The effect of 5,6 – O,O – ethylene – L-ascorbic acid and it's complexes on erythrocyte catalase in chronic renal failure patients

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Abstract:

In order to study the activity of human erythrocytes catalase a well-known enzymes uses H_2O_2 as substrate, in chronic renal failure with complication, hypertension, diabetes, hypertension-diabetes and renal failure with out any complication 5,6 – O,O – ethylene –L-ascorbic acid (L) and it's complexes with Cu, Hg, Ca after it characterized by H^1, C^{13} NMR and Fourier transform infrared (FT-IR) and C,H analysis, were used to study the effect on hemolized erythrocyte catalase after and before hemodialysis in chronic renal failure patients.

Introduction:

Chronic kidney disease (CKD), also known as chronic renal disease(CRD), is a progressive loss in renal function over a period of months or years. Severe CKD requires one of the forms of renal replacement therapy; this may be a form of dialysis, but ideally constitutes a kidney transplant(1) Hypertension is a very common feature of renal parenchymal and vascular disease and is an early feature of glomerular disorders. Renal mechanisms are also likely to be important in essential hypertension, and most inherited disorders of blood pressure have been attributed to salt and waterhandlingbythe\kidney(2).

Diabetic nephropathy is an important cause of morbidity and mortality, and is now among the most common causes of end-stage renal failure (ESRF) in developed countries. As it is found with other microvascular and macrovascular complications, management is frequently difficult and the benefits of prevention are substantial. (3) is a common enzyme found in nearly all living organisms that are exposed to oxygen, where it functions to catalyze the decomposition of hydrogen peroxide to water and oxygen[4][5]. Catalase has one of the highest turnover numbers of all enzymes; one catalase enzyme can convert 40 million molecules of hydrogen peroxide to water and oxygen each second[6][7]. Catalase can also oxidize different toxins, such as formaldehyde, formic acid, phenols, and alcohols [8][9]. In doing so, it uses hydrogen peroxide according to the following reaction:

$$2H_2O_2 + H_2R \longrightarrow 2H_2O + R$$

Vitamin C and its derivatives are reducing agents[10][11], can also act as pro-oxidants. Vitamin C has antioxidant activity when it reduces oxidizing substances such as hydrogen peroxide[12], however, it will also reduce metal ions that generate free radicals through the Fenton reaction[13], [14]

$$2\ Fe^{3+} + \text{Ascorbate} \rightarrow 2\ Fe^{2+} + \text{Dehydroascorbate}$$

$$2\ Fe^{2+} + 2\ H_2O_2 \rightarrow 2\ Fe^{3+} + 2\ OH\cdot + 2\ OH^{-}$$

The objective of this study effect of this ligands and complex were evaluated for their effects on RBC catalase from. Patients with CRF on hemodialysis in order to get on an idea for their application on the enzyme.

Experimental:

Instrument

U.V-Vis spectrophotometer

Centrifuge

Chemicals

All reagents were of highly analytical grade

Samples were collected from patients treated in Al-Kahdmia hospital, during period November 2010 to April 2011.

About 5 ml venous blood was taken from each individual, of the groups . All patients with C.R.F were diagnosed by laboratory tests which included age, sex and hypertension: systolic Bp ≥ 140 and diastolic Bp ≥ 90 mmHg and serum blood glucose, and were treated by hemodialys. About 5 ml venous blood was taken from each individual, of the groups. The blood was placed in tube and left min at room temperature, centrifuged at 2500 rpm for 10 min, to separate serum, which was stored at 20 ^{o}C unless used immediately. The separated RBC was washed immediately four times with 0.9 % NaCl (Normal Saline), and centrifuge. The supernatalnt was with drawn while the left RBC, were used immediately.

Hemolysate was prepared by adding four parts volume of distilled water to one sediment volume of erythrocyte. A chloroform-ethanol extract was prepared by adding (0.5 ml) hemolysate to (3.5 ml) ice cold D.W, followed by (1.0 ml) ethanol and (0.6 ml) chloroform. After centrifugation, 1:500 dilution of this concentrated hemolysate was prepared with phosphate buffer immediately before the assay. and in

Patient Groups:

Four groups of patients of chronic renal failure without and with complication, were enrolled Diabet-mellites type-2 (T2DM), hypertension, hypertension-diabets and chronic renal failure have been included in this study. The diagnosis was performed by specialist doctors

Control group: consisted of (20) healthy group (10 male, 10 female) range age (19-38) years.

Group 1-A (G1A): consisted of (20) patients (12 male 8 female) prehemodialysis-hypertension C.R.F range age (70-20) years.

Group 1-B (G1B): consisted (20) patients (12 male 8 female) post hemodialysis-hypertension C.R.F age range (70-20) years.

Group 2-A (G2A): consisted of (20) patients (10 male and 10 female) prehemodialysis diabet C.R.F, age range (60-18) years.

Group 2-B (G2B): consisted of (20) patients (10 male and 10 female) post hemodialysis-diabet C.R.F, age range (60-18) years.

Group 3-A (G3A): consisted of (20) patients (13 male and 7 female) prehemodialysis-hypertension-diabet C.R.F (60-18).

Group 3-B (G3B): consisted of (20) patients (13 male and 7 female) posthemodialysis-hypertension-diabet (60-18).

Group 4-A (G4A): consisted of (20) patients (10 male and 10 female) prehemodialysis without any complication.

Group 4-B (G4B): consisted of (20) patients (10 male and 10 female) post hemodialysis without any complication.

Determination of Erythrocyte Catalase (CAT) Activity according to (15) assay:

Catalase activity can be determined using assay method that depends on its ability decompose H_2O_2 to give H_2O and O_2. This assay was based on the reduction in the absorbance of hydrogen peroxide H_2O_2 at 240 nm. The difference in the absorption (ΔA_{240}) per unit time is a measure of Catalase activity.

Fig.(1) show preparation of different concentration of H_2O_2 to evaluate k_m value ($K_m = 1\times10^{-3}$, $V_{max} = 8$) to be used in the preparation of ligands and complexes to find their effect on catalase.

And get the percentage inhibition or activation according to the equation:

% Inhibition = 100 (activity with inhibitor/activity with out inhibitor) ×100

Distal water was used as diluent,so no effect on catalase activity.

Determination of Hb:

Darbkin reagent used for Hb determination(16).

Preparation of L, and its complexes stock solution:

The amounts of synthesized derivative (L) and its complexes used for the preparation of the stock solution (10^{-2} M) in (10 mL) are shown in table (1). Stock solution (1 mL) was diluted in (10 mL) volumetric flask to give (10^{-3} M).

Table (1): The molecular formula and weight of L and its complexes used for the preparation of the stock solutions (10ml of 10^{-2} M) in distail water.

Compound No.	*Molecular formula*	*Weight (mg)*
1	$C_8H_{10}O_6$ (L)	0.0202
2	$C_8H_{10}O_{6.\ CUCl2}$	0.033 4
3	$C_8H_{10}O_{6.}$cacl2	0.0330
4	$C_8H_{10}O_{6.}HgCl_2$	0.0473

Ligand and it's complexes (Cu, Hg, Ca) where identified using different techniques i.e., H^1, C^{13} NMR and Fourier transform infrared (FT-IR) and C, H analysis according to A.A. Muklus, et al.(17).

Result and discussion:

The table(2) show the inhibition percent of L and its complexes with copper, mercury and calcium were tested on erythrocyte catalase activities for G_1, G_2, G_3 and G_4 pre and post hemodialysis. The result revealed an inhibitory effect on G_1 pre, G_2 pre, G_2 post, G_3 post and G_4 pre, post for complexes of ligand with copper with percentage inhibition values (74.01, 34.8, 50.01, 52.25, 148.1, 93.65). 55.51) % while thecomplex of L with calcium was found to inhibit erythrocyte catalase only G_1 pre dialysis on the other hand L with copper mercury, calcium showed an activitory effect with virous percentage of activation for the rest with the highest percentage activation for G_3 pre (441, 303.8, 571.4), G_2 pre (260.14), G_3 post (380), G_4 pre (201.4) L without any metals shows inhibitory effects for pre G_1, G_2 post, G_3 post and G_4 post with percentage inhibition (59.14, 0.0, 80.1, 0.0, 27.20), respectively and acts as an activator for erythrocyte catalase for G_2 post, G_3 pre and G_4 pre with percentage activation (107.8, 60.8,171.9) respectively

The effect of hemodialysis of erythrocyte catalase activity was studied in CRF patients in all studied groups. This enzyme was analyzed invitro, and its activity was found to be lower than healthy people.

The influence of L, with its complexes with (Cu, Hg, Ca) showed inhibitor and activitory effect with different percentage with activation and inhibition values. A study conductance of erythrocyte catalase activity showed that many metals including copper decrease the catalase activity of CRF under dialysis[18],[19] science no literature data was found concerning the effect of ascorbic acid derivative as ligand and its complexes which were synthesized during this study was modulators of erythrocyte catalase activity, so the inhibitory and activitory effect on catalase could be due to the modulating the binding affinity of the enzyme toward its substrate in the presence of synthesized complexes through changing the steriostructure of the active site or due to the competition with the natural substrate binding to form the enzyme substrate complex (ES).

Table (2): Show inhibition percent of L and its complexes on catalase in sera of hemodialysis patients and control.

	L	*L $CuCl_2$*	*L. $HgCl_2$*	*L. $CaCl_2.H_2O$*
G1A	–59.14	–74.01	–80.6	–3.5
G1B	0.0	+5.8	55.51	+201.4
G2A	+107.8	–34.8	+260.1	+39.9
G2B	–80.1	–50.01	+73.2	–5645.3
G3A	+60.8	+441	+303.8	+517.4
G3B	–0.0	–52.25	+212.4	+380
G4	+171.9	–148 1	+124.1	+198.01
G4B	27.28	–93.65	+134.2	201.4

+ activation, – inhibition value comparable w

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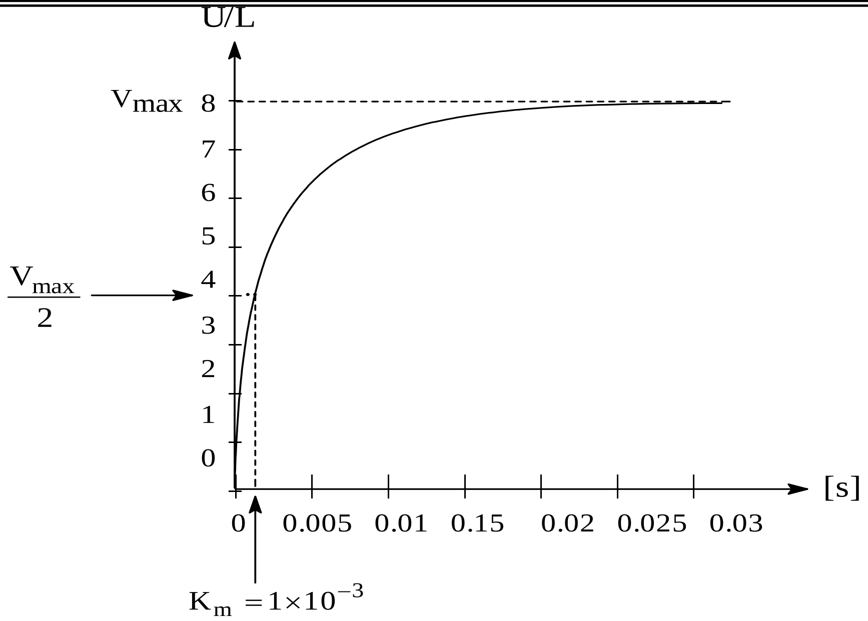

Fig.(1) Michaelis-Menten plot for erythrocyte catalase activity in control group.

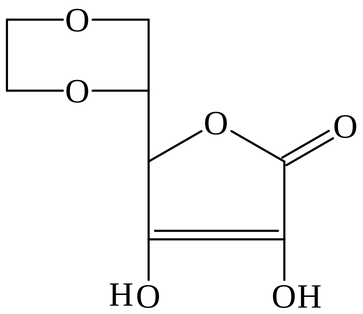

Fig.(2) Proposal structure for L.

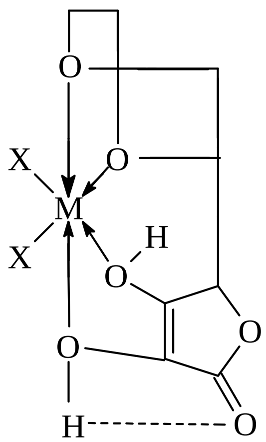

Fig.(3) Proposed structure for L with Ca^{+2}, Cu^{+2}, Hg^{+2}, Pb^{+2}.

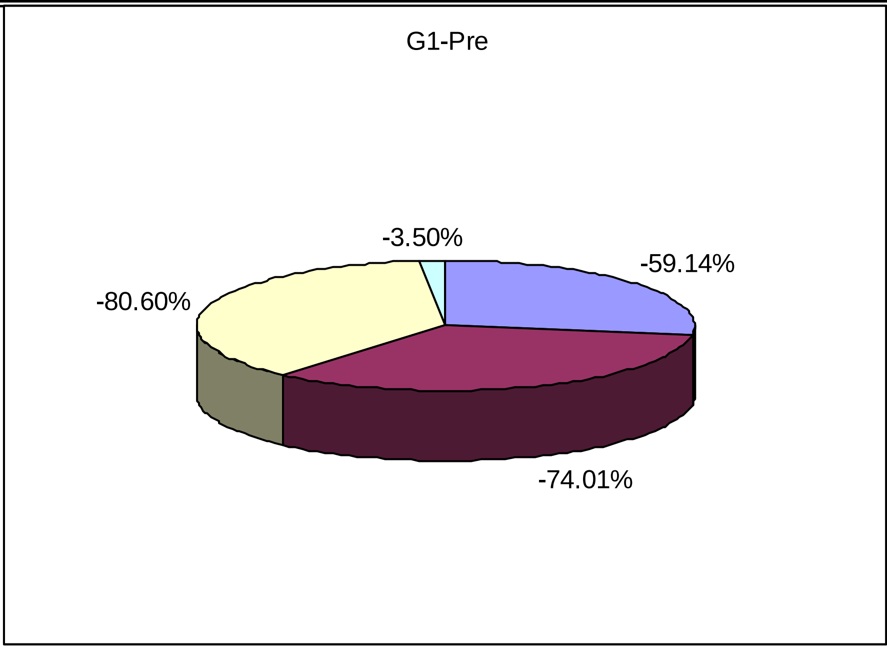

Fig. (4) Show inhibition % inG1A.

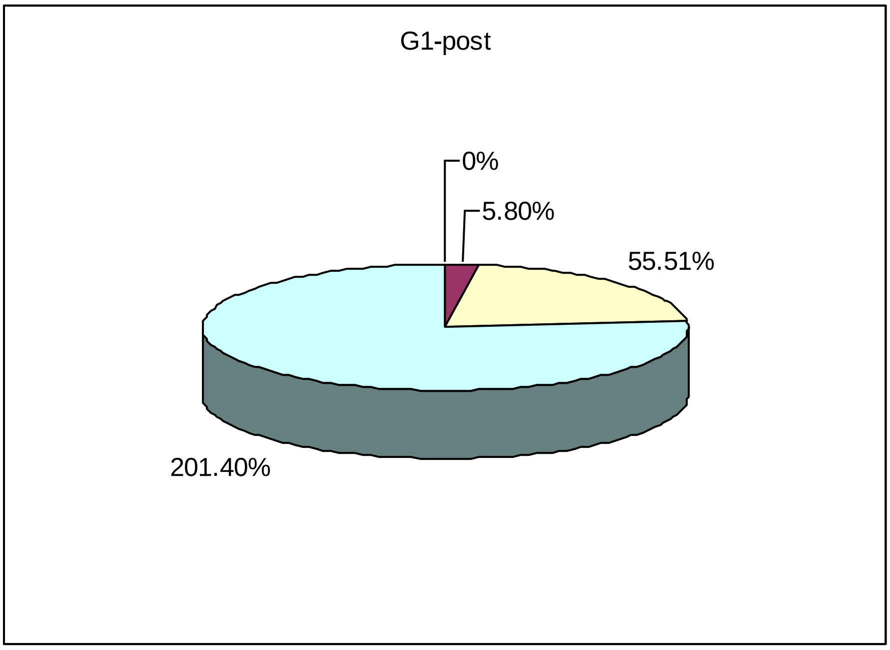

Fig.(5) Show inhibition % of G1B.

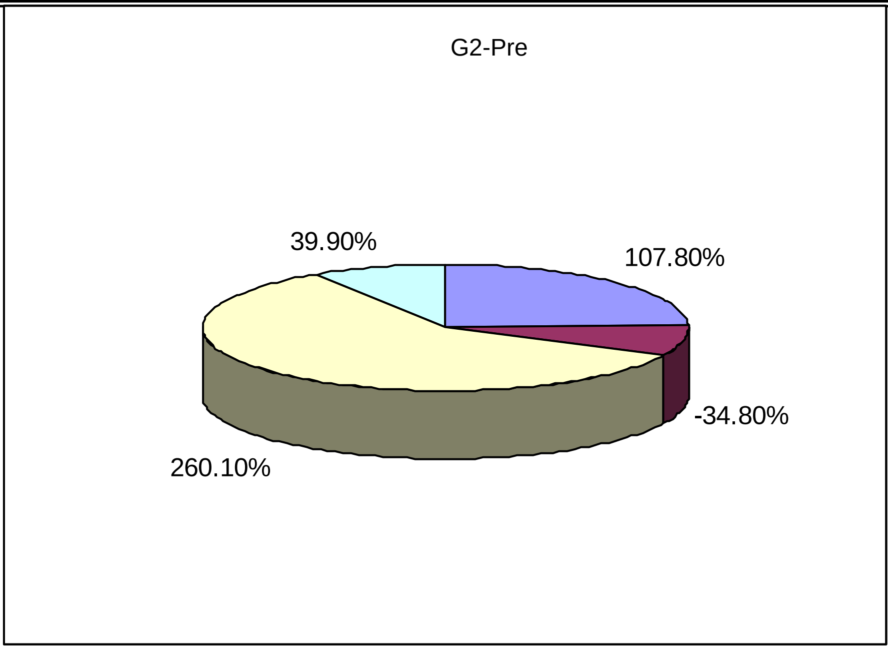

Fig.(6) Show inhibition % ofG2A.

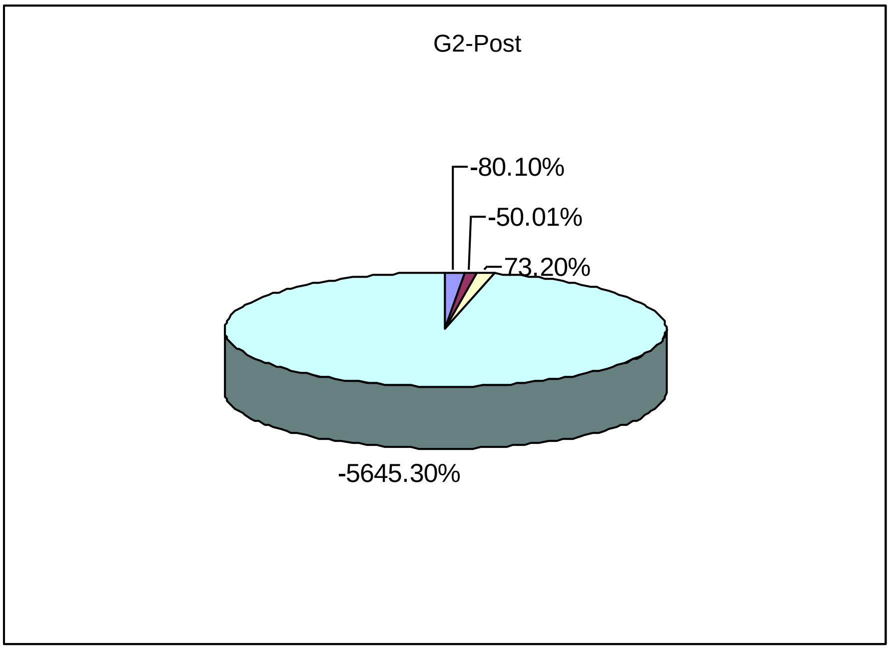

Fig.(7) Show inhibition % of G2B.

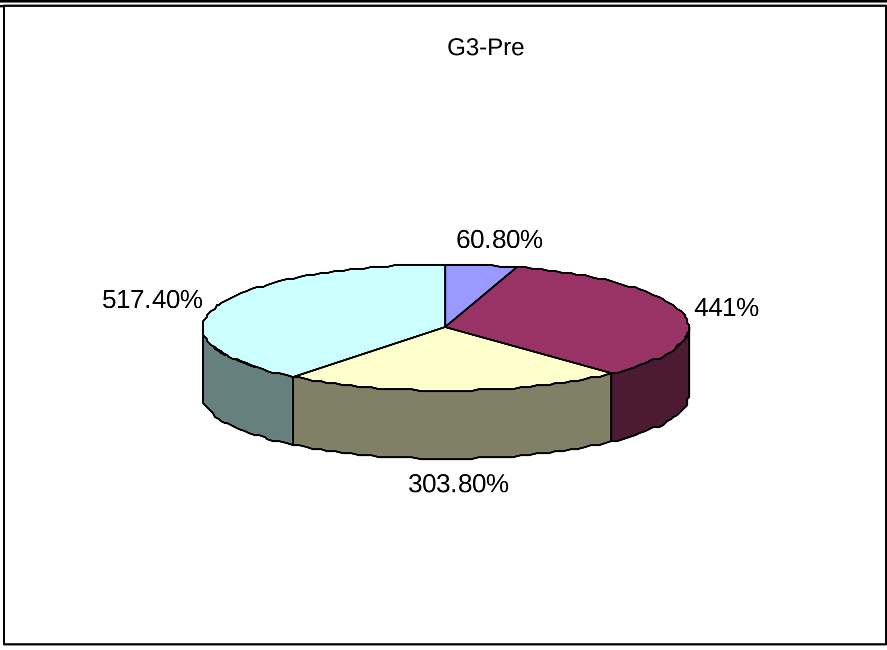

Fig.(8) Show inhibition % of G3A.

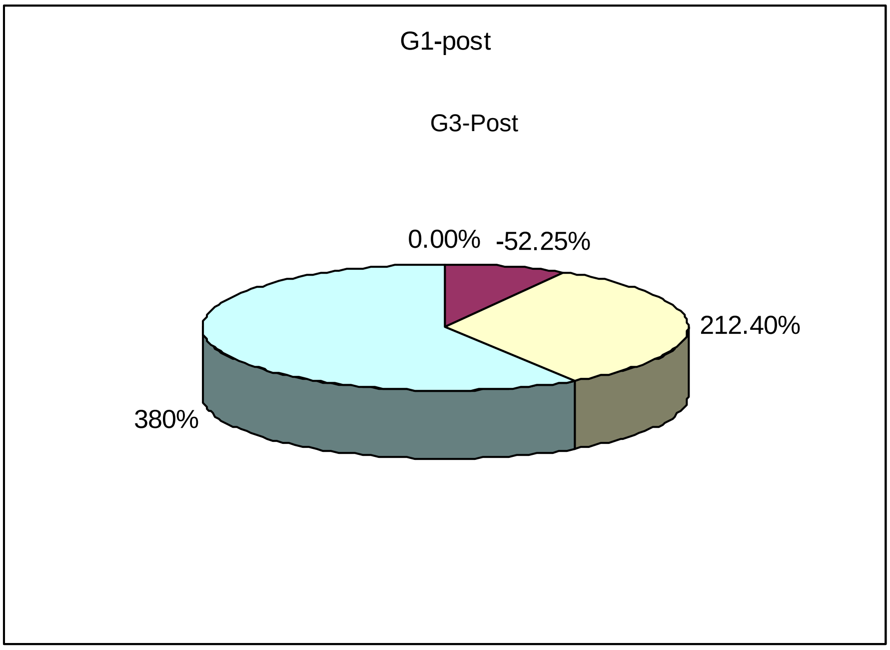

Fig.(9) Show inhibition % ofG3B.

a

Fig.(10) Show i G4-Pre ***% of G4A***

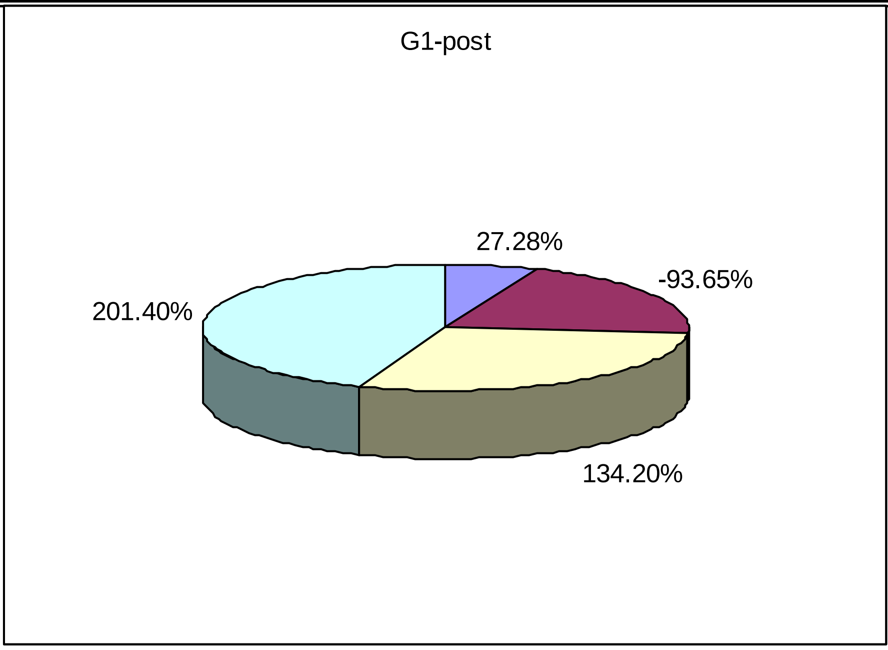

Fig.(11) Show inhibition % of G4B.

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تأثير لـ 5 ,6 ,O ,O اثيلين -L– حامض اسكلور بيك وبعض معقداته على انزيم الكتاليز لمرضى الفشل الكلوي

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العراق – بغداد

الخلاصة

تمت دراسة فعالية انزيم الكتاليز في كريات الدم الحمر لمرضى الفشل الكلوي الذي يعانون من مضاعفات الضغط،السكر،الضغط والسكر ومرضى الفشل الكلوي الذي لا يعانون من هذه المضاعفات واستخدم (5 ,6 ,O ,O – اثيلين –L– حامض اسكلوربيك ومعقداته مع (Ca, Hg, Cu)

بعد ان شخصت بواسطة التقنيات الاتية: تحليل العناصر (H,C)، والاشعة الحمراء والاشعة فوق البنفسجية – المرئية مع طيف الر نين النووي المغناطيسي – البروتون H^1 والكاربون C^{13} ودراسة تاثيرهم على فعالية انزيم الكتاليز في كريات الدم الحمراء لمرضى الفشل الكلوي قبل وبعد الديلزة