

Serum Antioxidant Enzymes and Purification of Blood Cells Cu-Zn Superoxide Dismutase in Chronic Childhood Diarrhea

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ABSTRACT:

This study included two parts: the firstly, measurements the antioxidant enzymes activities [Superoxide Dismutase (SOD), Catalase (CAT), Reduce Glutathione (GSH) and Glutathione –S- Transferase (GST)] and lipid peroxidation product (LPO) levels in sera of children healthy[control(n=35),male=16,females=15] and children with chronic diarrhea less than two years[patients (n=41), male=21,females=20].The results revealed that highly significant increasing in the SOD activity and LPO in sera of children with chronic diarrhea ($p<0.01$) in comparison with that of controls subjects. The results show depletion in the CAT, GSH and GST activities in sera of children with chronic diarrhea ($p<0.01$) to compared with control group. This increase and decrease in this enzyme in male more than female. The correlation between the level of GSH and SOD, GST.MDA. The results show highly negative correlation (significant) between GSH and (SOD, MDA) while weak positive correlation (no significant) between GSH and (GST, CAT).

The secondly part from this study included the purification of erythrocytes Cu-Zn superoxide dismutase from control and children with chronic diarrhea by ion exchange chromatography, on a column (7×0.7 cm) of DEAE-Sepharose-CL-6B. The purification of SOD had a specific activity of 2956 U/mg protein(control) and 6502.6 U/mg protein for patients, and gave a single band on polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS) and each of its to subunit has a molecular weight about[(18500 Daltons, control) , (18300 Daltons, children with chronic diarrhea)].

الخلاصة:

هذه الدراسة تضمنت جزئيين : الأول قياس فعاليات الأنزيمات المضادة للأكسدة

[superoxide dismutase (SOD), reduce glutathione (GSH), catalase (CAT) وفعاليتها بيروكسيد الدهون(LPO) في مصول كل من الأطفال الأصحاء(n = 35, ذكور=16, إناث = 15) ومرضى الإسهال المزمن (أطفال اقل من سنتين, n = 41, ذكور=21, إناث = 20). النتائج أظهرت ارتفاع معنوي في فعالية إنزيم ال SOD و مستوى LPO لمرضى الإسهال المزمن ($P<0.01$) مقارنة مع مصول الأطفال الأصحاء. كذلك تم قياس فعاليات كل من الإنزيمات CAT, GSH , GST في نفس المجاميع وأظهرت النتائج انخفاض معنوي ($P<0.01$) لمرضى الإسهال المزمن مقارنة مع الأطفال الأصحاء وان الارتفاع والانخفاض في هذه الإنزيمات في الذكور أكثر مما في الإناث. العلاقة بين مستوى أنزيم GSH وSOD, MDA, GST, CAT. النتائج أظهرت وجود علاقة عالية المعنوية(سالبة).

بين GSH و (SOD, MDA) بينما علاقة غير معنوية(موجبة) بين GSH و(GST,CAT) الجزء الثاني من هذه الدراسة تضمن تنقية إنزيم Cu-Zn superoxide dismutase من الأطفال الأصحاء ومرضى الإسهال المزمن بواسطة تقنية كروماتوغرافية التبادل الأيوني على عمود أبعاده (7×0.7) سم وهلام. DEAE-Sepharose-CL-6B إنزيم ال SOD المنقى من الأطفال الأصحاء ومرضى الإسهال المزمن يملك فعالية نوعية مقدارها (6502.6 u / mg) و (2956 u / mg) على التوالي. وكل منهما أعطى حزمة مفردة بواسطة الترحيل الكهربائي بوجود ال SDS وكل وحدة ثانوية تملك وزن جزيئي مقدارها [سيطرة (دالتون) 18500) ومرضى الإسهال المزمن(دالتون) 18300].

INTRODUCTION:

Diarrhea is defined as an increase in stool weight (>200 gm/day) that may be associated with increased liquidity, stool frequency, perianal discomfort and urgency, with or without fecal incontinence. The following abnormalities may lead to diarrhea : (1) a decrease in the normal absorption of solute in water; (2) increase secretion of electrolytes obligating water to the intestinal lumen; (3) the presence of poorly absorbed, osmotically active solutes in the gut lumen; (4) increase intestinal motility; and (5) inflammatory disease producing blood, pus, or mucus⁽¹⁾.

Chronic diarrhea can be subdivided into multiple categories: (a) Secretory diarrhea, usually caused by drugs, hormones, bile acids, or fatty acids. (b) Osmotic diarrhea, generally caused by drugs, laxatives, or malabsorption. (c) Inflammatory diarrhea due to ischemic colitis, parasitic infection such as that caused by amebae, or inflammatory bowel disease (IBD) (ulcerative colitis, Crohn's disease). (d)

Motility disorder such as irritable bowel syndrome, progressive systemic sclerosis and the autonomic neuropathy of diabetes. (e) Disorders of lost absorptive surface, as is seen in postsurgical diarrhea syndromes.

Antioxidants are agents that scavenge reactive oxygen species (ROS), prevent their formation, or repair the damage they cause⁽²⁾. This complex system consists of antioxidant enzymes (Superoxide Dismutase (SOD, convert O_2^- to form O_2 and H_2O_2), Catalase [(CAT), convert H_2O_2 to O_2), Reduced Glutathione (GSH), Glutathione γ -S-transferase (GST), etc.^(2,3,4)), the ROS term is often used to refer to free radicals and other oxygen-related reactive compounds, such as singlet oxygen, hydrogen peroxide (H_2O_2) and hydroperoxides (ROOH)⁽⁵⁾.

The main intracellular source of ROS in the mitochondrial respiratory chain, also many exogenous agents, such as hyperoxia, ozone, mineral dusts and ionizing radiation^(6,7). ROS are formed during normal cellular metabolism such as: oxidative phosphorylation, the mitochondrial cytochrome oxidase enzyme system, the oxidation-reduction reactions (such as xanthine oxidase, aldehyde oxidase, peroxidases and others), and Fenton reaction in which an interaction between hydrogen peroxide and superoxide anion to form hydroxyl radical occurs⁽⁸⁾. The antioxidant functions are suspected to favor the neoplastic growth of initiated cells⁽⁹⁾.

Lipid peroxidation products and ROS have been found very active in binding to DNA, to cause mutations and to initiate cancer⁽¹⁰⁾. They are also involved in the mechanisms of myocardial ischemia⁽¹¹⁾, inflammatory and rheumatic disease⁽¹²⁾ and some other diseases, e.g., diabetes⁽¹³⁾. The ROS will damage proteins, cause breakage of DNA strands and initiate lipid peroxidation. The lipid radical formed⁽⁸⁾ when contacts with transition metal ions will produce more radicals that are highly toxic aldehydes, which will cause more damage⁽¹⁴⁾. ROS require a transition metal such as iron for their formation, and it was reported that superoxide radical promotes the release of iron from ferritin^(15,16). Many studies have shown an elevated antioxidant capacity and a reduced potential for lipid peroxidation^(9,17). However, significant variation in antioxidant capacity was recorded to vary from one tumor type to another^(18,19,20).

The Aim of This Study:

1-The aim of our study is to evaluate the antioxidant enzymes (SOD, CAT, GSH and GST) and lipid peroxidation product (LPO) levels in sera of control and children with chronic diarrhea.

2-Study the relationship of oxidative stress associated with children with chronic diarrhea

3-Separation and purification of erythrocytes Cu-Zn superoxide dismutase from healthy children and children with chronic diarrhea.

MATERIALS AND METHODS:

1-Samples:

Two groups of samples were included in this study. Group (1) contained (41) patients children with chronic diarrhea less than two years (21 males,20 females).Group (2) consisted of (35) healthy children (control subjects) (16 males,15 females). Admitted to Babil children hospital in Hilla city ,over a period of about (7) months from March 2006 to September 2006.Serum used to assay antioxidant enzymes activities, while erythrocytes (packed cell) used to separate and purification of Cu-Zn superoxide dismutase enzyme.

2-Determination of Superoxide Dismutase(SOD) Activity by Riboflavin/NBT Method:

SOD enzyme activity was measured by the using modified Nitroblue tetrazolium-HCL (NBT) method described by Byer and Fridovich⁽²¹⁾,employing a photochemical generator of ($O_2^{\cdot-}$).

3-Determination the Activity of Serum Catalase (CAT):

CAT activity was determined by the decrease in absorbance due to H_2O_2 consumption⁽²²⁾.Take (2ml) from diluting serum (Dilute 50 μ l of serum with 5 ml phosphate buffer solution 50 mM,pH 7)and add (1ml) from H_2O_2 to sample tube ,the blank tube contain (1ml) from phosphate buffer and (2 ml) from diluting serum .Mix immediately and read the absorbance at (15) seconds and after(30) seconds at 240 nm.

4- Determination of the Serum Reduced Glutathione (GSH) :

According to the Burtis and Ashwood⁽²³⁾ the level of serum GSH was determined by using a modified procedure utilizing Ellman 's reagent (DTNB), to 100 μ l serum sample add the followings:800 μ l double distilled water (DDW) , 100 μ l trichloroacetic acid 50 %,mixed well by vortex for 10-15 minutes, and centrifuged for 15 minutes at $3000 \times g$, then take 400 μ l from supernatant, add 800 μ l Tris-EDTA buffer and 20 μ l DTNB reagent . Mixed well by vortex ,in the same way prepare blank (DDW) and standards tubes (0.0307) g of GSH in a final volume of 100 ml of 0.4 M EDTA solution).Read the absorbance of standard and sample within 5 minutes of the addition of DTNB at 412 nm.

5-Determination the Activity of Serum Glutathione -S- transferase(GST):

GST activity was analyzed by measuring the conjunction of glutathione (GSH) and 1-chloro-2,4-dinitrobenze(CDNB) as substrate⁽²⁴⁾. To (100 μ l)serum sample added the flowing:(2.7 ml) phosphate buffer pH 6.25 ,(100 μ l) CDNB solution and after (3) minutes add (100 μ l) glutathione solution (29.93)mM ,use another tube to the blank, then mix and read the absorbance after each (1)minutes for (10)minutes at (340)nm.

6-Determination of the Lipid Peroxidation Activity :

Malondialdehyde (MDA) indicative for lipid peroxidation was measured using Hirayama *et al* method⁽²⁵⁾.

7-Determination of Total Protein:

The protein content of all samples was determined by Bradford method⁽²⁶⁾using bovine serum albumin as standard protein for all samples.

8-Determination of Hemoglobin Concentration:

According to the Drabkin`s method by using hemoglobin kit.

9-Separation of Cu-Zn Superoxide dismutase from erythrocytes:

According to the Djalali *et al* method⁽²⁷⁾.

10-Ion Exchange Chromatography:

Ion exchange chromatography used to separate the erythrocytes Cu-Zn Superoxide dismutase from erythrocytes according to the Gartner *et al*⁽²⁸⁾ was performed on a column(7 \times 0.7 cm)of DEAE-Sepharose CL-6B.

11-Separation of Proteins by SDS-PAGE:

The separation of proteins was conducted by using SDS lab gel electrophoresis by using modification of Lammeli method⁽²⁹⁾. Gel were stained with 0.05 (w/v) Commassie Brilliant Blue R 250.

12-Molecular Weight Determination:

The molecular weight of purified Cu-Zn superoxide dismutase was determined by SDS-PAGE using standard protein (bovine serum albumin, ova albumin, lactate dehydrogenase and lysosyme).

13-Chemicals :

All laboratory chemical and reagent in this work were of analar grade and imported from BDH Co. and SIGMA Co. Nitrotetrazoliumblauschlorid (NBT)(Fluka) K_2HPO_4 , KH_2PO_4 , EDTA, L – Methionine, Sodium Syanid, CDNB Riboflavin, Na_2HPO_4 , Glutathione, Triton x -100 Phosphate buffer, H_2O_2 .

Statistical Analysis :

The results of SOD, CAT, GSH, GST, MDA and protein values were analyzed statically by values expressed as mean \pm standard deviation (SD). Student's t-test was used to estimate differences between the groups and differences were considered significant when the probability was high significant at ($p < 0.01$).

RESULTS:

A-Antioxidant Enzymes:

The mean values \pm SD of sera superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH) and glutathione -S- transferase (GST) activities and malondialdehyde (MDA) concentration in control and patients (children with chronic diarrhea) were measured in table (1, 2 and 3).

The results shown in table (1) revealed a highly significant increase ($p < 0.01$) in the SOD activity in sera children with chronic diarrhea in compared to the control group (1.7 fold, 79.68%). While highly significant decrease in CAT activity was found between children with chronic diarrhea and control ($p < 0.01$, 3 fold, 67.1%).

In table (2), the results revealed a significant decrease ($p < 0.01$) in sera of GSH and GST activities of children with chronic diarrhea group when compared with that of control group (3.7 fold, 73% and 2.2 fold, 54.6 %).

Levels of MDA in sera of children with chronic diarrhea were show highly significant increase when compared with control ($p < 0.01$, 2.8 fold, 64.9 %).

Table(1):Superoxide Dismutase (SOD) and Catalase (CAT)Activities in Sera of Controls and Children with Chronic Diarrhea (M = male ,F = females ,** significant (p < 0.0\)).

Groups	Sex	No.	Mean	±SD	Upper Value	Lower Value
<i>Control No. = 35</i>						
SOD (U/ml)	M	16	0.316	0.761	0.651	0.161
	F	15	0.315	0.861	0.591	0.134
<i>Children with Chronic Diarrhea No. = 41</i>						
SOD (U/ml)	M	21	0.541**	0.543	0.78	0.314
	F	20	0.541**	0.496	0.791	0.322
<i>Control No. = 35</i>						
CAT (K/ml)	M	16	0.443	0.861	0.642	0.296
	F	15	0.436	0.791	0.731	0.196
<i>Children with Chronic Diarrhea No. = 41</i>						
CAT (K/ml)	M	21	0.194**	0.65	0.34	0.961
	F	20	0.194**	0.543	0.31	0.678

Table(2):Reduced Glutathione (GSH)and Glutathione -S-transferase (GST) Activities in Sera of Healthy Controls and Children with Chronic Diarrhea (M = male , F = females , ** significant (p < 0.0\)).

Groups	Sex	No.	Mean	±SD	Upper Value	Lower Value
<i>Control No. = 35</i>						
GSH mM/L	M	16	28.67	4.61	29.61	26.87
	F	15	27.87	4.78	30.41	25.78
<i>Children with Chronic Diarrhea No. = 41</i>						
GSH mM/L	M	21	7.36**	3.46	8.64	6.79
	F	20	7.89**	3.26	8.75	6.86
<i>Control No. = 35</i>						
GST (U/L)	M	16	3.152	1.316	3.75	2.64
	F	15	3.025	0.967	3.65	2.66
<i>Children with Chronic Diarrhea No. = 41</i>						
GST (U/L)	M	21	1.471**	0.961	1.673	1.23
	F	20	1.362**	1.024	1.531	1.13

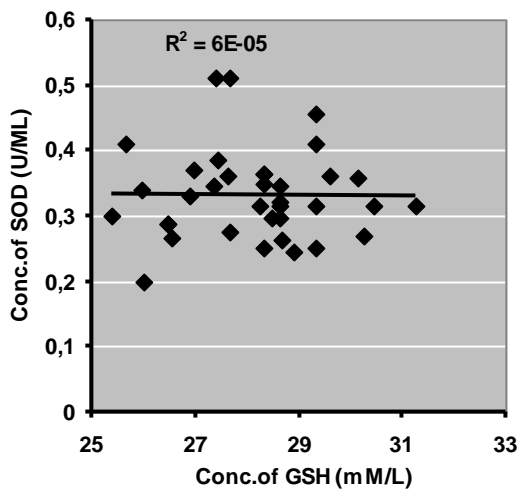
Table(3):Malondialdehyde (MDA) Concentration in Sera of Healthy Controls and Children with Chronic Diarrhea (M = male ,F = females , ** significant (p < 0.0\)).

Groups	Sex	No.	Protein g/dl	MDA nmol/mg	±SD	Upper Value	Lower Value
<i>Control No. = 35</i>	M	16	5.43	0.034	0.013	0.052	0.0296
	F	15	5.36	0.035	0.012	0.055	0.0289
<i>Children with Chronic Diarrhea No. = 41</i>	M	21	5.013	0.192**	0.014	0.167	0.096
	F	20	5.012	0.142**	0.013	0.177	0.089

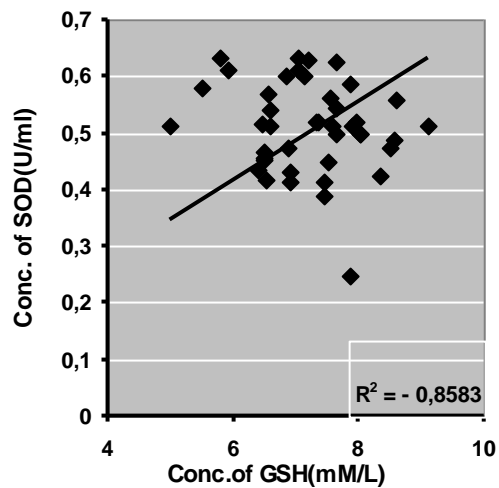
The correlation between the level of reduce glutathione and several biochemical markers determined in this study ,from result .its clear that present highly negative correlation(significant) between reduce glutathione and (superoxide dismutase , malondialdehyde),while weak positive correlation (no significant) between reduce glutathione and (Glutathione -S- transferase, Catalase)figure (1)table(4).

Table(4): The Correlation Between the Level of Reduce Glutathione and Several Biochemical Markers Related to Oxidative Stress (Control and Chronic Children Diarrhea).

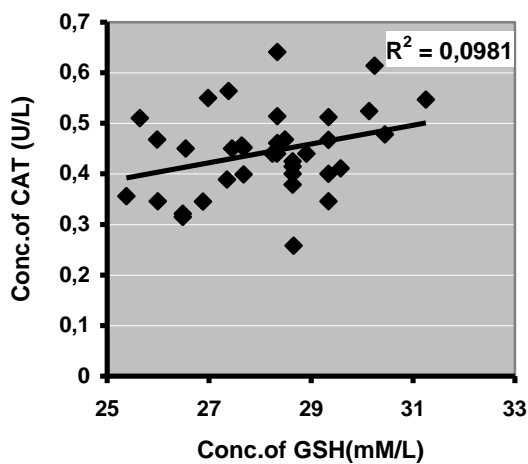
Correlation of GSH(mM/L)	(r ²)	P Value	Correlation of GSH(mM/L)	(r ²)	P Value
CONTROL			CHRONIC DIARRHEA		
vs. SOD	6E-05	significant	vs. SOD	-0.8583	significant
vs. CAT	0.098	no significant	vs. CAT	-0.1032	no significant
vs. GST	0.014	no significant	vs. GST	0.0002	no significant
vs. MDA	8E-06	significant	vs. MDA	4E-06	significant



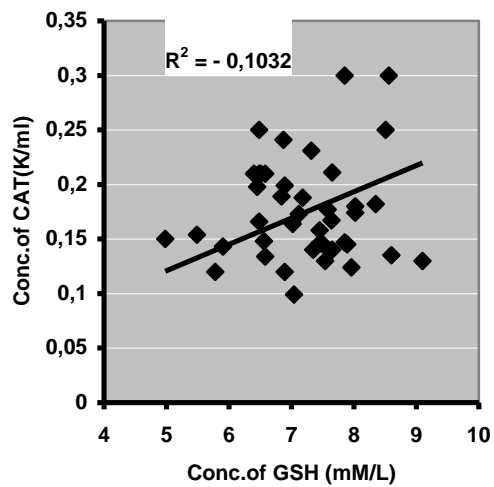
A



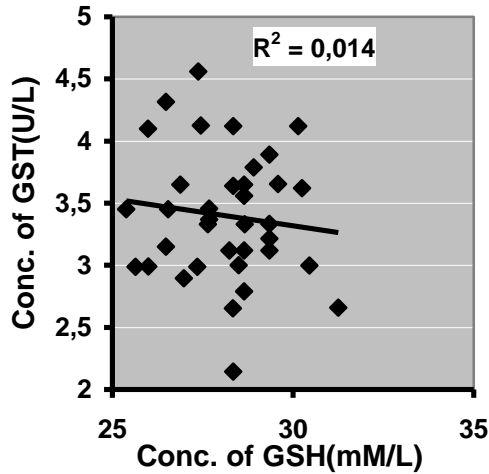
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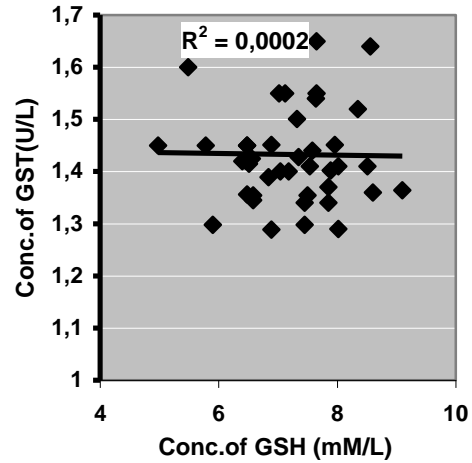
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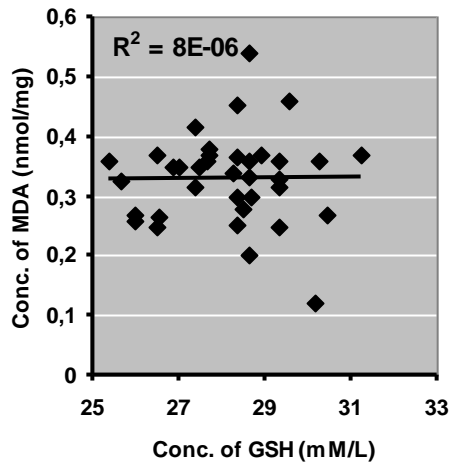
D



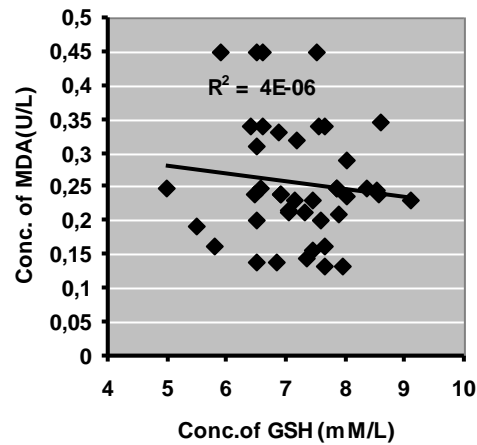
E



F



G



H

Figure(2):The Correlation Between the Level of Reduce Glutathione(GSH) and SOD,CAT,GST and MDA [Control (ACEG)and Children with Chronic Diarrhea(BDFH)].

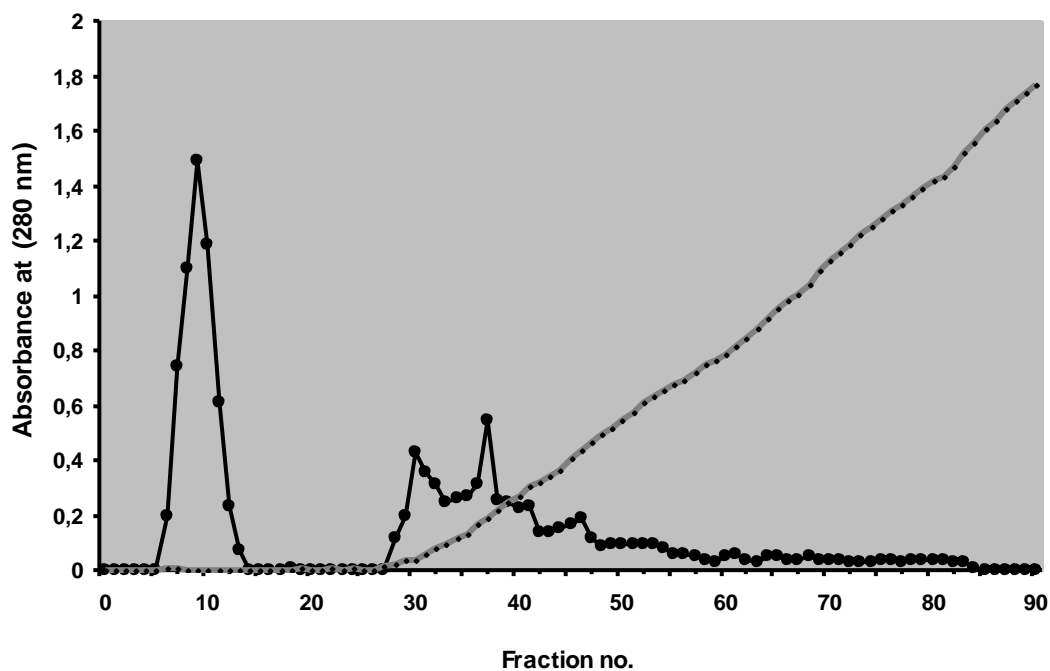
B-Separation and Purification of Erythrocytes Cu-Zn Superoxide Dismutase :

The superoxide dismutase was purified from human erythrocytes of control and patients' children with chronic diarrhea .The purification steps for SOD are summarized in table (5) and (6) respectively.

All of hemoglobin (8.34 g) and (7.64 g) were eliminated from the hemolysate at the first step by ethanol-chloroform (figure 3, 4).

Table(5):Purification Steps of Human Erythrocytes SOD from Control.

Sample	Vol. ml	Total SOD (U)	Total Protein (mg)	Specific Activity (U/mg)	Yield %	Fold of Purification	Total Hb. (g)
Hemolysate	190	6734.2	24310.4	0.277	100	1	8.34
After ethanol-chloroform treatment	202	6125.33	276.5	22.153	90.95	79.97	-
After K ₂ HPO ₄	65.5	5603.54	54.14	103.5	83.21	373.64	-
After acetone	9	5549.6	4.21	1318.19	82.409	4758.8	-
After ion exchange chromatography	18	4936.5	1.67	2955.98	73.3	10671.4	-

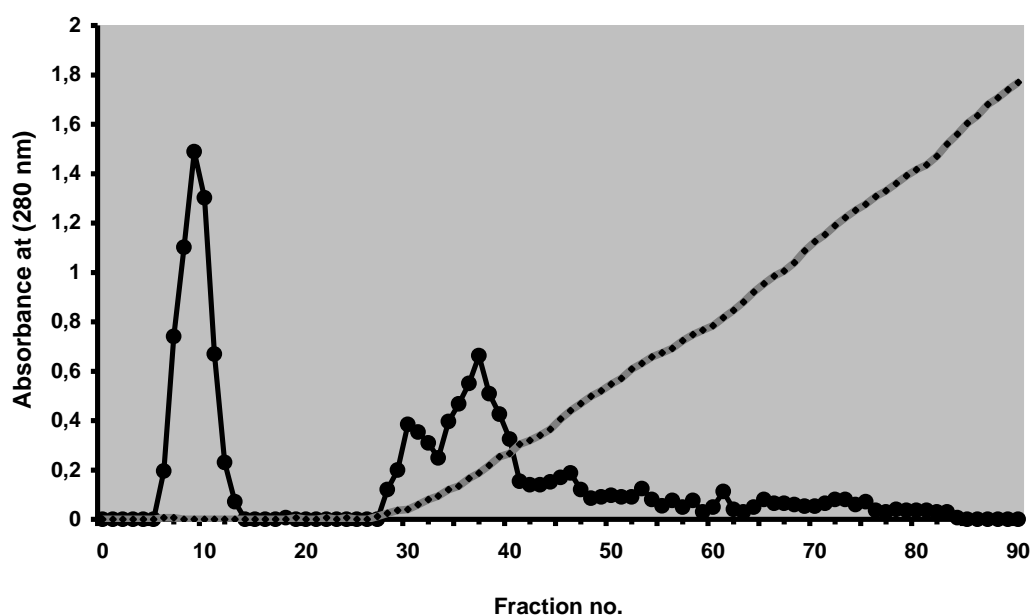


Figure(3):Fraction Absorbance at 280 nm after DEAE ion Exchange Chromatography (Control).

SOD from human erythrocyte (control) was purified 10671.4 fold with a yield of 73.3% and its specific activity was 2955.98 u/ml of protein (table7),while SOD from chronic children diarrhea was purified 16256.67 fold with a yield of 83.469 and its specific activity was 6502.67 U/ml of protein (table 6).Ion exchange chromatography ,the [(34-45),(20 ml)] and [(35-49),(20 ml)] eluted fractions detected at 280 nm presented an SOD activity and corresponds therefore to the SOD fraction (figure 3, 4) control and chronic children diarrhea respectively.

Table(6):Purification Steps of Human Erythrocytes SOD from Children with Chronic Diarrhea.

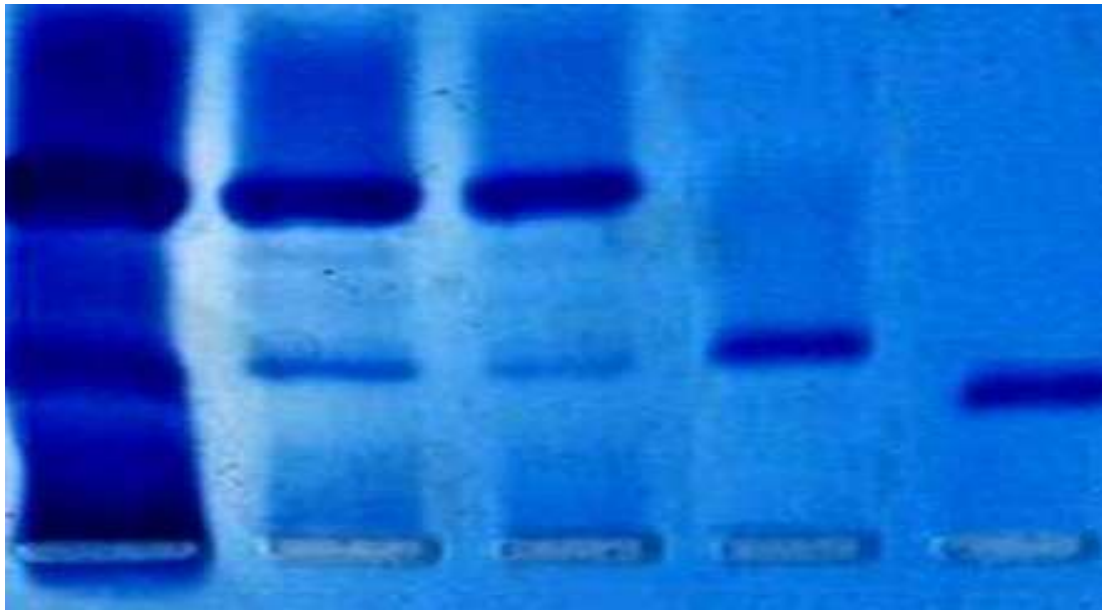
<i>Sample</i>	<i>Vol. ml</i>	<i>Total SOD (U)</i>	<i>Total Protein (mg)</i>	<i>Specific Activity (U/mg)</i>	<i>Yield %</i>	<i>Fold of Purification</i>	<i>Total Hb. (g)</i>
Hemolysate	195	9613.4	24031.2	0.4	100	1	7.64
After ethanol-chloroform treatment	200	9024.6	265.4	34.0	93.87	85	-
After K ₂ HPO ₄	70.5	8350.4	49.61	168.32	86.86	420.8	-
After acetone	8	8260.6	4.02	2054.8	85.92	5137	-
After ion exchange chromatography	15	8024.3	1.234	6502.67	83.469	16256.67	-



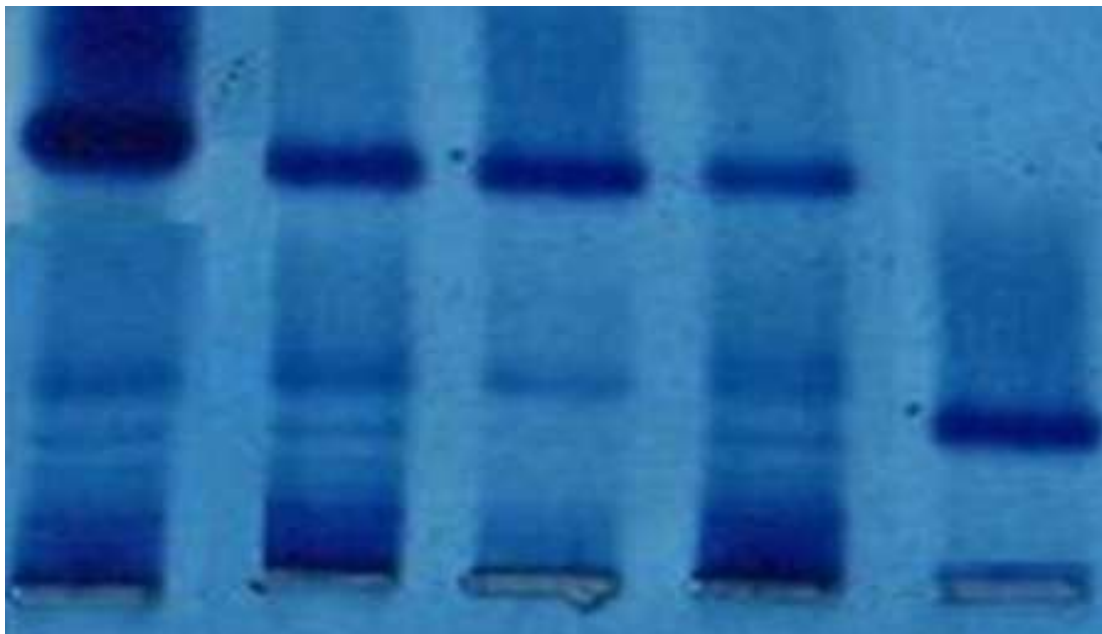
Figure(4):Fraction Absorbance at 280 nm After DEAE ion Exchange Chromatography (Children with Chronic Diarrhea).

C-Purification of Erythrocytes Cu-Zn Superoxide Dismutase by SDS- PAGE:

The separation of Cu-Zn superoxide dismutase from erythrocytes of control and children with chronic diarrhea was conducted by using SDS lab gel electrophoresis for (figure 5).Subunit has a molecular weight about [(18500 Daltons, control), (18300 Daltons, children with chronic diarrhea)].



Crude(A) After ethanol- After After After ion exchange
Control chloroform K₂HPO₄ Aceton chromatography



Crude(B) After ethanol- After After After ion exchange
Patients chloroform K₂HPO₄ Aceton chromatography

Figure(5):SDS-PAGE ,Profile of Crude Serum Control(A) , Children with Chronic Diarrhea(B) and Partial Purified steps. The Gel was Stained for Protein with CBB R-250.

DISCUSSION :

The superoxide dismutase activity was assessed by the riboflavin /nitroblue tetrazolium assay. The values of superoxide dismutase activity for both healthy children and children with chronic diarrhea are illustrated in table (1), as compared to the activity of SOD in healthy children. There was an increment in the serum SOD activity in patients' children with chronic diarrhea. The superoxide ion radicals might effect the enzyme activity only in susceptible organs. Therefore; this suggests that the enzyme activity in the blood sample may reflect the antioxidtive defense of the whole organism. The enzyme SOD catalase the breakdown of superoxide anion and provides the first line of defense against oxygen toxicity⁽³⁰⁾.

In this work, serum GSH,CAT and GST were significantly decreased in children with chronic diarrhea when compared with control (table 1,2).Depletion of serum GSH causes slightly increases risk of another disease in those patients .The decrease in serum GSH activity and another antioxidant enzymes in different diseases and tumors^(31,32,33).Higher levels of lipid peroxidation product is associated with reduction of antioxidant activity and increase oxidative stress^(34,35,36).Lipid peroxidation is a radical-mediated chain reaction initiated by abstraction of a hydrogen atom from a polyunsaturated lipid so abundant in the cell membranes, and this amplifies the damage caused by the initiating event, and terminated by chain-breaking antioxidants. Prevention of lipid peroxidation is an essential process in all the aerobic organisms, as lipid peroxidation products can cause DNA damage. Increased lipid peroxidation and decreased antioxidant protection frequently occurs⁽³⁷⁾.

All cells develop several lines of defenses against oxidative attack to keep free radicals under control at physiological levels. It is suggested that oxidation-antioxidation potential may be partuculary important in determine the permissiveness of the enzyme for development, in that an oxidizing environment may be more conducive for development⁽³⁸⁾. Oxidative stress, resulting from the imbalance between prooxidant and antioxidant states. Many recent studies have concluded that reactive oxygen species related oxidative stress in an important factor in cancer formation^(39,40).

Cu-Zn superoxide dismutase separation and purification from erythrocytes of control and children with chronic diarrhea by ion exchange chromatography, on a column(7×0.7 cm)of DEAE-Sepharose-CL-6B.The use of an ethanol-chloroform mixture in early stages of purification of erythrocytes Cu-Zn superoxide dismutase removes hemoglobin almost completely and facilitates future purification⁽⁴¹⁾.

Precipitation with ammonium sulfate and sometimes heavy metal salts could be connected to the fractionation with chloroform-ethanol and acetone. It was found that if hemoglobin was present in the extract, it could inhibit the reaction of superoxide radicals with NBT and interfered with the assy. According to the results in the first step of precipitation, the hemoglobin was undetectable(table 5,6).

Another study observed that exposure treatment with ethanol –chloroform leads to an increase in the degree of heterogeneity⁽⁴²⁾,also Stansell and Deatch show increased in sedimentation constant, and the loss of about 8 % of the copper⁽⁴³⁾.K₂HPO₄ used to increase the pH of the solution and since this pH is higher than the pHi of SOD only the contaminant proteins are precipitated .The precipitation by K₂HPO₄ is very important, where the purification factor increase (4.6 fold,4.9 fold) and specific activity(4.6 fold,4.9 fold)in control and children with chronic diarrhea respectively (table 5 ,6) .Also in this step total protein content was (5.1 fold,5.3 fold) decreased.

The use of acetone important to eliminate contaminants and concentrated the protein solution(4.21,4.02).After ion exchange chromatography ,the final recovery (73.3%,83.4%)that

is better than obtained by Fridovich⁽⁴⁴⁾ and Djalali *et al*⁽²⁷⁾. Cu-Zn superoxide dismutase appeared as a single band upon analyses by SDS –PAGE showing the purity of the enzyme (figure 5).The enzyme obtained by this method are nearly identical in the control and patients children with chronic diarrhea and to those native protein⁽⁴⁵⁾.

Conclusions:

- 1-Chronic childhood diarrhea elicited increase in SOD activity and lipid peroxidation product associated with free radical mediated oxidative stress demonstrated by increased level of MAD and decrease of GSH, CAT and GST in the serum, therefore this results it is regarded us a good indicator to another complication.
- 2-Chronic childhood diarrhea, I suggest that receive antioxidant vitamin to prevent further future complication.
- 3-It may be possible that oxidative stress plays a larger role in children with chronic diarrhea at long time, and that risk factor.
- 4-Cu-Zn superoxide dismutase purification from erythrocytes of control and children with chronic diarrhea with M.wt.18500 and 18300 Daltons respectively.

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