Activity of Peroxidase in Free Radicals, Human Blood Cells and Effect of the pH and Temperature on Peroxidase Activity and Stability

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Abstract

The previously studies of enzymes were focused only on the extraction and purification of enzyme and increase the toxic effect of drugs and supplement of free radical scavenging. So, the aim of the study was to extend purified peroxidase from *Pseudomonas aeruginosa* application and to abundant new enzymatic drug of free radicals scavenging with low side effect. Peroxidase is categorized as oxido-reductases, which were used for catalyzing various oxidative reactions that protect cells from the harm effect of free radicals .Peroxidase was tested as antioxidant and test their toxicity on human cells to overcome the problem of the side effect of drug and supplement that used as an antioxidant. The enzyme was applied as H_2O_2 scavenging activity antioxidant by used three concentration of enzyme and triplicate (10, 40, 60 µg/ml). The enzyme was show higher activity at 60 µg/ml reached to 40% activity. The cytotoxicity of peroxidase was tested on whole healthy human blood by C.B.C by using equal amount 5µg of (enzyme substrate, only substrate and enzyme without substrate). The result showed that the enzyme only had no effect on human blood as compare with H_2O_2 and enzyme with H_2O_2. The characterization of enzyme was done by using more than one temperature and pH value and the result were shown that the optimal temperature for enzyme activity and stability rang (40-45°***C***) and the optimal pH for enzyme stability and activity were 7.

Keywords: Antioxidant, Activity, Characterization, Cytotoxicity, Peroxidase, Stability.

1- Introduction

An enzyme is a type of protein found within a cell, they actually speed up the rate of a chemical reaction to help support life, the enzymes in body perform very important tasks include building muscle, destroying toxins and breaking down food particles during digestion, The enzymes became more important products for human need to increased profit in many industrial such as food and drug industrials because of their source and their price [1]. The enzyme is protein and consists of amino acid chains so they contain functional group and any improper pH or temperature in vitro can cause denaturation of protein or enzyme because of ionic state and lose their function [2]. To overcome this problem can test more than one pH and temperature value for the enzyme to reach the optimal pH and temperature that keep the function and structure of protein or enzyme[3].Free radicals are atoms or groups of atoms (unpaired) can be formed when oxygen interacts with certain molecules, Once formed these highly reactive radicals can start a chain reaction, Their chief danger comes from the damage they can do when they react with important cellular components such as DNA, or the cell membrane , to prevent free radical damage the body has a defense system of antioxidants such as vitamins and enzymes but the body cannot able to synthesis essential vitamin beside the toxic effect of drug and supplement such as interfering with anticancer drug and reduce the effect of drug, ulcer, nausea and cost [4]. To overcome this problem using Peroxidase enzyme which is naturally occur, cheap, less effect on body and categorized to oxidoreductase kind of enzymes and usually catalyzes reaction between H_2O_2 as electron acceptor and many kinds of substrates by resources of O_2 release from H_2O_2, Hurt molecules such as super oxide and hydroxide radicals can be occurred in cells because of the presence of oxygen, Peroxidase is an enzyme which has iron that catalyzes the transport of oxygen from hydrogen peroxidase to an appropriate substrate and thus takes about oxidation of the substrate [5]. In our study, we reached to synthetically and cheap peroxidase with antioxidant scavenging activity and low side effect and long term storage without loss their function or structure.

2-Materials and Methods

2-1 Peroxidase H_2O_2 scavenging activity

The free radical scavenging activity of the peroxidase enzyme was evaluated by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH method) according to Shin [6]. In Briefly, an 0.1mM solution of DPPH in methanol was prepared and 1mL of this solution was added to at different enzyme concentration [20,40,60, µg/mL , Ascorbic acid (control)].The mixtures were shaken vigorously and allowed to stand at room temperature for 30 minutes. Then the absorbance was measured at 517 nm using a UV-VIS spectrophotometer. Ascorbic acid was used as the control. Lower absorbance values of reaction mixture indicate higher free radical scavenging activity. The capability of scavenging the DPPH radical was calculated using the following formula DPPH scavenging effect (% inhibition) = (A0 –A1)/A0) x100

2-2 The toxic effect of peroxidase on blood cells using C.B.C

This study was designed to determine the relationship between the heamatological parameters [WBC,RBC, and Platlet] by automated hematology analyzer[C.B.C] with the manual methods, 8 subjects were randomly selected from both apparently healthy subjects (four from male and four from female) from the University of Technology. 5 mL of venous blood sample was collected aseptically from each subject into EDTA tubes for the analysis and then 5µ from testing compounds [enzyme without, enzyme with H2O2, H2O2, only blood (control)] were additions to tubes followed by incubation for 1 minute [7].

2-3 Characterization of peroxidase enzyme

Optimal pH for peroxidase activity

To determine the optimum pH for the peroxidase activity, sodium acetate C2H3NaO2 was prepared with a concentration of 0.1 M and pH ranged between (4.5-5) , Phosphate buffer saline with 0.1 M concentration and pH ranged from (6-7.5) and Tris-HCl 0.1 M concentration and pH ranged between (8-9). Then equal volumes of these buffers were mixed with the solution of the hydrogen peroxide at 0.1 M concentrations was 1: 1. After that, 0.1 ml of the purified enzyme was added to 0.9 ml substrate. Solution of substrate with pH values, then plotted the relationship between enzymatic activity and pH to determine the optimal pH of enzyme activity [8].

2-4 Optimal temperature for peroxidase activity

To determine the optimum temperature for peroxidase activity, 0.9 mL of the substrate solution with 0.1M concentration was added to 0.1 mL of purified enzyme solution and then incubated for one minute in a water bath at different temperatures (30, 35, 40, 45, 50, 55, 60, 65, and 70 ° C) and then determined the enzyme activity for each temperature .Then plotted the relationship between enzymatic activity and temperature to determine the optimal temperature for enzyme activity [8].

2-5 Optimal pH for peroxidase stability

To determine the optimal pH for enzyme stability, equal volumes of pure enzyme (0.4) ml were mixed with each buffer with pH rang between (from 3 to 9) at 0.1M concentration and substrate. The solutions were incubated in a water bath at 37 ° C for one minute and then piped to ice bath. The absorption was then measured with the optical spectrometer at a wavelength of 625 nanometers, calculated and then the relationship was plotted Percentage of residual activity and optimal pH for enzyme stability [8].

2-6 Optimal temperature for peroxidase stability

To determine the optimum temperature for enzyme stability, 0.5 ml of purified peroxidase was incubated in a water bath at different temperatures (10, 20, 30, 40, 50, 60, 70 ° C) for one minute. The enzyme-containing tubes were then transferred directly to an ice bath. Estimation of residual activity, the relationship between temperature and percentage of residual activity was determined to determine the optimal temperature for enzyme stability [8].

3-Results and Discussion

3- 1 Peroxidase free radicals scavenging activity:-

When the solution of DPPH mixed with peroxidase enzyme that can donate hydrogen atom this can give rise to reduce form and decolorize red color of DPPH stain to yellow and measurement the absorbance at517 nm**.** The percentage of scavenging activity was 20% at peroxidase concentration 10 µ/mL, while 30% of scavenging activity at 40 µ/mL of enzyme concentration, the higher scavenging activity recorded at 40% in 60 µ/ml of enzyme concentration and compare the result with ascorbic acid, the result was agreed with [9].Shown in the figure 1 and figure 2.

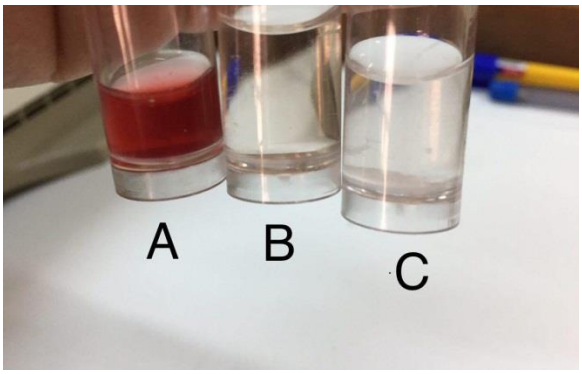

Figure 1 A: DPPH stain, B: DPPH stain with enzymes, C: DPPH stain with ascorbic acid (control)

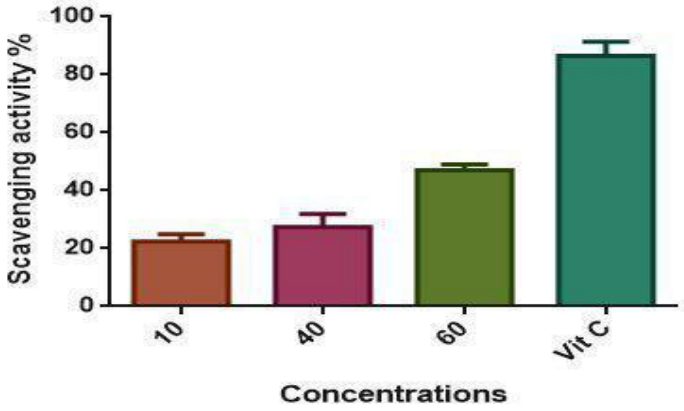

Figure 2 The relationship between enzyme concentration and scavenging activity

3-2 The totoxicity effect of peroxidase on blood cells using C.B.C

The result show higher hemolysis of W.B.C and R.B.C and platelet by enzyme with H_2O_2 (orange color) (2cells/mm^3 of RBC, 4.5cells/mm^3 of WBC and 40 cells/mm^3 of PLT) and show less effect with only H_2O_2 (blue color) (3 cells/mm^3 of RBC, 7 cells/mm^3, and 70^3 cells/mm^3 of PLT), while show very slightly effect when used enzyme without H_2O_2 (red color)(53 cells/mm^3 of RBC,6cells/mm^3 of RBC and 1903 cells/mm^3 of PLT) and control the result with the control of 8 healthy persons, and the result agreed with [10]. Shown in the figure 3.

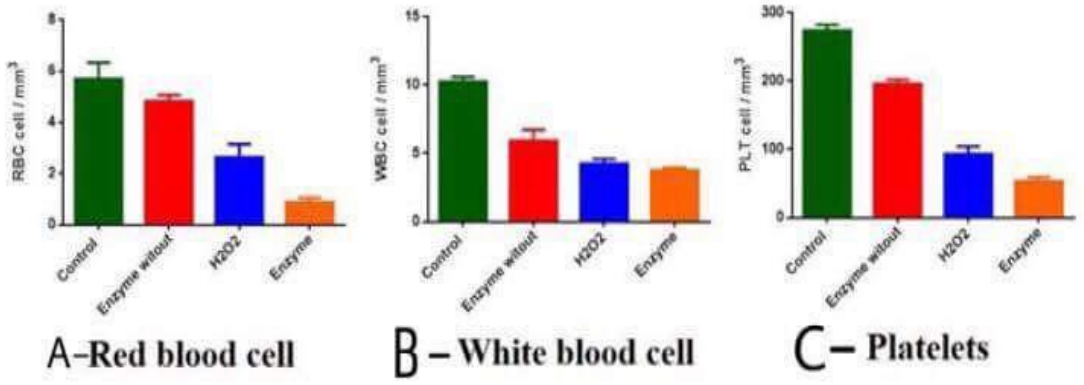

Figure 3 the cytotoxicity effect of enzyme on human blood components, A-Red Blood Cells, B-White Blood Cells and C-Platelets.

3-3 Optimal pH for peroxidase activity

The activity of enzyme is increases by increasing the pH value to reach maximum activity 1.15U/ml in pH=7, using H_2O_2 as a substrate of enzyme, then it began to decrease in a higher pH values. It observed that the peroxidase active in neutral pH and lowering of activity nearby acidic and alkaline pH .The differences in peroxidase activity with the different pH values return mainly to changes that occur an ionic state of enzyme molecule and substrate with another compound. The results come closer to [11] when they found that the optimum pH of peroxidase purified from *Broccoli* spp. was 6.That Shown in figure 4.

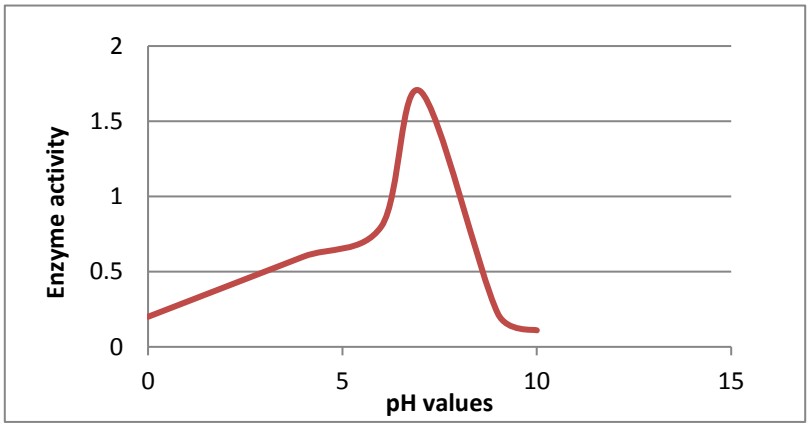

Figure 4 Effect of pH range (5-15) on the peroxidase activity

3-4 Optimal temperature for peroxidase activity

The results shown in figure 5 the peroxidase activity is increase by increased the temperature when the remaining activity reached to maximum 100% in 45˚C, then it begins to decline with increasing temperature until remaining activity reached to 9% in 70˚C. This study was agreed with [12], when he has shown the highest activity of peroxidase purified from *litchi pericarp* was in 40˚C for 10 minutes, after this temperature the activity of peroxidase started to reduce until reach to 58.8 and 76.6% at 60˚C and 70˚C respectively. Shown in the figure 5

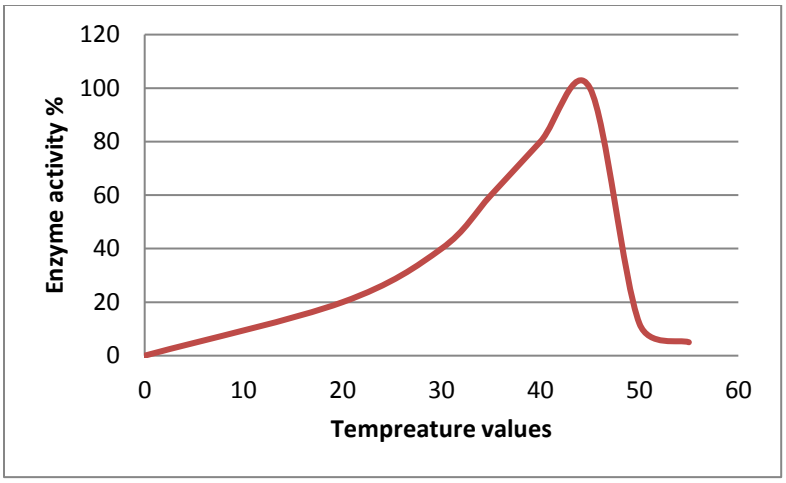

Figure 5 Effect of different temperature (30- 70) ˚C on peroxidase activity

3-5 Optimal pH for peroxidase stability

The figure 6 shows the optimum pH of stability ranging between (7-7.5) and this stability was decreased in extreme acidic and alkaline medium. The enzyme was kept 87% of its activity in pH 6.5 while kept75% of activity in pH 7, the activity was declining in pH 4.5 and 9 to 54% and 51% respectively. This agreed with [13], that showed the optimum pH of peroxidase stability purified from *Helianthus tuberosus* ranging between (5.0- 6.0).

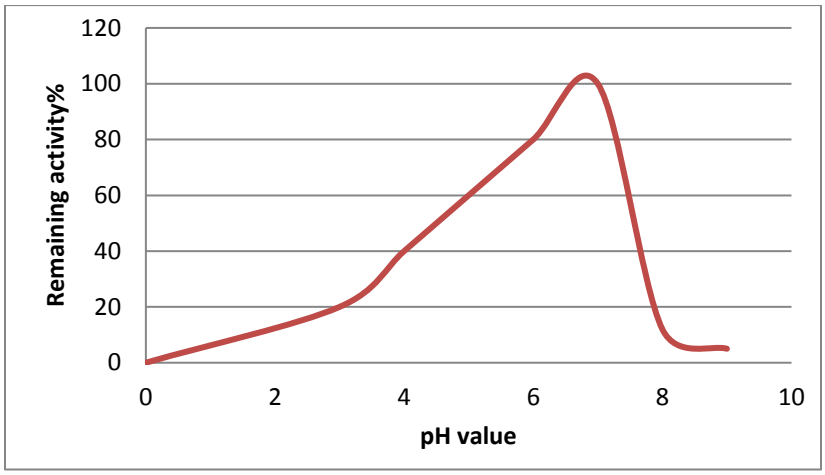

Figure 6 pH ranges (4-10) for peroxidase stability

3-6 Optimal temperature for peroxidase stability

Figure 7 shown the results of incubation enzyme with different temperature ranging between (30-70 ˚C) for 15minutes, the enzyme was maintained the activity when it incubated into (30-40) ˚C. While keeping 95% of its activity in temperature 45˚C, while its keep only 43% in 70˚C. These results were close to [14].

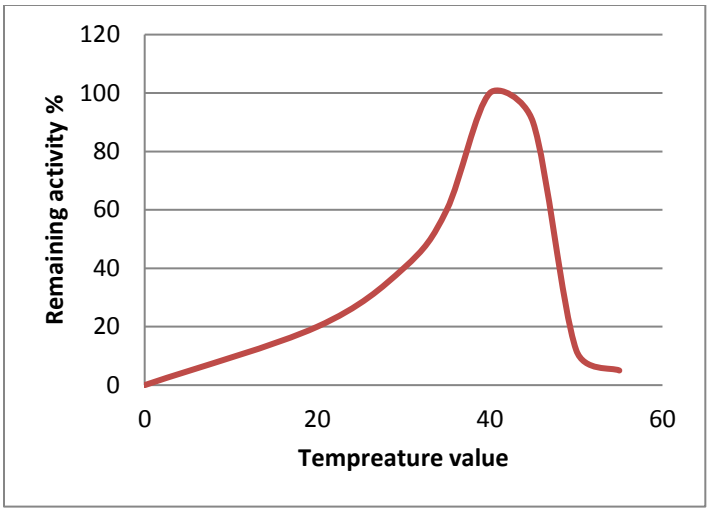

Figure7 The optimum temperature (20-60 ˚C) of peroxidase stability

4- Conclusion

The peroxidase enzyme show only slightly affected on human blood components compared with hydrogen peroxide as well as the enzyme with hydrogen peroxide together. The peroxidase enzyme was significantly suppressed free-radical, which was confirmed by DPPH dye and the results were compared with vitamin C. The optimum temperature for enzyme activity was 45 ° C and the optimum temperature for enzyme stability was 40 ° C .The optimum pH for enzyme activity was 7 and the optimal pH for enzyme stability was also 7.

CONFLICT OF INTERESTS.

- There are no conflicts of interest.

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فعالية انزيم البيروكسيديز في كبح الجذور الحرة، خلايا دم الانسان وتاثير الاس الهيدروجيني ودرجة الحرارة في فعالية واستقرار الانزيم

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الخلاصة

تركزت الابحاث السابقة للانزيمات على استخلاص وتنقية الانزيمات والمؤثرات الجانبية لاستخدام الادوية والمكملات الغذائية الكابحة للجذور الحرة. لذلك، الهدف من الدراسة هو توسيع تطبيقات إنزيم البيروكسيديز المنقى من الزوائف الزنجارية والقدرة على اكتشاف علاج انزيمي متوفر ورخيص الثمن وقليل التاثيرات الجانبيه. انزيم البيروكسيديز من الانزيمات المؤكسدة المختزلة والتي تعمل على تحفيز العديد من تفاعلات الاكسدة لتخليص الخلايا من خطر الجذور الحرة. اختبر البيروكسيديز في قدرته على كبح الجذور وسميته على خلايا الدم للإنسان لحل مشكلة الادوية والمكملات الغذائية. لذا استخدمت ثلاث تراكيز لانزيم البيروكسيديز (60,40,10 مايكروغرام/مل). حيث اظهر البيروكسيديز فعالية عالية في كبح الجذور الحرة عند تركيز (60 مايكروغرام/مل) وصلت الى 40%.درس تاثير الخلوي السام للبيروكسيديز على جميع خلايا الدم للانسان باستخدام جهاز عد الخلايا وباستخدام كميات متساوية من (الانزيم،المادة الاساس،الانزيم مع المادة الاساس) حيث لم يظهر الانزيم بدون المادة الاساس سوى تغيرا طفيفا باعداد الخلايا مقارنة بالمادة الاساس والمادة الاساس والانزيم. توصيف انزيم البيروكسيديز أجرى باستخدام أكثر من درجة حرارة واحدة وأكثر من اس هيدروجيني واحد حيث اظهرت النتائج ان أفضل اس هيدروجيني لفعالية الانزيم وثباتيته كانت عند اس هيدروجيني 7 وافضل درجة حرارة عند (40–45) درجة مئوية.

الكلمات الدالة:– مضاد للاكسدة، الفعالية، توصيف، الخلايا السمية، بيروكسيديز، ثباتية.