

## Relationship Between Conductivity Of Erythrocytes Suspension And Some Variable After And Before Treatment With New And Used Antioxidants For Rabbits With Induced Diabetic

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### Abstract

Diabetes mellitus (DM) is associated with hyperglycemia and oxidative stress. Oxidative stress which indicates the imbalance between reactive oxygen species (ROS) and defensive antioxidants system. In this work we study the relation between the conductivity of washing solution of erythrocyte membrane with induced diabetic rabbits (n=3) compare with rabbits control (n=3) after and before treatment with following antioxidants aspirin (n=3), GSH (n=3), Vitamin C (n=3) and with new derivative 4(1,2-di hydro -4,6-dihydroxy pyrimidine -2- yH thio)-3-hydroxy-5-(2,2-dimethyl-1,3-dioxolan-4-yl) furan-2(5H)one (3A) which believed as antioxidants. The results shows the conductivity of washing solution of erythrocyte membrane in diabetic rabbits a significant increased ( $p < 0.000$ ) as compare with control group and a significant decreased in treatment groups with aspirin, GSH, Vit C, and 4(1,2-di hydro -4,6-dihydroxy pyrimidine -2- yH thio)-3-hydroxy-5-(2,2-dimethyl-1,3-dioxolan-4-yl) furan-2(5H)one .

### الخلاصة

يرتبط مرض السكر مع ارتفاع سكر الدم ، دلالة على ارتفاع جهد الاكسدة والذي يؤدي الى حالة عدم التوازن بين اصناف الاوكسجين الفعالة ودفاعات مضللات الاكسدة . تم في هذا البحث دراسة علاقة التوصيلية لعالق كريات الدم الحمراء للارانب المستحث بها مرض السكر (n=3) مقارنة مع مجموعة السيطرة (n=3) قبل وبعد العلاج بمضادات الاكسدة والتي هي الاسبرين (n=3)، الكلوتاثايون وفيتامين C والمشتق المحضر 4(1,2-di hydro -4,6-dihydroxy pyrimidine -2- yH thio)-3-hydroxy-5-(2,2-dimethyl-1,3-dioxolan-4-yl) furan-2(5H)one (3A) ، اذ بينت النتائج في هذا البحث ، ان التوصيلية لعالق كريات الدم الحمراء للارانب المستحث فيها السكر تزداد زيادة ملحوظة مقارنة مع مجموعة السيطرة وتنخفض (انخفاض ملحوظ) بعد العلاج بالاسبرين ، و كلوتاثايون، وفيتامين C و المشتق 4(1,2-di hydro -4,6-dihydroxy pyrimidine -2- yH thio)-3-hydroxy-5-(2,2-dimethyl-1,3-dioxolan-4-yl) furan-2(5H)one (3A).

### Introduction

Diabetes Mellitus(DM) :is not one disease but rather is a heterogeneous group of syndrome characterized by elevation of fasting blood glucose caused by inadequate release in insulin are aggravated by an excess of glucosones (Ameerha *et al.*,2011). Type 1 diabetes is the consequence of an autoimmune-mediated destruction of pancreatic  $\beta$ -cell ,leading to insulin deficiency. Patients require insulin treatment for survival (Ahamed,2005). Type 2 diabetes is characterized by insulin resistance and/or abnormal insulin secretion .Individuals with type2 are not dependent on exogenous insulin , but may require it for control of blood glucose levels if this is not achieved with diet alone or with oral hyperglycemic agents (Masur *et al.*,2008). Long term diabetes mellitus, usually associated with a state of chronic hyperglycemia, results in dysfunction and failure of various organs especially the eyes,kidneys,nerves,heart and the blood vessels. This causes dreaded complications which increases the mortality among the diabetes (Ayas *et al.*,2011). During diabetes persistent hyperglycemia causes increased production of free radicals especially reactive oxygen species(ROS)(Moussa ,2008). Oxidative stress defined as a measure of a steady levels of oxygen species (ROS) or oxygen radical in the biological system(Ayas *et al.*,2011) ,Reactive oxygen species (ROS) are products of natural oxygen metabolized oxygen .The balance between production and disposal oxidant molecules is essential for tissue homeostasis. Increased rate of free radical production or decreased rate of removal leads to free radical accumulation and cellular damage (Ahmed *et al .*, 2010). Erythrocytes are particarly sensitive to oxidative damage due to presence of fatty acid

content in their membranes and high cellular concentrations of oxygen and hemoglobin .Erythrocyte damage includes changes in membrane protein and lipid structure which in turn induced alterations in external surface of the cell (Jihan *et al.*, 2011). This study is subjected to evaluated the conductivity in diabetic mellitus after and before treatment with aspirin , GSH, Vitamin C and 4(1,2-di hydro -4,6-dihydroxy pyrimidine -2- yH thio)-3-hydroxy-5-(2,2-dimethyl-1,3-dioxolan-4-yl) furan-2(5H)one (3A) new prepared compound, which are used as antioxidant agent in this work (Fairs *et al.*, 2010).

## Material and Method:

### 1-Experiment Animals:

- 1- Eighteen rabbits (male & female) were selected for the study
- 2-The rabbits were used in the experiments have weight on an average weight of 1.5 Kg.
- 3 -Induced Diabetes Mellitus : the rabbits were injected with alloxane ((2,4,5,6-tetraoxyhexa hydro pyrimidine Alloxane was freshly prepared (150 mg/Kg), into the vein to induced the diabetes in the rabbits after fasting 12 hr . Alloxane was freshly prepared (150 mg/Kg). The rabbits given oral with 15% glucose solution after (4-6) hr dose of alloxane and the animals had taken 5% glucose with tap water for the first day only . Then left to relief and to eat enough after 72 hr . The rabbits had diabetes were indicated by the positive glucose test in blood glucose more than 200 mg/dL .

### 2-Experiment Stratage

The rabbits were divided into six groups after to injected with alloxane.

- G1 : control group (n=3) was fed to the control diet .  
 G2 : untreatment group (n=3) with induced diabetic .  
 G3 : treatment (n=3) with aspirin(300mg/kg) .  
 G4 :treatment (n=3) with GSH(50mg/kg).  
 G5 : treatment (n=3) with vitamin C(50mg/kg).  
 G6 : treatment (n=3) with 4(1,2-di hydro -4,6-dihydroxy pyrimidine -2- yH thio)-3-hydroxy-5-(2,2-dimethyl-1,3-dioxolan-4-yl) furan-2(5H)one(3A) <sup>(8)</sup> . (50mg/kg).  
 Blood samples were collected after 8 weeks from the beginning of experiment ,with fasting condition and treatment.

### 3-Determination of Conductivity (Nabila ,2010)

Blood is the most accessible sample one can obtain for analysis. Its consists of 45% cells and 55%plasma. Red blood cells constitute 99% of its cellular components .They mainly govern the blood behavior either rheologically or electrically . RBC is a biconcave enucleated cell containing hemoglobin molecules. Several types of analysis can be used to measure the dielectric properties of cell suspensions.

#### Procedure:

- 1-Red blood cells were prepared by centrifugation at 500 X g for 5 min .The plasma and Buffy coat were removed by aspiration
- 2-The above solution were washed twice with normal saline (pH=7.4) and separated by centrifugation at 500 X g for 10 min .
- 3- The red blood cells were resuspended in buffered saline .
- 4- The samples were incubated in water bath at 37 C° for ( 10 min. ), then the conductivity was measured by using the instrument of conductivity (WTW Inolab 740).
- 5- The conductivity of using conductivity (WTW Inolab 740) the suspension of erythrocyte was measured.

## Results and discussion :-

There was listed in table 1 show the conductivity of erythrocyte suspension before and after treatment with aspirin ,GSH, Vitamin C and 4(1,2-di hydro -4,6-dihydroxy pyrimidine -2- yH thio)-3-hydroxy-5-(2,2-dimethyl-1,3-dioxolan-4-yl) furan-2(5H)one(3A).

**Table 1**

The level of Conductivity in diabetic rabbits as compare with control and treatment rabbits

Groups	Mean $\pm$ S.E	P-value
Group 1*	0.4333 $\pm$ 0.18559	0.000
		0.007
Group 2**	11.333 $\pm$ 1.85592	0.047
		0.000
		0.000
		0.001
Group 3	6.9000 $\pm$ 2.1221	
Group 4	0.7000 $\pm$ 0.25166	
Group 5	0.8333 $\pm$ 0.11798	
Group 6	2.1667 $\pm$ 0.96751	

\* : mean a significantly related of group 2 or group 3 with group 1.

\*\* mean a significantly related of group 3 or group 4 or group 5 or group 6 with group 2.

Table 1 shows the level of suspension erythrocyte conductivity of diabetic rabbits comparison with control and treatment groups . There were a significant increased (  $p < 0.000$  ) as compare with control group and a significant decreased (  $p < 0.047$  ) , (  $p < 0.000$  ) , (  $p < 0.000$  ) , (  $p < 0.001$  ) in treatment groups with aspirin , GSH ,Vitamin C and with 4(1,2-di hydro -4,6-dihydroxy pyrimidine -2- yH thio)-3-hydroxy-5-(2,2-dimethyl-1,3-dioxolan-4-yl) furan-2(5H)one (3A) respectively as compared with diabetic rabbits. In other study illustrate that the conductivity is significantly increased in the diabetic patients (IDDM,NIDDM) when compared with control subjects. The higher level of conductivity in diabetic rabbits than in control group due to the oxidation of hemoglobin molecules leads to unfolding of the globular protein with the formation of a new group exposed to the surface besides the polar hydrophilic groups and consequently increasing electrical conductivity .This indicates the increase in the overall charge of hemoglobin molecule due to the increase in the free radical production (Moussa,2008) . In 1999 Hillier et al. (Desouky,2009) reported that hypo and hyperglycemia excursion lead to changes in the electrolyte balance in blood cells and interstitial fluid in the healthy subjects and in patients with diabetes. These variations cause changes in the red blood cells membrane potential which can be estimated by determining the conductivity of the cell membranes. (Desouky,2009) , The same results was reported by (Tura et al.,2007) who found that glucose is able to directly affect the impedance of the blood. In this study we use in the treatment GSH, Vitamin C , aspirin and 4(1,2-di hydro -4,6-dihydroxy pyrimidine -2- yH thio)-3-hydroxy-5-(2,2-dimethyl-1,3-dioxolan-4-yl) furan-2(5H)one(which believed as antioxidant according to the results in this study) , which repair the permeability of cell membrane by decreasing the oxidants that is significantly clear in

decreased the level of conductivity after treatment with antioxidants according to the ability of its to scavenger free radicals. Treatment with acetyl salicylic acid (ASA) is accepted therapeutic approach for preventing vascular damage in diabetic mellitus. Antiplatelet agent (ASA) has been documented to exert beneficial effects in the evolution of diabetic retinopathy and to protect against cataract . Anti-inflammatory and Haemostatic (antithrombotic) effects of ASA may be due to inhibition of cyclo oxygenase , increased production of lipoxides ,protein acetylating and antioxidative action. In both human and experiment diabetes, ASA has been shown to inhibit protein glycation by acetylating of free amino groups, ASA lower concentration of glucose and attenuated oxidative stress (Zavodnik *et al.*, 2011) . In other study was indicated the beneficial effect of ASA on type 1 comparison with untreated rats , ASA treatment was improved blood glucose , insulin secreting ,weight , antioxidants system ,lipid profile , liver enzyme and resportiary chain complexes ( I ,II) (Ashori *et al.*, 2009). In this study we found that ASA a significantly decreased ( $p<0.047$ ) administration conductivity of suspension erythrocytes compare with untreated group.

Vitamin C plays a central role in the antioxidant protective system, protecting all lipids undergoing oxidation and diminishing the number of apoptotic cells .Furthermore , vitamin c regenerates the oxidized vitamin E (Aysel *et al.*, 2009). In other study Borcea et al,(Nadar et al.,2009), have demonstrated that vitamin c supplementation improves the consumption of glucose in diabetic patients . Additionally ,it has been proposed that vitamin c therapy improves  $\beta$ - cell function and tissues, insulin resistance, increase insulin plasma and can lower blood glucose and glycated hemoglobin levels. The vitamin c supplementation may improve the antioxidant defense due to improve the level of blood glucose , (Nadar *et al.*,2009) . In our study we found the treatment with vitamin c lead to a significant decreased ( $p<0.000$ ) in the level of conductivity of suspension erythrocytes compare with untreated group. Reduced glutathione (GSH): is one of the major constituents of the erythrocytes and plays an important role in providing protecting against oxidative damages. The antioxidant function of the tripepted is related to oxidation of the thio group of its cysteine residue with the formation of disulfide (GSSG) . Diabetic state has been shown to be associated with an increased oxidative stress (Rizvi *et al.* , 2001).In this study we found that treatment with GSH was lead to a significantly decreased( $p<0.000$ ) in the level of conductivity of suspension erythrocytes of diabetic rabbits compared with untreated group. Also in this study we used 4(1,2-di hydro - 4,6-dihydroxy pyrimidine -2- yH thio)-3-hydroxy-5-(2,2-dimethyl-1,3-dioxolan-4-yl) furan-2(5H)one , in treatment of diabetic rabbit which lead to a significantly decreased ( $p<0.001$ )the conductivity of suspension erythrocytes compare with untreated group.

## Conclusion:

The observation in diabetic rabbits increasment the level of Conductivity is the powerful indicators to evaluate the oxidative stress syndromes in diabetic rabbits induced with alloxane compare than the control rabbits, also we examined in this study we use aspirin ,GSH , vitamin C, and 3A as antioxidant for free radicals in the treatment of diabetic rabbits groups, and the level of conductivity is improved after treatment.

## Reference

- Ahamed (2005). The Physiological and Biological Effects of Diabetes on The Balance Between Oxidative Stress and Antioxidant Defenses System . Medical Journal of Islamic World Academy of Sciences. vol.15 , NO.1, 31-42.
- Ahmed W.M, Ghada M.Nabil , El-Khadrawy H.H, Hanafi E.M, Abd El- Moes S.I., and Amal .R .Abd El- Hameed (2010). The Relationship between Oxidant/Antioxidants Imbalance and Fertility in Buffalo -Cows . Int .J.OF Academic Research, Vol.2, NO.1.
- Ameerha M.Jasim , Mufeed J Ewadh , and Oda M. Al Zamely .(2011). Estimation Influence of Green Tea As a Medical Herb For Treating. International Journal of Applied Biology And Pharmaceutical Technology. Vol.2 ,Issue-1:Jan-May
- Ayas K.Mallick ,Ravindra Maradi , Gaurav Shorey and Marya Ahsam.(2011). A Study On Malondialdehyde As A Marker of Lipid Peroxidation in Male andFemale Patients with Type 2 Diabetes Mellitus. Int. J of Pharmaceutical Sciences Review and Research. Vol.8.
- Aysel Kukner , Neriman Colakoglu ,Mustafa Naziroglu and Tulin Firat (2009). The effects of combined vitamin C and E in Streptozotocin -induced diabetic rat kidney. journal of African studies and development vol.1(2) pp.029-036.
- Ashori, Zahra Bathaie and Jafame Jad (2009). The effect of ASA on the respiratory chain complexes of type 1 diabetic rats . J .Iran.chem.soc. vol.6, pp.s20-s27.
- Desouky (2009) . Rheological and electrical behavior of erythrocytes in patients withdiabetes mellitus . Romanian j biophys., vol. 19, No. 4,p.239-250.
- Fairs H., Sadown Al - Shmary , and Oda M .Al- Zamely (2010) Study of some nitrogen and sulfur derivative on vitamin c molecule unpolished.( thesis). collage science . Babylon university. Jihan Hussein , Ehab Mostafa , Zakarya El- Khayat and Dalia Medhat (2010). Effect of Omega -3 Fatty Acids on Erythrocytes Membrane in Diabetic Rats . Macedonian Journal of Medical sciences 0180 , 1857-5773.
- Masur K, Thévenod F ,Zänker KS (2008). Pathophysiology of Diabetes Mellitus Type2 :Role of Obesity ,Insulin Resistance and  $\beta$ -cell Dysfunction . Diabetes and Cancer .Epidemiological Evidence and Molecular Links. Front Diabetes. Basel,Kargen., Vol.19, 1-18
- Moussa (2008).Oxidative Stress in Diabetes Mellitus, Romanian J Biophys . Vol.18 ,NO. 3 ,225-236 .
- Nabila S. Selim(2010) . Comparative Study on The Effect of Radiation on Whole Blood and Isolation Red Blood Cells . Romanian J .Biophys., Vol.2, P. 127-136 .
- Nadar Shakrokhi , Muhammad Khaksari Hadad and Muhammad Shabani(2009). Effects of aqueous extract of water cress on glucose and lipid plasma in Streptozotocin induced diabetic rats. Pak j physiol . 5(2).
- Rizvi and Zaid (2001) . Intracellular reduced glutathione content in normal and type 2 diabetic erythrocytes : effect of insulin and (-) epicatechin . Journal of physiology and pharmacology. 52 , 3 , 483-488.
- Tura , Sbrignadello , Barison , Conti and Pacint (2007). Dialectical properties of water and blood samples with glucose at different concentrations in : T. Jarm , P Kramar, A . Zupanic (Eds.). Medicon , 16 , 194-197.
- Zavodnik I. , Lukivskaya O. , Lapshina E. and Buko v (2011) .Antioxidant correlation of diabetic complications . Advances in molecular mechanisms and pharmacology of diabetic complications.263-283 ISBN:978-81-7895-499-8-Editor:Milan stefek.