Effect of Antibiotics, Anesthetics, Sugars and Saltson The Oxidation Activity of Human Ceruloplasmin in *vitro*

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ABSTRACT

The optimum conditions for Ceruloplasmin (CP) activity have been measured in serum samples of a healthy individual without any detectable diseases. CP activity was evaluated by measurement of its p-phenylenediamine oxidase activity. This study showed the optimum pHforCP activity of a healthy human serum was 6.0 and the optimal temperature for CP activity was 45°C using *in vitro* conditions. The maximum value of oxidation activity was in incubation period70 min using optimal substrate concentration 60 mmol/l.

Several types of antibiotics were experimented in order to detect their effect on ceruloplasmin oxidation activity; these are consisted of Cephalexin, Amoxicillin, Erythromycin, Ampicillin, Ampicillin & Cloxacillin, Streptomycin Sulfate, Chloramphenicol, Cefrtriaxone, Benzathine, Pencilline and Aspegic. Results showed that Ampicillin and Streptomycin Sulfate had maximum influence on ceruloplasmin activity. Different types of sugars, salts and anesthetics were used to evaluate their effect on p-phenylenediamine oxidation by serum ceruloplasmin. Results appeared that starch, $FeSo_4$ and lidocaine 4% were activator for oxidation process. This study aimed to measure the optimal conditions of ceruloplasmin activity and to evaluate effect of different antibiotics and anesthetics onproteins from the serum of a healthy individual using *in vitro* conditions.

Keywords: Ceruloplasmin, p-phenylenediamine, Antibiotics, Anesthetics, Oxidase activity.

INTRODUCTION:

Ceruloplasmin (Cp) is one of the major copper binding proteins of serum and it has appeared that one of its roles is to act as a ferroxidase enzyme by oxidation of Fe^{2+} to Fe^{3+} for the efficient uptake of iron by transferrin. Further support for the function of Cp in iron homeostasis as an indicator in patients with hereditary Cp deficiency who have a high iron deposition in brain and liver. The primary site of Cp production is the liver where it is synthesized as an apoprotein Copper and then incorporated into the newly synthesized ceruloplasmin, if copper impaired to incorporate into the newly synthesized ceruloplasmin as a result accompanied by a failure to excrete copper ions into the bile leading to copper accumulation in toxic amount in the liver, brain and kidney. The process of CP deficiency in patients refers to Wilson's disease [1].

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Ceruloplasmin is β2 glycoprotein has a 132-kDa that is synthesized and secreted by hepatocytes as a holoprotein. It is a member of a multicopper oxidase group contains more than 95% of the copper ions in serum [2].
Ceruloplasmin level estimated according to the oxidation of p-phenylenediamine dihydrochloride C_6H_4 $(NH_2)_2$.2HCl substrate and the color intensity determined at 530 nm [3].
Wilson's disease is an autosomal disorder of copper overload with impaired biliary excretion of copper which led to hepatic disease in young adults and children. Deficiency leads to copper accumulation in the liver and other tissues. It is considered one of the few fatal diseases in the liver and central nervous system for which a specific therapy and a diagnosis are available. It is usually based on laboratory and clinical findings. Low level of serum ceruloplasmin, serum copper and increased copper excretion in urine are characteristic biochemical findings of the disorder [4].
Aceruloplasminemia is unlike to Wilson's disease, the hepatic architecture is normal and no abnormal copper accumulation, it suggests as a clinical disorder and represents a disruption of iron homeostasis due to the mutation of the ceruloplasmin gene and loss the ferroxidase activity of ceruloplasmin [2]. It is a neurodegenerative disease type result from the inherited loss in the function mutations of the ceruloplasmins gene, this disorder revealed the physiological role for ceruloplasmin in determination the iron efflux rate from cells with iron stores and it has provided new insights of human irons metabolism [5].Ferroxidase activity inhibited by adding of sodium azide, absorbance deficiency led to the rate of the negative control sample [6].
Ceruloplasmin executes its ferroxidase activity at the cell surface by interaction of irons and transferrin and this is the first step in the conversion of Fe^{2+} to Fe^{3+}. Previous experiments appeared that serum CP levels increased during sport, estrogen supplement and pregnancy, as well as in other cases such as malignities, infections, cholangitis and Hodgkin's disease. On the other side a decrease in the CP level was recorded in the cases of malnutrition and malabsorption states, primary biliary cirrhosis and nephrotic syndrome [7, 8].

Materials and Methods:

Reagents:

a. Acetic acid solution, 0.2M.
b. Sodium acetate solution, 0.2M.
c. Acetate buffer solution, 0.1M,pH 5.5 at 37°C.
d. Sodium azide solution, 1.5 M.
e. PPD buffer solution,27.6 mM.

Procedure:

Ceruloplasmin activity was estimated according to Sunderman and Nomoto method [9] with some modification.
a) Two test tubes were taken and labeled with R (Reaction tube) and B (Blank tube),then 2ml of acetate buffer solution were transferred into each tube.
b) 0.1 ml of serum was added to each tube.
c) 1 ml of warmed PPD buffer was added into both tubes and kept in water bath at 37°C.
d) 50µl of sodium azide were added to tubes B, R after 5,30 min respectively.
e) The absorbances of samples were measured at 530nm.
Ceruloplasmin (g/l) = $0.752(A_R-A_B)$
A_R represented the absorbance of sample R, A_B represented the absorbance of sample B.

Estimation the optimal conditionsof ceruloplasmin activity:

Different pH values of Acetate buffer solutions were used in order to estimate the optimum pH of CP activity andIn order to determine the optimal incubation temperature of CP activity,serum and substrate were incubated in a water bath at different temperature values.
Serum and substrate were incubated in a water bath at different incubation time to determine the optimal incubation period of CP activity.
PPD substrate solutions were prepared at different concentrations ranged between (20-70 mmol/l), to estimate the optimal substrate concentration for the oxidation reaction of serum ceruloplasmin.
The effect of local drugs was assessed by using different types of antibiotic comprised of (Cephalexin, Amoxicillin, Erythromycin, Ampicillin, Ampicillin & Cloxacillin, Streptomycin Sulfate, Chloramphenicol, Cefrtriaxone, Benzathine Pencilline and Aspegic) at a rate of 0.3 mg to each1ml of the PPD solution and the effect of local lidocaines and valipams were assessed at a concentration 0.3 mg/ml of the PPD solution.
Different types of sugars and salts were experiments in order to estimate the more suitable activating factors for CP activity, which were prepared in a concentration 0.3 mg/ml of PPD solution.

Results and Discussion:

Different values of pH were examined to estimate the optimum pH for ceruloplasmin activity using *in vitro* conditions ,the pH of the reaction was measured at 37°C, results in figure (1) illustrates the optimum pH which was 6.0 for PPD oxidation by serum ceruloplasmin and gave PPD oxidase activity ratio 35 %. The previous study appeared the optimum PH for ceruloplasmin activity in the human source was 5.45 [9] .Other study found the maximum enzyme activity at pH 5.4 in partially purified ceruloplasmin [10] .The decrease in CP activity at low pH may be referred to the effect of the pH environment of reaction on the ionic state of enzyme active site or changing the ionic strength of substrate or enzyme -substrate complex at substrate concentration above than K_m [11].
Serumwas incubated with p-phenylenediamine substrate at different temperature values using *in vitro* conditions. The results showed that 45°C was more suitable for CP action and the ratio of ceruloplasmin activity reached 51% as in figure (2). The previous study showed that ceruloplasmin activity increases according to incubation temperature until it reached maximum at 35°C [10].

The oxidation reaction of PPD by ceruloplasmin rised with the increasing of incubation period, results in figure (3) showed that 70 min period after addition the substrate was the optimal time for ceruloplasmin activity and gave PPD oxidase activity ratio 94 %. Previous research found the optimum incubation period for ceruloplasmin activity was 30 min using *in vitro* conditions [9].

In order to detect the best substrate concentration for ceruloplasmin activity, different concentrations of PPD were examined; results in figure (4) showed that the optimal substrate concentration was 60 mmol/l, ceruloplasmin activity ratio reached 98%.The previous study showed the optimal PPD concentration was 40mmol/l in iron deficient rats [12].

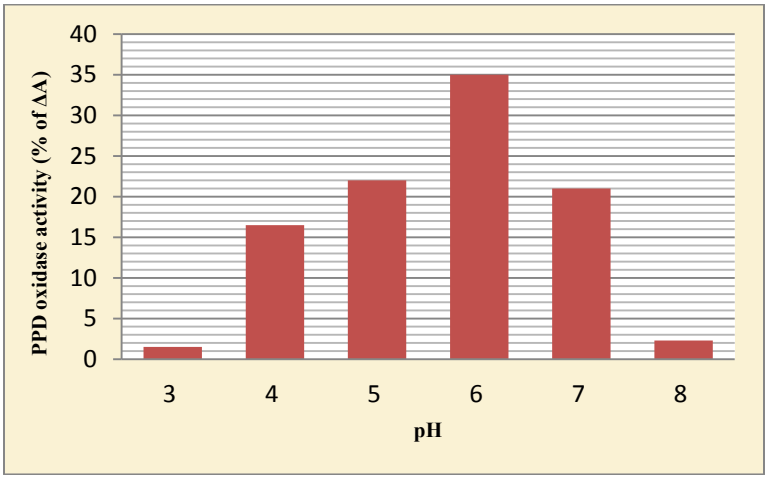
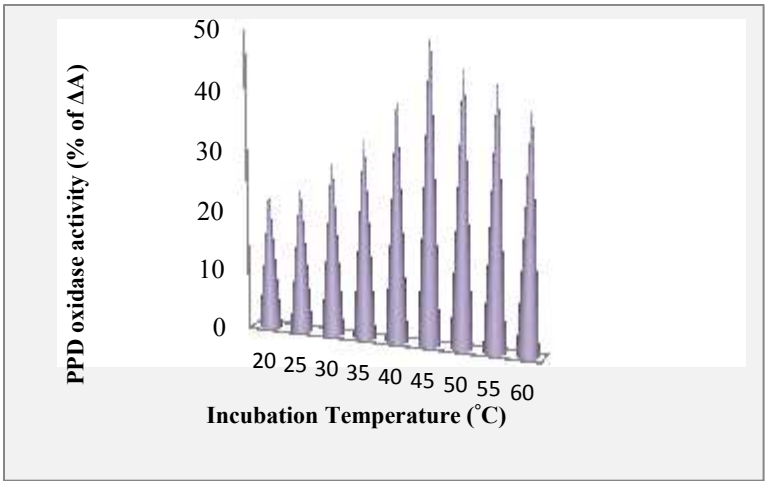


Figure (1): The optimal pH for CP activity.



Figure(2):The optimal incubation temperature for CP activity.

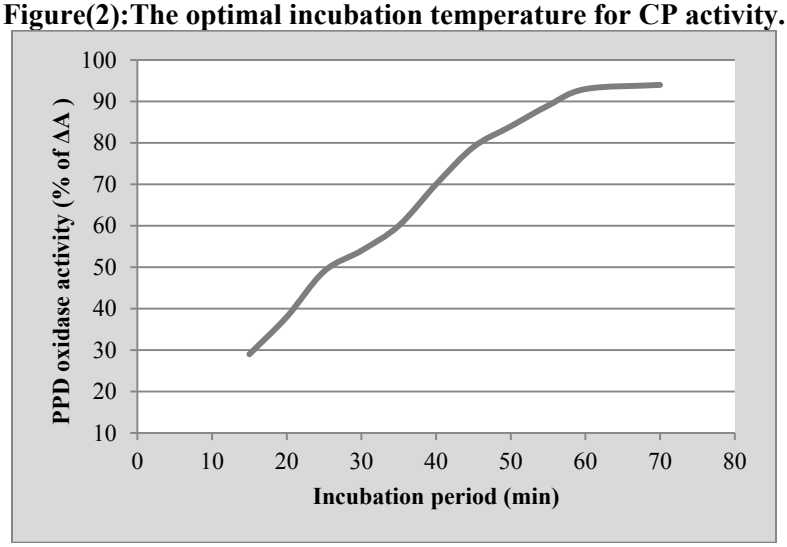


Figure (3):The optimal incubation period for CP activity.

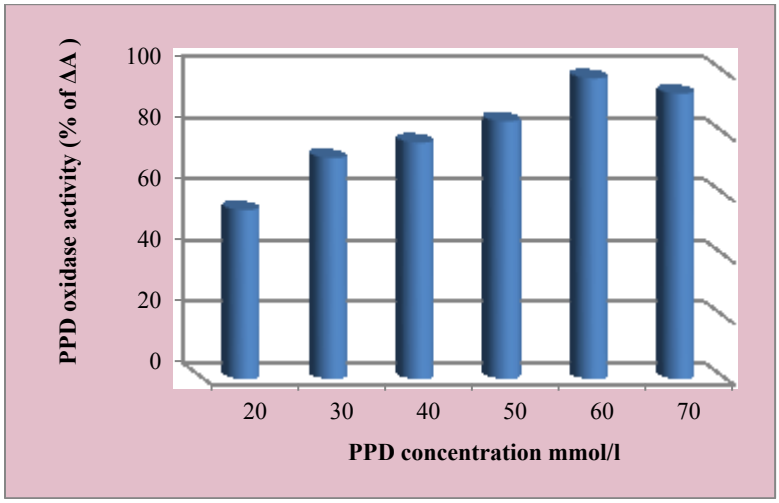


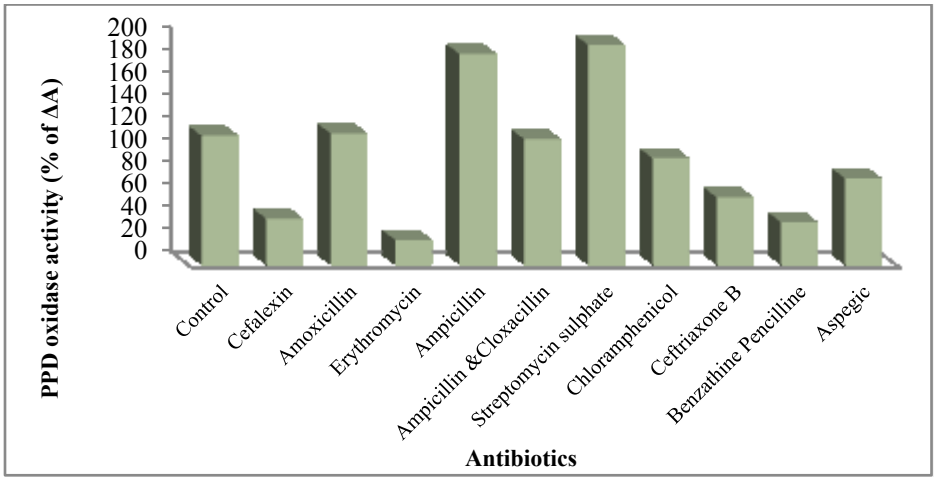
Figure (4): The optimal PPD concentration for CP activity.

Different types of locally available antibiotics were tested to study their effects on ceruloplasmin activity *in vitro* conditions ,results in figure(5) showed an enhancement in CP activity in the presence of Ampicillin and Streptomycin sulfate and gave activities ratios188 and 196% respectively. Other researchers studied The effect of protein binding in serum of different eight cephem antibiotics which were (ceftazidime, ceftizoxime, cefotiam, cefmetazole, cefpiramide, cefazolin, cefuzonam and ceftriaxone) on their therapeutic effects were examined in mice with experimentally induced pneumonia or intraperitoneal infections [13]. Previous studies had shown when lactoferrin (LF) and myeloperoxidase (MPO) were added to ceruloplasmin (CP) lead to a CP-LF-MPO triple complex forms, these complexes were used for patients with pleurisies of various etiology [14]. Lidocaines and valipams were selected to study their ability to enhance ceruloplasmin activity *in vitro* condition. These are consisted of lidocaine 2% lidocaine 4% and valipams as shown in figure (6), the highest CP activity appeared in lidocaine 4%, PPD oxidase activity ratio reached to 139%.

Different additional sugar sources were tested for their capability to enhance CP activity, these sources consisted of glucose, maltose, sucrose and starch .Results in figure (7) showed that starch was more active to enhance oxidation reaction of ceruloplasmin using *in vitro* conditions, PPD oxidase activity ratio reached to134%.

In order to study the effect of salts on CP oxidative activity, various types of salts were used which consisted of NaCl,$FeSo_4$,$MgSo_4$,$MnSo_4$ and $CuSo_4$. Results in figure (8) showed that $FeSo_4$ was more suitable for CP activity than other salts, PPD oxidase activity ratio was 142 %.

The concentrations of copper, silver, zinc and iron are relatively active for genes of copper transporting protein and copper enzyme which were measured in the cerebellum, cortex, amygdala, hippocampus, hypothalamus and pituitary gland. These changes were accompanied by a low concentration of copper and high level of iron and zinc contents. The activity of genes for copper transport enzymes which were formed in the pathway of intracellular secretory did not decrease in rats brain with copper deficiency [15].The gram quantities of different copper salts have been taken in many attempts and appeared copper toxicity in human as a result of redox cycling and generation of reactive oxygen which damage DNA [16] .Moreover the corresponding values of copper salts were 30 mg/kg of body weight which was toxic to animals [17].



Figure(5):Antibiotics effect on ceruloplasmin activity.

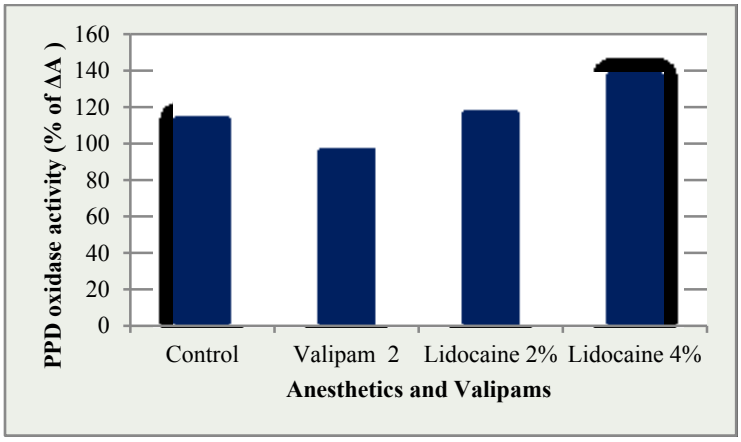


Figure (6): Effect of anesthetics and valipams on the CP activity.

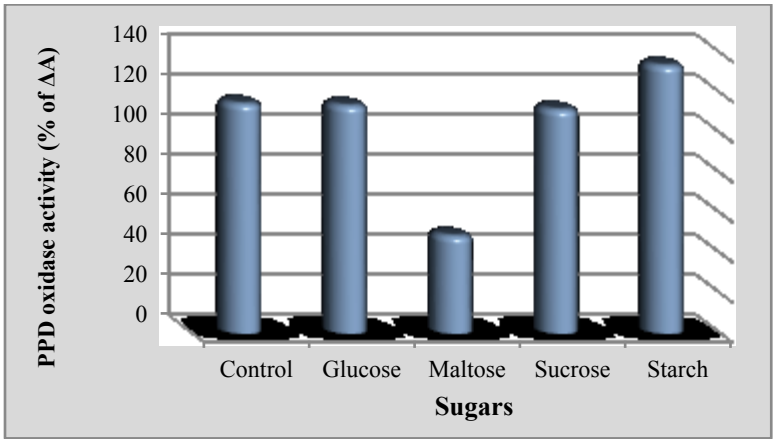


Figure (7): Effect of additional sugar sources on CP activity.

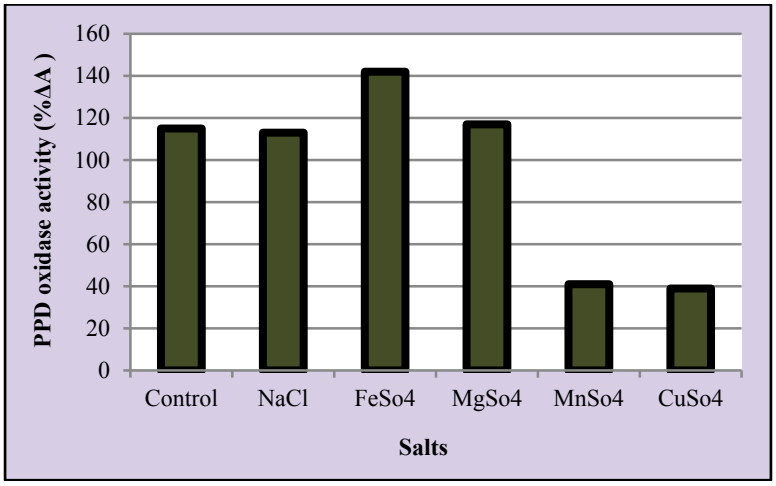


Figure (8): Effect of additional salts on CP activity.

CONCLUSIONS:

The method describedhere uses Spectrophotometer apparatus to determine Cp oxidation activity from the serum of normal person without detectable Wilson's disease or ceruloplasminea. It was achieved by studyingthereactionof serum CP proteins with PPD as a specific substrate, we demonstrated that we can measure CP concentration and oxidase activity.

In this study,we had tended to determine the optimal conditions of ceruloplasmin activity.The optimal pH was 6.0 that mean the CP reaction prefers low acidic condition to achieve oxidation process, as well as the optimal temperature was 45°C this indicated that CP activity increases with increasing the body temperature more than normal value 37°C.

Ceruloplasmin activity increased when rising incubation period and substrate concentration, also we had studied that Ampicillin and streptomycin sulfate as antibiotics could beused to treat or prevent Wilson's disease by increasing CP activity, lidocaines effects on CP activity and can be used to prevent Wilsons disease by increasing oxidation activity in human serum. The effects of different sugars and salts were evaluated to determine their roles in CP oxidation reaction from serum of a healthy individual using *in vitro* conditions.

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