت المستويات منخفضة (P≤0.05)في المرضى مقارنة مع السيطرة حيث كانت النسب على التوالي أوكسيد النتريك في المرضى (1.9±30.0) مقارنة مع السيطرة (5.3±44.2) وفيتامين D3 في المرضى (0.99±11.04) اما السيطرة (3.45±15.78)نستنتج من النتائج أعلاه ان هناك بعض المتغيرات ترتفع مع تسمم الحمل مثل هرمونADH وهرمون EPO لكن المتغيرات الأخرى مثل ALD وفيتامين D3 والنتريك أوكسايد تنخفض مع زياد ضغط الحمل أي مع تسمم الحمل إضافة الى ان الهدف الأساسي من البحث تنقية انزيم البرولايديز في مصل النساء المصابات بتسمم الحمل ومعرفة مدة تأثير هذا الانزيم على النساء الحوامل وربطه في دراسة أخرى مع مواد لإيجاد علاج جديد.

Introduction

Hypertension in pregnancy involved a spectrum of conditions, most notably preeclampsia, a form of hypertension unique to pregnancy that occurs de novo or may be superimposed on chronic hypertension. The other forms, chronic and gestational hypertension, usually have more benign courses **[1].** Preeclampsia, a pregnancy-specific disorder characterized by hypertension (≥ 140/90 mm Hg) and proteinuria (≥ 300 mg in a 24-hour urine), affects 3% to 4% of all pregnancies worldwide. Risk factors include primiparity, previous preeclampsia, increased maternal body mass index (BMI) before pregnancy, ethnicity (black women are more at risk), multiple gestations, and underlying medical conditions such as renal disease and diabetes mellitus [2]. Beginning early in the first trimester, there are surges of estrogen, progesterone, and relaxin, leading to systemic vasodilation.[3]**.** Prolidase (E.C. 3.4.13.9) is a cytosolic exopeptidase that cleaves imido dipeptides and imido tripeptides with C-terminal proline or hydroxyproline it may have a role in various disorders such as Chronic liver disease, osteoporosis, osteoporosis, uremia, hypertension [4]. The disorder in this enzyme causes irregular levels of collagen in the body, which can be considered a diagnostic tool for many diseases. There is a relationship linking collagen formation to the proportion of prolidase. The deficiency in this enzyme is a genetic condition that occurs due to mutations in the PEPD gene on the nineteenth chromosome and in some pathological cases. The enzyme prolidase is present in a modified form, so it does not have the ability to dismantle some bonds, which causes its levels to rise and be excreted with urine [5]. The work of this enzyme requires special conditions to work, as it needs a narrow range of pH from (6-8) and a temperature within the range (55-35) degrees Celsius, Gly-Pro although effective with Ale-Pro, Met-Pro and Phi-Pro [6].

The aim of the study: - The aim of the research is to purify the proliase enzyme from the serum of women with preeclampsia and to study some variables in patient addition to using the purified enzyme in another study.

- Experimental

The study was conducted in، Salah al-Din Governorate - Salah al-Din General Hospital 40 samples From the Preeclampsia patients were collected (aged 16 to 45), as well as 60 samples From the healthy control subjects (aged 18 to 45). blood was drawn of the vein using a 5 ml plastic syringe. The blood was put into a clean, free anticoagulant Tubes, coagulate at room temperature. the blood Then the serum was quickly separated by centrifuge 3000 g for 15 minutes to ensure proper serum extraction. Enzyme activity It was measured directly and the study was conducted indirectly.

-Biochemical parameter:

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Hormones, include Erythropoietin (EPO) Fine Test Biotech-China, hormone, (ADH), (Aldosterone) (ALD), vitamin D and (Nitric oxide) (NO) Bioassay technology Laboratory-China in serum. prolidase activity in serum was measured by use a base material for the enzyme glycine - proline with different concentrations of solutions of specific compounds to form the used kit.

-Determination of serum prolidase activity:- The enzyme prolidase reacts with a dipeptide (glycine-proline) as a basic substance for the enzyme. To give the amino acid proline and glycine freely, the amount of chromatically released proline is measured after its interaction with ninhydrin at the wavelength (515) nm. Relying on proline as standard.

Assay Procedure- The following steps were used to estimate the enzyme's activity:

1- The serum was diluted six times by adding 250 µl of dilution solution for every 50 µl of serum, then the mixture was incubated for 24 hours at 37° C, then (100 µl) was added to the control samples.

2- Test tubes were prepared as shown in the following table:

Solutions	Test	Control
Basic material solution	100 µl	…….
Dilute serum solution	100 µl	100 µl

The tubes were incubated for 30 minutes at 37°C, then the reaction was stopped by adding (1ml) of 0.45mol/L of TCA solution. After stopping the reaction, the tubes were placed in a centrifuge for 5 minutes at a speed of 2000 rpm, then (500) microliters of the clear solution were withdrawn from both the Test and Control tubes.

3-prepared four tubes as follows:

Solutions	Test	Control	Standard	Efficient
Chenard detector	1 ml	1 ml	1 ml	1 ml
glacial acetic acid	1 ml	1 ml	1 ml	1 ml
clear solution	0.5 ml	0.5 ml	…….	
Proline measurement solution	…….	…….	0.5 ml	…….
stop reaction solution	…….	…….	…….	0.5 ml

The above four tubes were incubated for 10 minutes at 90 °C in a water bath to complete the formation of the colored complex whose absorbance was measured at 515 nm after filtering the device by means of an adequate solution.

- Calculations

The enzyme activity in the blood of the women under study was calculated based on the following equation:

Prolidase Activity = Abs of taste –Abs of control/ Abs of standard ×2.4×{S}

The concentration of the substrate in mmol/L= 94 mmol/L.

- Separation and purification of Prolidase from serum of Preeclampsia patients.

Prolidase purified from women's serum Preeclampsia using the following Steps:

1- Adding ammonium sulfate (%65) 2- **Dialysis** a dialysis sac on taining prolidase preparation was placed is a large volume of 0.1 ammonium carbonated ,the solution was changed twice during dialysis the solution containing prolidase perpetration was lyophilized and the resulting power was stored in deep freeze for the next step. **3-Ion exchange** (DAEA-Cellulose) Chromatography the method of chromatographic analysis by ion exchange is based on the partition chromatography principle [7].

- Kinetics of Prolidas. The Kinetic study of Prolidase included:

1-Effect of the substrate concentration: by using different concentration of substrate (0.5,0.75.1.5.2,3,4,5,6) mmol/l .

2-Effect of pH: The pH effect of the prolidase reaction. Different pH solution (3.2, 5.2, 7.2, 9.2, 11.2).

3-Effect of temperature: using to measure the effectiveness of Prolidase. The reaction was conducted at different temperatures (7,17,27,37,47,57)C°.

-(SDS-PAGE)– Laemmli's method was used to prepare the separation gel in

determining the molecular weight of Prolidase[8].

-Results and Discussion

-Biochemical parameter

In Table (1), show the concentration of hormones (Erythropoietin, ADH, ALD) and (Vitamin D, NO) for preeclampsia patients Compared with the control, when Average calculation Nitric oxide(NO) and vitamin D.

Table 1: The value of (ADH, ALD, Vitamin D, NO and Erythropoietin) in patients with preeclampsia (mean ± SD).

Groups	ADH (pg./ml)	ALD (pg./ml)	Vitamin D (ng/mL)	NO (ng/ml)	Erythropoietin (pg./ml)
Patient	7.343 ±1.063**	143.6 ±33.8*	11.04±0.99*	30.0±1.9*	438.47 ±30.35*
Control	2.155 ±0.571	288.7 ±20.8	21.32±4.95	44.2±5.3	251.70 ±38.30

* significant at P≤0.05 ** significant at P ≤ 0.01

We found a high level of vasopressin in the patient Preeclampsia that was severe Significant (P≤0.01) increase high value for this hormone ADH and Erythropoietin hormone (P≤0.05)

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During fetal development, EPO synthesis gradually changes from hepatocytes that stimulate erythropoiesis in the liver to EPO-producing renal cells in the cerebral cortex and outer marrow that stimulate erythropoiesis in the bone marrow, with the timing of this transition dependent on species. Arterial hypo filling induces osmotic stimulation of arginine vasopressin and upregulation followed by trafficking of this water channel to the apical membrane of principal cells along the collecting ducts. In mid and late pregnancy, there is also a fourfold increase in vasopressin's, a cysteine aminopeptidase produced by the placental trophoblast, which enhances the metabolic clearance of vasopressin in the case of preeclampsia while Aldosterone, Nitric oxide, and Vitamin D decrease value ($P \leq 0.01$) Vitamin D decrease in the second and third trimesters of pregnancy compared to the control Vitamin D deficiency is considered a global epidemic, with a Prevalence ranges from 18% to 84% depending on Country of residence, ethnicity, local clothing customs and dietary intake [9,10]. decrease in the levels of Aldosterone in the blood serum of preeclampsia patients in third stage compared with second stage and control. Aldosterone causes an increase in salt and water reabsorption into the bloodstream from the kidney thereby increasing the blood volume, restoring salt levels and blood pressure Studies indicate vascular endothelial growth factor as a major stimulator of aldosterone secretion independent of renin. This would explain the altered response of aldosterone to salt in take, It is preferable to have high aldosterone levels and a high amount of salt during pregnancy[11].Nitric oxide decrease in pregnancy pressure compared to the control .in which nitric oxide mediates many endothelial functions, including vasodilation and inhibition of platelet aggregation. Where preeclampsia is associated with nitric oxide deficiency, agents that increase nitric oxide may inhibit preeclampsia [12].

- Partial purification of prolidase from serum Preeclampsia patients.

Enzyme isolation and purification of biological molecules (protein, carbohydrate, nucleic acids and lipid) can be under special laboratory techniques of cell lyses tissue homogenization, filtration. centrifugation, chromatography and salt or organic solvent precipitation and concentration.

Table 2: steps of purifications of prolidase

Purification steps	Volume ml	ACTIVITY Iu/ml	Total Activity	Protein Concentration mg/ml	Specific Activity IU/mg	Recovery Yield %	Fold of purification	Total protein mg
Crude	10	268.7	2.68	56	0.0047	100	1	560
Precipitation	8	206.1	1648	48	0.00429	61.4	0.91	384
Dialysis	8	0.111	0.888	17	0.0065	33.1	1.38	136
Ion exchange (DAEA-Cellulose)	5	71.11	0.385	8	0.888	13.2	1. 88	40

-Precipitation of the protein:

The most common used salt is ammonium sulfate which has high water solubility the results predicted that a 65% saturation with ammonium sulfate to curd preparation produced maximum protein precipitate was dialyzed to remove the small molecular compound.

-Ion exchange:

The technique was applied to separate the materials, Which obtained by ammonium sulfate precipitation method and dialysis for crude preparation form patient human serum the results of elution show in fig (1) indicated one peak A was obtained with high prolidase activity (70) µmole /min fig (1) and peak B Protein concentration with low activity (50)µmole /min fig (1)the specific activity was higher (8.88).

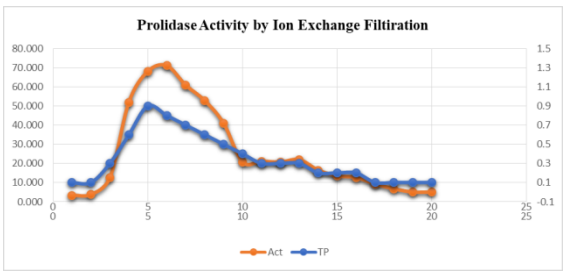

Fig. 1: Isolation of prolidase with Ion Exchange Filtration

protein conc, Enzyme activity

-Determination the Molecular Weight of Prolidase: Enzyme by Electrophoresis on Polyacrylamide Gel (SDS-PAGE) SDS. In order to investigate the purity of prlidase which was purified from serum, Polyacrylamide gel electrophoresis sample was the purified prolidase when the gel is immersed in Comassie brilliant blue G-100, several protein bands appeared with different molecular weights along the gel. While the purified enzyme sample demonstrated one clear band only. The appearance of many protein bands along the gel is imputed to that crude extract which contains a large number of different proteins with different molecular weights. The purified enzyme sample gave only one band, this means that there is no contamination from other proteins, as referred to by Roy and Kumar (2014)[13]. Molecular Weight of Prolidase is (54KD).

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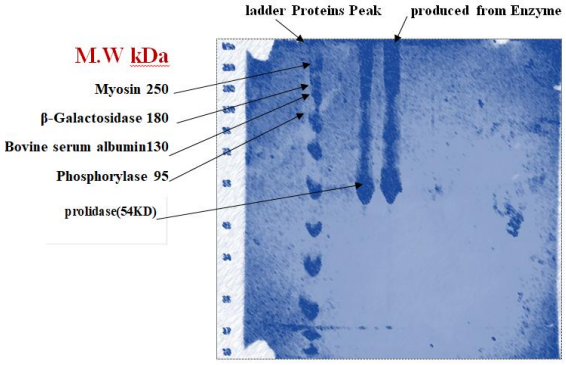

Fig. 3: SDS-PAGE analysis bands of the purified prolidase respectively and the standard ladder proteins

3.3. Kinetic study of partially purified prolidase:

- Concentration of substrate.

The activity of enzyme was measured in the presence of different concentrations of partially purified enzyme from normal human between (0-6) µg/ml as shown in figure(4). The result indicated that enzyme activity increased linearity with increasing the concentration of the protein as a source of the enzyme the optimum substrate 5.

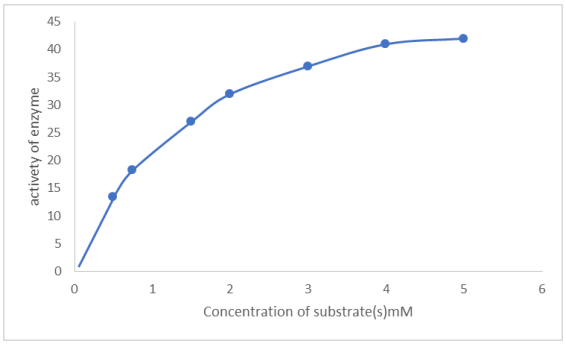

Fig. 4: Effect of Concentration of substrate on the activity of prolidase

-Effect of temperature: The optimum temperature of the enzyme activity was 37Cº, the results varied with the other studies may be happened because of partial glycyl proline hydrolysis at higher temperatures the effect of several divalent cations on prolidase activity. This modified assay is agree with study that done by Mr. wilk and Mr. Kalms (2017) for the optimum temperature [14].

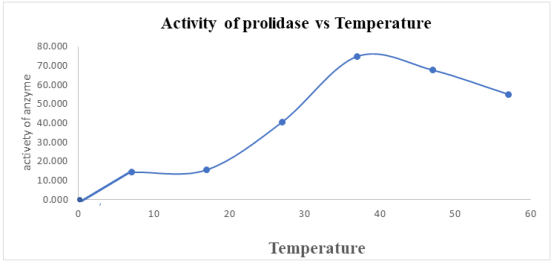

Fig. 5: Effect of Temperature on Prolidase activity

-Effect of pH :-The influence of pH upon the activity of prolidase was investigated using buffer solution containing Tris –HCl with a pH rang (3-12)in this case conditions were conducted in the same manner as described earlier except that the p H was wearied as indicated in fig(6)the optimum pH for prolidase was found to be (9)in Tris –HCl buffer, The result of pH varied with other studies ,the optimal pH for erythrocyte prolidase is also at pH 9.0 [15].

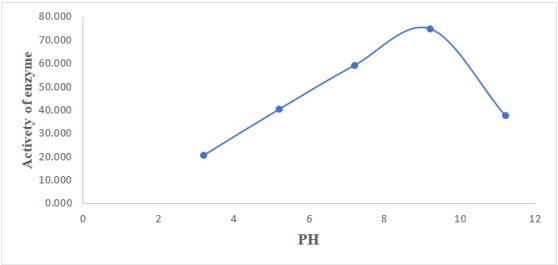

Fig. 6: Effect of pH on prolidase activity

-Effect of substrate concentration on the enzyme activity:

For many enzymes, the rate of catalysis varies with the substrate concentration the rate of catalysis is defined as the number of moles product formed per second at a fixed concentration of enzyme almost lineally proportional to (s)is small at high (s)is nearly independent of (s).to determine the effect of substrate concentration on the enzyme activity as series of concentration on the were performed where the concentration of substrate was varied fig (7). The optimum concentration of the substrate was ()mmol/l and the constant value of the Michaels – minten of substrate was (1.25mM) and Vmax was (71.42 U/L).

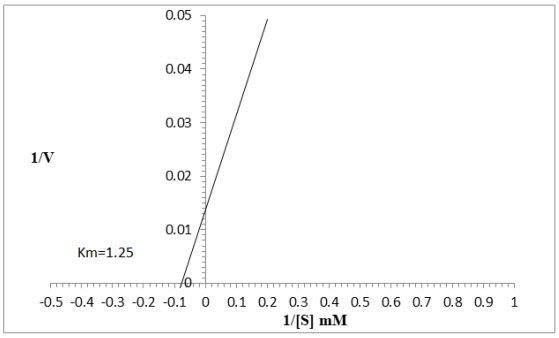

Fig. 7: Lineweaver Burk Plot

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