Partial Purification and Some Kinetic studies of Glutathione Peroxidase (GPx) in Normal Human Plasma and Comparing with Primary Infertility Female

Luay A. Al-Helaly Obeda A. Al-kado
Department of Chemistry ,College of Science , Mosul University , Mosul , Iraq
(Received: 10 / 9 / 2012 ---- Accepted: 8 / 1 / 2013)

Abstract
This study attempts to isolate the glutathione peroxidase(GPx) from normal human plasma compared to infertility female. Three proteinous components had been isolated by gel filtration chromatography from the precipitate produced by ammonium sulfate. It was found that only the first peak had a high activity for GPx. The apparent molecular weight of the isolated GPx using gel filtration (Sephadex G-100) was (88131) dalton respectively.
Maximum activity for GPx was obtained using (2.5) mmol/l of glutathione (GSH) as substrate, phosphate buffer (0.3 M/l) as a buffer at pH (6.5) for (14) minutes in incubation at (40)°C. Using Line Weaver–Burk plot, the values of maximum velocity (V_{max}) and Michaelis constant (K_m) were (1.56 µmol/ min) and (1.1 mmol/l) respectively. The study showed the effect of some inhibitors on the enzyme activity. Paracetamol and copper sulfate possessed a noncompetitive inhibition while zinc acetate showed a competitive inhibition .
Keywords: Purification, Glutathione peroxidase(GPx), Infertility, Inhibitors.

Introduction
The Glutathione peroxidases (EC 1.11.1.9) are selenoprotein residues present in cytoplasm and mitochondria of cells for liver, red blood cells, plasma and sperm, as it exists enzyme in spermatids in the form of soluble peroxides either in the sperm mature is present in the body is active enzymatically (Protein insoluble) in the segment average mid piece of mature sperm in the form of a template-like Keratin embedded in a helix or spiral Mitochondrial helix as it serves to protect the movement of sperm[1].
The important roles of glutathione peroxidases plays in the organization of the level of peroxides different by accelerating transformation reduced glutathione (GSH) to oxidized glutathione (GSSG) after removal of peroxides such as hydrogen peroxide H_2O_2 or peroxides fat LOOH or organic peroxides ROOH as indicated in equations below, as a part of the GPx system protecting cells against oxidation and products also reduces the cell damage caused by the increase of free radicals (oxidative stress)[2].

$$2GSH + H_2O_2 \xrightarrow{GPx} GSSG + 2H_2O$$
$$2GSH + LOOH \xrightarrow{GPx} GSSG + LOH + H_2O$$
$$2GSH + ROOH \xrightarrow{GPx} GSSG + ROH + H_2O$$

Glutathione peroxidases contains four units each unit has a single atom of selenium which associated with cysteine is selenocysteine, glutathione reduced the selenium in the glutathione peroxidases enzyme and then glutathione reductase(GRd) enzyme reacts with hydrogen peroxide[3].
Oxidized form of glutathione reduced to GSH by glutathione reductase enzyme which needs coenzyme NADPH (Nicotinamide adenine dinucleotide phosphate) reduced as below[4,5]:

$$GSSG + NADPH + H^+ \xrightarrow{GRd} 2GSH + NADP^+$$

The enzyme glutathione peroxidases plays a role in protecting cells and tissues against lipid peroxidation by removing hydrogen peroxide, as well as reduce the configuration of the hydroxyl radical and thus prevent one way to start lipid peroxidation that can occur in unsaturated fatty acids in the reproductive system[6].
In recent years, there has been growing interest in the roles of ROS and oxidative stress in female reproduction[7,8]. Accumulating evidence demonstrates that ROS are key signals in the initiation of apoptosis in antral follicles and granulosa cells of antral follicles by diverse stimuli, such as gonadotropin withdrawal, exposure to exogenous toxicants, and exposure to ionizing radiation, and that antioxidants protect against these stimuli. The aim of the research is to provide a detailed study of (GPx) involving isolation, characterization and purification from human plasma in normal and infertility female using different biochemical techniques and show the effects of ROS on partial separation of GPx for non-infertility and infertility female.

Materials and methods
Assay the activity of plasma Glutathione peroxidase (GPx)
Glutathione peroxidase (GPx) activity was measured by the method of Rotruck *et al*., 1984 [9] .Briefly, the reaction mixture contained 0.2 ml 0.4 M sodium phosphate buffer, pH 7.0, 0.1 ml 10 mM sodium azide, 0.2 ml plasma, 0.2 ml of 2 mM GSH, and 0.1 ml 0.2 mM hydrogen peroxide . The contents were incubate at 37°C for 10 min, the reaction was stopped with 0.4 ml 10% TCA and centrifuge. The supernatant was assayed for GSH content using Ellman reagent (19.5 mg DTNB [5,5-- dithio bis (2-nitrobenzoic acid)] in 100 ml 0.1% sodium citrate).
Purification of Glutathione peroxidase (GPx) from Human Plasma.
The method given here has yielded an enzyme preparation acceptable for human plasma. All steps were performed at 4 °C unless stated otherwise.
Step I: Ammonium Sulfate Fractionation
A human fresh plasma was obtained from the plasma of non-infertility female (age: 26 year) and protein

was precipitated using 50% ammonium sulfate $(NH_4)_2SO_4$ saturation or protein salting out[10,11].

Step II: Cooling ultracentrifuge separation

The suspension was centrifuged at 9,000 Xg for (45) min. The protein in precipitate and supernatant are determined using the modified Lowry method, and the GPx activity is determined in each fraction.

Step III: Dialysis

Dialysis was made using a semi permeable cellophane dialysis membrane with M.Wt. cut off (<10000) dalton. The dialysis sac containing the suspension in (Step I) was dialyzed against 0.1M ammonium bicarbonate, was stirred with a magnetic stirrer overnight at 4 ^oC. The solution of dialysis was changed three times only per 3 hours during dialysis for 24 hour[10]. The protein of the dialyzed enzyme was estimated by modified Lowry method[12].

Step IV: Gel Filtration Chromatography (Sephadex G-100)

The sephadex gel G-100 supplied as a powder was suspended in adequate distilled water so that when it was stirred incorporated air bubbles that escape rapidly to the surface. It was then allowed to swell for 3 hours at 90 ^oC in a complete swelling. This procedure was used for column packing and sample application[10].

In the present study, the column of dimension 2.5×120 cm which contained a gel sephadex G-100 to height of (105) cm. The exclusion limit for this type of the gel is (150,000) Dalton[10]. Depending on the volume of this column which was 590 ml, it was packed with a slurry of the gel in water .

A concentrated sample (2) ml of the protein material, which was obtained in (Step II), was applied on the top of a bed sephadex G-100, followed by distilled water.

Elution of the protein materials was carried out at a flow rate (54)ml/ hour with a definite time (8) min, using distilled water, as eluant, The fractions were collected using a fraction collector apparatus which is worked on minute system. The protein compounds in each fraction collected were detected by following the absorbance at wave length (280) nm using UV/Visible Spectrophotometer. Peak was combined separately from the plot of an absorbance versus elution volumes and GPx was determined in each fraction[13].

Step V: Freeze-Dryer (Lyophilization) Technique

The enzyme fraction which was obtained from gel filtration was dried using a freeze-dryer (Lyophilization) technique to obtain a powder or a concentrated protein. The enzyme was kept in a deep freeze at -20^oC in a tight sample tube to be used in further investigations.

Results and discussion

Enzyme Purification

The results predicted that the enzyme activity was found in the (50)% of a saturation ammonium sulfate precipitates .

As shown in Table (1), the specific activity was increased after dialysis. This might be due to the removal of the small molecules and increasing the purification of GPx.

Table 1: Partial purification steps of Glutathione peroxidase (GPx) from the plasma of non-infertility female.

Purification stage	Volume taken (ml)	Protein conc. (mg/ml)	Total protein (mg)	Activity (U*/ml)	Total activity (U)	Sp.activity (U/mg protein)	Folds of Purification	Recovery %
Plasma	100	80.9	8090	0.573	57.3	0.0071	1	100
Precipitate by $(NH_4)_2SO_4$(50%)	45	92.3	4153.5	0.855	38.47	0.0093	1.31	43.6
Supernatant	60	70.9	4254	0.501	30.06	0.0071	0.99	31.5
Dialysis	62.5	60.1	3756.25	0.91	56.87	0.0151	2.13	99.2
Sephadex G-100 (Fractions)								
Peak A	62.3	9.1	566.93	0.793	61.86	0.0871	12.3	86.2
Peak B	50.4	15.7	791.28	0.136	6.85	0.087	1.23	11.9
Peak C	16.8	15.1	253.68	0.095	1.6	0.0063	0.887	2.79

U*: a mount of Glutathione peroxidase (GPx) catalyzing the formation of one micromole of product per min under optimum conditions.

Table 2: Partial purification steps of Glutathione peroxidase (GPx) from the plasma of infertility female .

Purification stage	Volume taken (ml)	Protein conc. (mg/ml)	Total protein (mg)	Activity (U*/ml)	Total activity (U)	Sp.activity (U/mg protein)	Folds of Purification	Recovery %
Plasma	10	68.0	680	0.379	3.79	0.0056	1	100
Precipitate by $(NH_4)_2SO_4$(50%)	4.2	98.0	411.6	0.595	2.499	0.0061	1.1	65.9
Supernatant	6	78.0	468	0.19	1.14	0.0024	0.43	30.0
Dialysis	4.5	89.0	400.5	0.726	3.267	0.0082	1.5	86.2
Sephadex G-100 (Fractions)								
Peak A	25	5.6	140	0.089	2.225	0.0158	2.8	58.7
Peak B	14.8	9.7	142.59	0.009	0.133	0.00093	0.17	3.5
Peak C	10.4	7.7	80.08	0.007	0.0728	0.00091	0.16	1.9

U*: a mount of Glutathione peroxidase (GPx) catalyzing the formation of one micromole of product per min under optimum conditions.

Gel Filtration Separations

This technique was applied to separate the protein as a source of enzyme, which was obtained after dialysis using a column containing sephadex G-100 gel as shown in((step III (A)). The result (Figure 1) indicated that there were mainly three peaks. The elution volume of peak (A) was (169.7) ml, while the elution volume of peak (B) was (293) ml and the elution volume of peak (C) was (460.1) ml. The specific activity of the enzyme peak (A) was (0.0871 U/mg protein) and 12.3 folds of purification compared to initial extract Table (1). Peak (B) and (C) were neglected for the time, because of their low activity.

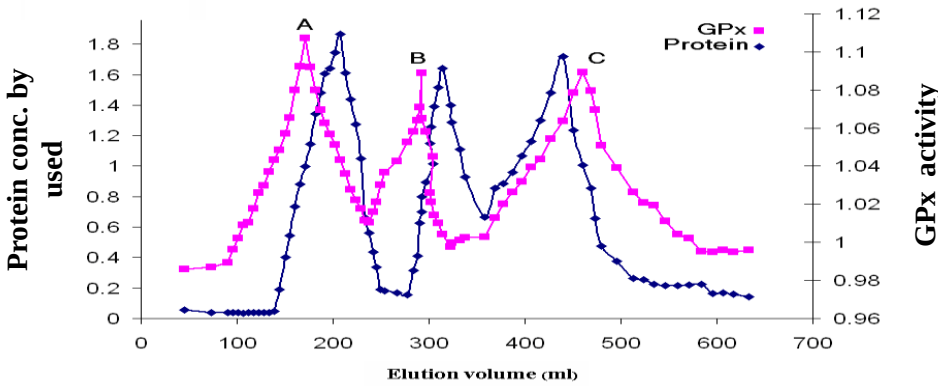

Figure 1: Elution profile Glutathione peroxidase (GPx) for plasma of non-infertility female on sephadex G-100.

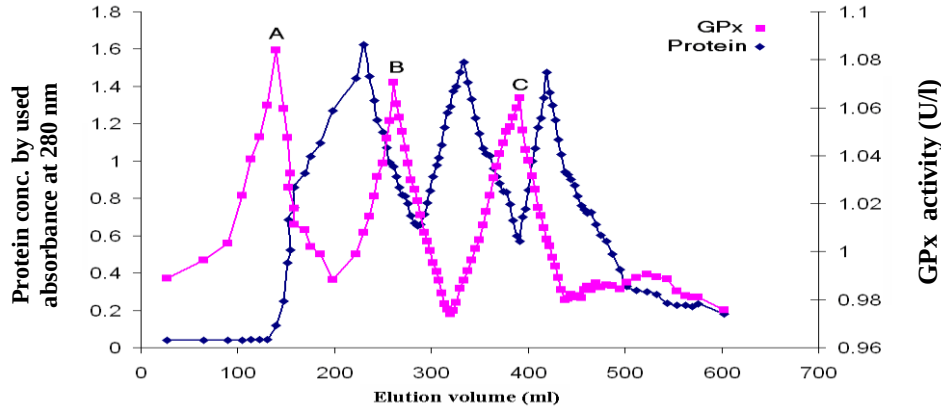

Figure 2: Elution profile Glutathione peroxidase (GPx) for plasma of infertility female on sephadex G-100.

Concentrations of ROS may play a key role both in the implantation and fertilization of oocytes[14]. Oxidative stress(OS) operates in follicular development, normal cycling ovaries, and cyclical endometrial changes. Several probes of oxidative stress such as decreased activity of GPx (Figure 2). On the other hand, the pathological effects are exerted by various mechanisms including lipid damage, inhibition of protein synthesis and depletion of ATP[15]. Excess ROS may hinder the endometrium, which normally functions to support the embryo and its development[16]. OS may induce luteal regression and insufficient luteal hormonal support for the continuation of a pregnancy[17]. The association of OS with various gynecologic and obstetric conditions related to infertility suggests a potential role for oral antioxidant supplementation (Figure 3).

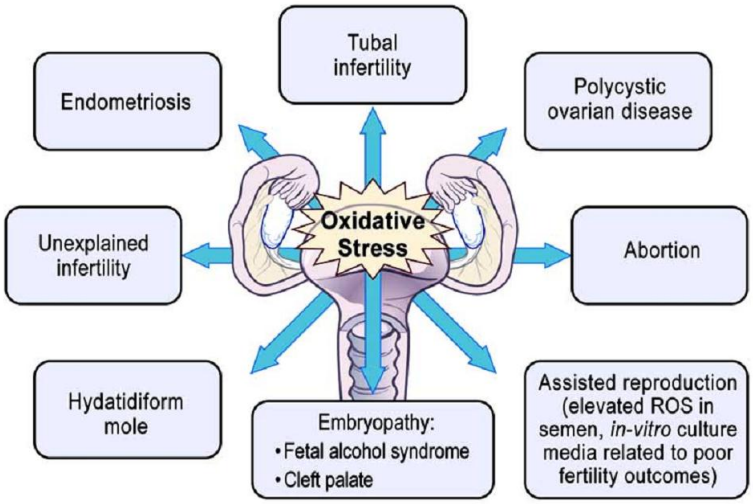

Figure 3: The role of oxidative stress in obstetric and gynecologic conditions that contribute to infertility [7].

Molecular Weight Determination of GPx by Gel Filtration

The molecular weight of first peak (A) as a source of GPx was determined by gel filtration chromatography using sephadex G-100 column (2.5 x 120) cm calibrated with known molecular weight proteins that were listed in Table (3).

Table 3: Elution volumes of known molecular weight materials on sephadex G-100.

Materials	Molecular weight (Dalton)	Elution volume (ml)
Blue dextran	2000000	122.8
Hexokinase	100000	144.3
Bovine serum albumin	67000	171.4
Egg albumin	45000	202.9
Pepsin	36000	223.9
Papain	23000	382.7
Tryptophan	204	410.6
Unknown (peak A)	88131	*169.7

*This value was obtained from Figure (1).

A plot of logarithmic molecular weight of each material versus the elution volumes indicated in Table (3) gives a straight line as illustrated in Figure (4).

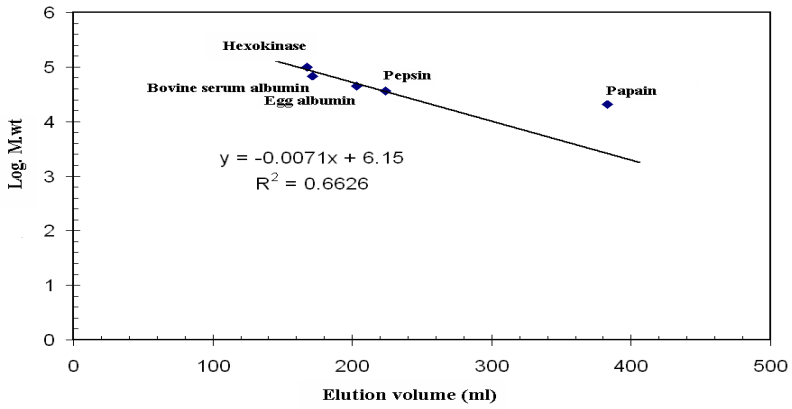

Figure 4: A plot of the logarithm molecular weights of known proteins versus elution volumes on a sephadex G-100.

The molecular weight of unknown protein compound separated by the same column chromatography as shown in (step III) was determined from the standard curve, which was represented by Figure (4). The comparative molecular weight of peak (A) as a source of GPx is approximately equal to (88131) dalton. This finding was in a agreement with the previous results where it was reported that the molecular weight of GPx was (88000) dalton from different tissues[18,19].

Optimum Conditions for GPx Activity

To develop assay conditions where GPx from normal female (Non-infertility) shows a maximum activity, a series of experiments were performed. These included enzyme concentration, pH of the assay conditions, incubation time, incubation temperature and substrate concentration[20].

1.Effect of Enzyme Concentration on GPx Activity:

It is important to establish that the activity varies linearly with enzyme concentration. The activity of enzyme was measured in the presence of different concentrations of partially purified enzyme from plasma between (10-100) µg/ml as shown in Figure (5).

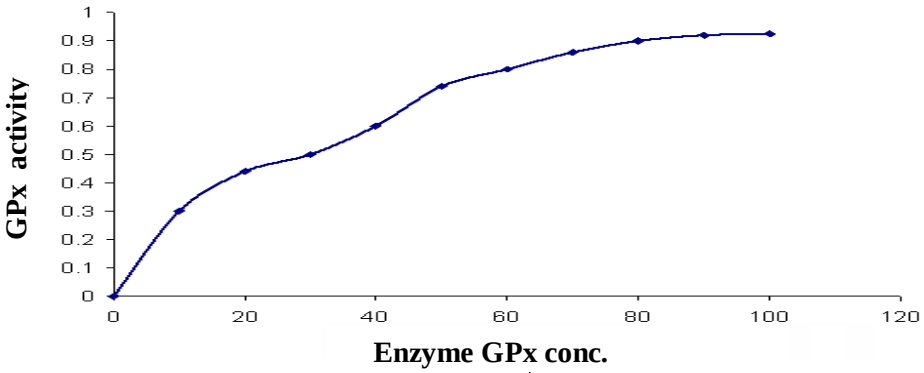

Figure 5: Effect of different protein concentrations on GPx activity

The result indicated that the enzyme activity increased with increasing the concentration of protein as a source of the enzyme. For the next experiment (50)µg/ml, as a source of the enzyme was selected for determination other optimum conditions.

2.Effect of Buffer Solution:

2.1.Effect of Buffer Concentration on GPx Activity:

The activity of enzyme was measured in the presence of different concentrations of buffer solution within the range (0.1-0.7) mol/liter of phosphate buffer at pH 7.0. Maximum activity was obtained using (0.3)mol/liter of phosphate buffer (Table 4).

Table 4: Effect of buffer concentrations on GPx activity.

Phosphate buffer (mol/liter)	GPx activity (U/l)
0.1	0.2
0.2	0.5
0.3	**0.84**
0.4	0.45
0.5	0.35
0.6	0.19
0.7	0.16

2.2.Effect of pH on the GPx Activity:

The influence of pH upon the activity of GPx was investigated by using (50 µg/ml) as a source for enzyme in (0.3)mol/liter phosphate buffer. The assay conditions were conducted in the same manner as described earlier at pH range of (2.5-9.0). Maximum GPx activity was obtained at pH (6.5) as indicated in Figure (6).

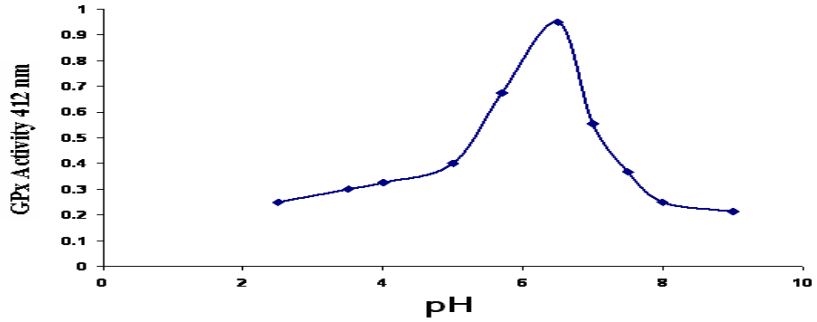

Figure 6: Effect of pH on GPx activity using (0.3)mol/liter phosphate buffer and (50µg/ml) as a source for the enzyme.

3. Incubation Time as a Function of Enzyme Activity

To determine the stability of GPx activity under assay conditions, a series of experiments were performed at different time intervals. The results indicated that maximum enzyme activity was obtained after (14) mint. in (25 °C) incubation(Figure 7).

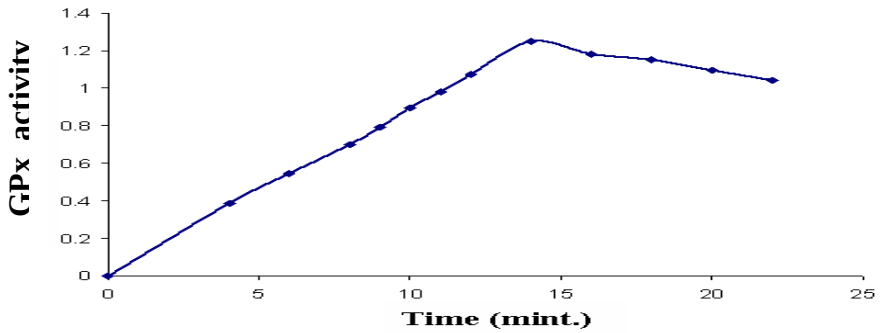

Figure 7: Effect of incubation time on GPx activity.

4.Effect of Temperature on GPx Activity:

It has been found that as the temperature increased, there was a concave up increase in the enzyme activity until it reached a maximum value at a temperature of (40 °C) then dropped gradually after that Figure (8).

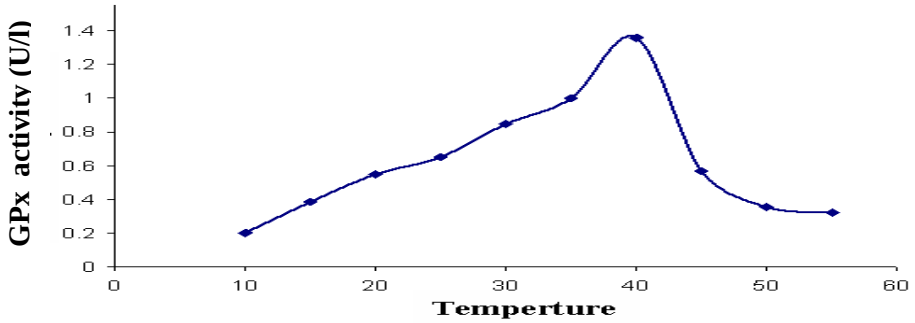

Figure 8: Effect of temperature (°C) on GPx activity.

5. Effect of Substrate Concentration on the Enzyme Activity:

To determine the effect of substrate concentration [GSH] on the enzyme activity, a series of experiments were performed where the concentration of the substrate was varied Figure (9).

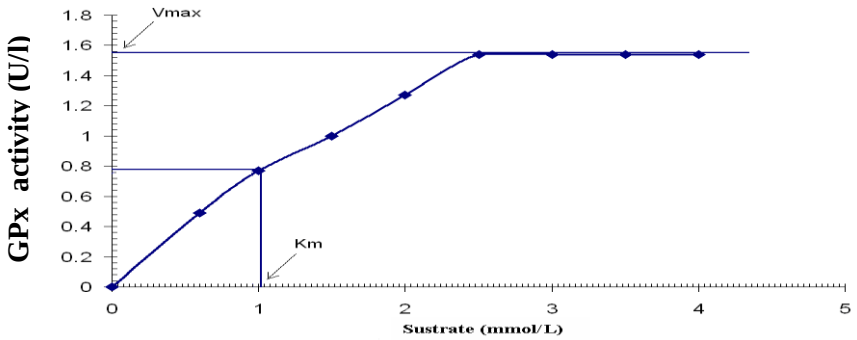

Figure 9: Effect of substrate concentration [GSH] on the activity of partially purified GPx.

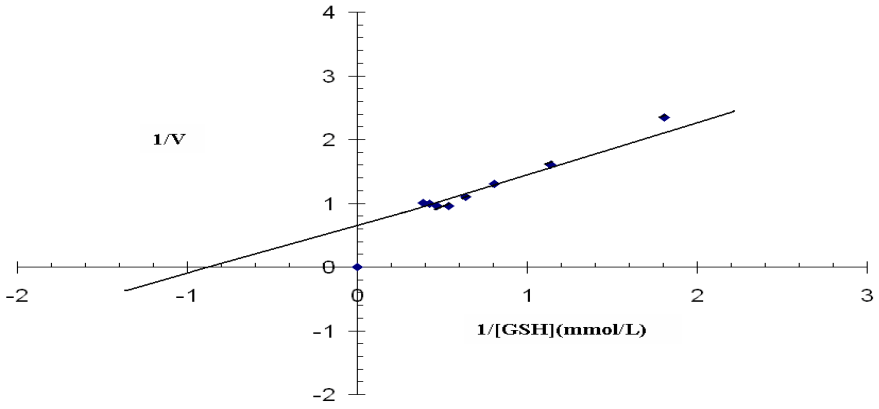

Figure 10: Line Weaver-Burk plot of partially purified GPx from plasma non-infertility female.

The line Weaver-Burk plot by plotting the reciprocal of the initial velocity versus the reciprocal of the substrate concentration. A linear relationship was obtained Figure (10) giving a K_m value of (1.1 mmol/liter) and V_{max} (1.56 µmol/min.).

6. optimum conditions of the partially purified Glutathione peroxidase:

The optimum conditions of the partially purified Glutathione peroxidase from plasma of non-infertility female were obtained in the following Table (5):

Table 5: Optimum conditions of the partially purified Glutathione peroxidase.

Substrate concentration (mmol/liter)	Temp.(°C)	Time (mint.)	pH	Buffer Conc. (mol/liter)	Enzyme Conc.(µg/ml)
50	40	14	6.5	0.3	50

7. Inhibition of GPx:

Many investigators observed that some chemical compounds have an inhibitory effect on GPx activity.

7.1. Inhibition copper sulfate

The results of adding copper sulfate on the activity of partially purified GPx were shown in Figure (11) . Copper sulfate decreased activity of enzyme with increased concentration.

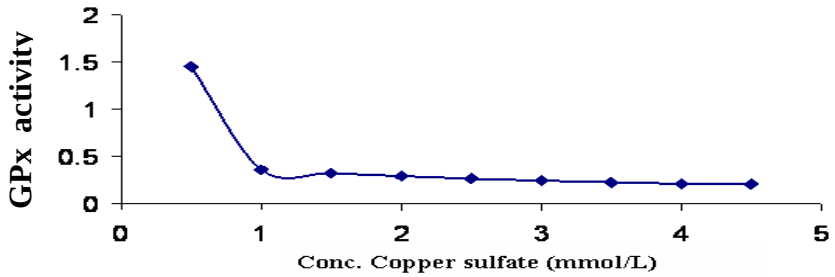

Figure 11: Effect of copper sulfate on the activity of partially purified GPx .

In this study, A line Weaver-Burk plot was performed as shown in Figure (12) The results showed that copper sulfate acted as a noncompetitive inhibitor. Noncompetitive-type inhibition was K_m appears unaltered and V_{max} was decreased proportionately to inhibitor concentration[20].

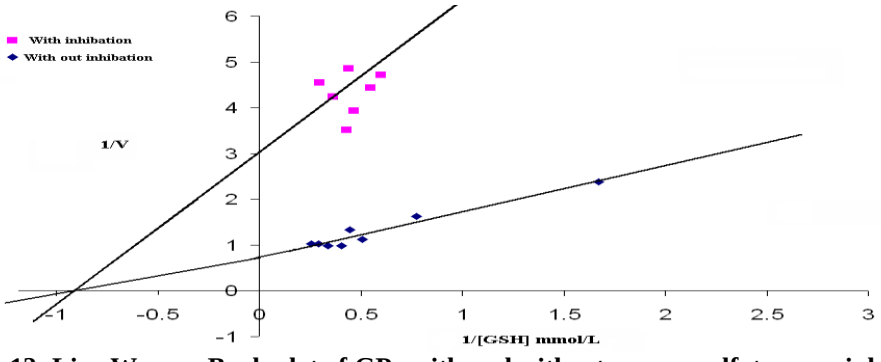

Figure 12: Line Weaver-Burk plot of GPx with and without copper sulfate as an inhibitor.

7.2. Inhibition zinc acetate

The results of adding zinc acetate on the activity of partially purified GPx were shown in Figure (13) . Zinc acetate decreased activity of enzyme with increased concentration.

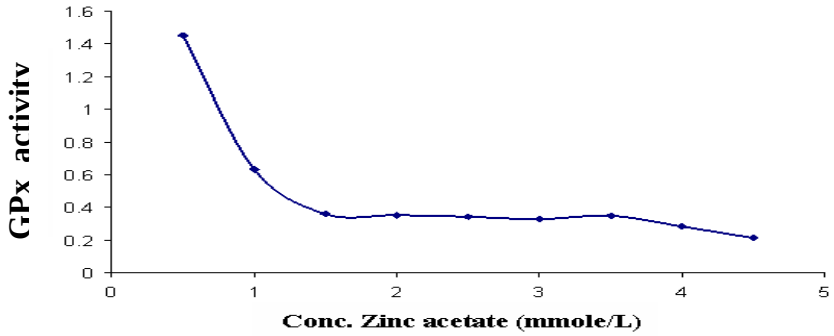

Figure 13: Effect of zinc acetate on the activity of partially purified GPx .

In this study, A line Weaver-Burk plot was performed Figure (14) The results showed that zinc acetate acted as a competitive inhibitor. competitive-type inhibition was K_m appears increased and V_{max} was unaltered proportionately to inhibitor concentration[21].

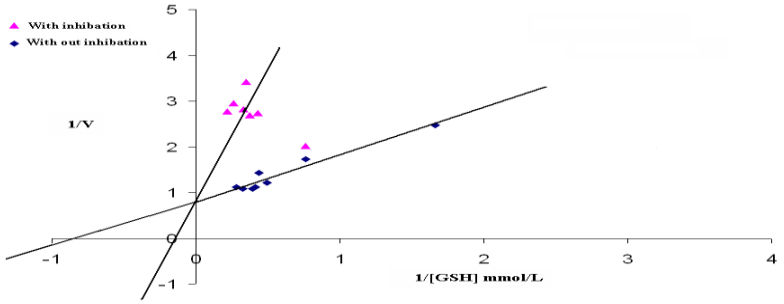

Figure 14: Line Weaver-Burk plot of GPx with and without copper sulfate as an inhibitor.

7.3. Inhibition paracetamol

The results of adding paracetamol on the activity of partially purified GPx were shown in Figure (15). Paracetamol decreased activity of enzyme with increased concentration.

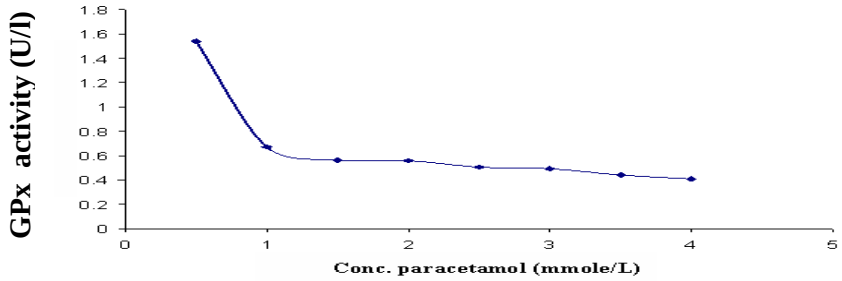

Figure 15: Effect of paracetamol on the activity of partially purified GPx .

In this study, A lineweaver-Burk plot was performed Figure (16) The results showed that paracetamol acted as a noncompetitive inhibitor. Noncompetitive-type inhibition was K_m appears unaltered and V_{max} was decreased proportionately to inhibitor concentration.

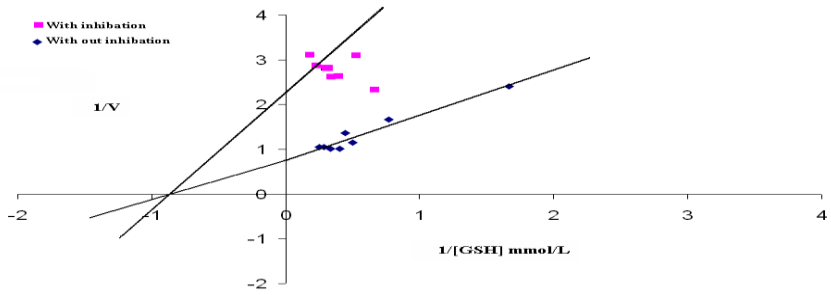

Figure 16: Line Weaver-Burk plot of GPx with and without paracetamol as an inhibitor

References

1. Ursini, F., Heim, S., Kiess, M., Wissing, J. and Flohe, L. 1999. Dual function of the selenoprotein GSH-PX during sperm maturation. *Science*, 27;285: 1393-1397.

2. Alberto, E. E., Nascimento, V., Braga, A. L. 2010. Catalytic application of selenium and tellurium compounds as glutathione peroxidase enzyme mimetics. *J. Braz. Chem. Soc.*, 21, 2032.

3. Banerjee, R., Becker, D., Dickman, M., Gladyshev, V., Ragsdale, S. 2008. "Redox Biochemistry". John Wiley and Sons, Inc., Hoboken, New Jersey. Canada.pp.201,209.

4. Delgado-Esteban M., Almeida A., Bolanos J.P.2000. D-Glucose prevents glutathione oxidation and mitochondrial damage after glutamate receptor stimulation in rat cortical primary neurons. *J. Neurochem* 75:1618–1624.

5. Nogueira, C. W., Rocha, J. B. T. 2011. Toxicology and pharmacology of selenium: emphasis on synthetic organo selenium compounds. *Arch. Toxicol.*, 85, 1313.

6. Halliwell, B., Gutteridge, J.M. 2000. Free radicals and antioxidant in the year 2000 : A Historical look to the future . *Ann. NY. Acad. Sci.* pp. 899:136-147.

7. Sekhon L. H., Gupta S., Kim Y., Agarwal A. 2010. Female Infertility and Antioxidants. *Current Women's Health Reviews* 6: 84-95

8. Devine P. J., Perreault,S. D. , Luderer U. 2012. Roles of reactive oxygen species and antioxidants in ovarian toxicity. *Biology of Reproduction*, 86(2): 1–10.

9. Rotruck, J. T., Pope, H. E., Ganther, A. B. 1973. Biochemical roles as a component of glutathione peroxidase. *J. Science*; 179,588-590.

10. Robyt, F. J., White, J. B. 1987. "Biochemical Techniques Theory and Practice". Books / Cole Publishing Com., USA.

11. David W. 2005. "Chapter 9: Protein expression, purification and characterization". *Proteins: Structure and Function*. Wiley, J., Ltd, p.320.

12. Chisti, Y., Moo-Young, M. 1991.Fermantation technology bioprocessing scale-up and manufacture .In: moses V and cape RE (eds), Biotechnology : The science and the Business. Harwood Academic Publishers. New York. PP. 167-209.

13. Mikkelsen, S.R. 2004 .Bioanalytical Chemistry. John wiley and sons Inc. Hoboken , New Jersey in the United states of America.P.26.

14. Sharma R.K., Agarwal A. 2004. Role of reactive oxygen species in gynecologic diseases. *Reprod. Med. Biol*. 3(4): 177-99.

15. Ray S.D., Lam T.S., Rotollo J.A. 2004. Oxidative stress is the master operator of drug and chemically-induced programmed and unprogrammed cell death: Implications of natural antioxidants *in vivo*. *Biofactors*. 21(1-4): 223-32.

16. Iborra A., Palacio J.R., Martinez P.2005. Oxidative stress and autoimmune response in the infertile woman. *Chem. Immunol. Allergy*. 88:150-62.

17. Agarwal A., Allamaneni S. 2004. Role of free radicals in female reproductive diseases and assisted reproduction. *Reprod. Biomed*. 9(3): 338-47.

18. Shulgin, K.K., Popova, T. N., Rakhmanova T.I. 2008 . Isolation and purification of glutathione peroxidase. *Biomedical and Life Sciences.* 44(3):247-250.

19. Lawrence, R. A., Burk, R. F. 1976. Glutathione peroxidase activity in selenium-deficient rat liver. *Biochem. Biophys. Res. Commun*. 71: 952-958

20. Berg, J. M., Tymoczko, J. L., Stryer, L. 2007. "Biochemistry". W. H. Freeman and Company. New York, USA. pp.138, 139, 145, 146.

21. Murray R. K., Bender D. A., Botham K. M., Kennelly P. J. , Rodwell V. W., Weil P. A. 2009. "Harper's Illustrated Biochemistry", 28ed. The McGraw-Hill Companies, Inc. Printed in China. Chapter 8. Enzymes: Kinetics. P.148.

تنقية جزئية ودراسة بعض الصفات الحركية لأنزيم كلوتاثايون بيروكسيديز من بلازما انثى سليمة ومقارنتها بعقيمة من النوع الأولي

لؤي عبد الهلالي ، عبيدة عبد الخالق القدو

قسم الكيمياء ، كلية العلوم ، جامعة الموصل ، الموصل ، العراق

(تاريخ الاستلام: 10 / 9 / 2012 ---- تاريخ القبول: 8 / 1 / 2013)

الملخص

الدراسة حاولت عزل أنزيم كلوتاثايون بيروكسيديز من البلازما أنثى سليمة ومقارنتها مع عقيمة من النوع الأولي. إذ تم فصل ثلاث حزم رئيسة من البروتينات بتقنية الترشيح الهلامي للراسب ألبروتيني الناتج من الترسيب بـ كبريتات الامونيوم، وأظهرت الحزمة الأولى فعالية عالية لأنزيم كلوتاثايون بيروكسيديز. قدر الوزن الجزيئي للأنزيم باستخدام تقنية الترشيح الهلامي باستخدام سفيدكس نوع G-100 ، ووجد أنه بحدود (88131) دالتون على التوالي.

تم أيجاد الظروف المثالية لفعالية أنزيم كلوتاثايون بيروكسيديز وكانت كالاتي: استخدام(2.5) ملي مول/لتر من مادة ((Glutathione (GSH) كمادة أساس و(0.3) مول/لتر من المحلول المنظم (Phosphate buffer) عند أس هيدروجيني (6.5) ودرجة حرارة تحضين(40) °م بفترة زمنية مقدارها (14) دقيقة.وباستخدام رسم لاين ويفر– برك تم ايجاد قيمة السرعة القصوى (V_{max}) وثابت مكيلس (K_m) والتي كانت مساوية لـ 1.56 مايكرومول/دقيقة و1.1 ملي مول/لتر على التوالي. كذلك تمت دراسة تأثير عدد من المثبطات على فعالية الإنزيم ودراسة تأثير عدد من الأدوية على فعالية الإنزيم وبينت النتائج أن كبريتات النحاس والباراسيتامول كانا من نوع التثبيط غير التنافسي وخلات الخارصين كان من نوع التثبيط التنافسي.

الكلمات الدالة: تنقية، كلوتاثايون بيروكسيديز، العقم، المثبطات.