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Verification of [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] Properties as Antioxidants in Experimental Animals Induced with Diabetes.

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Abstract:
Diabetes mellitus (DM) associated with Oxidative stress which indicates the imbalance between reactive oxygen species (ROS) and defensive antioxidants system. In this work it has been proved that compound [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) works as antioxidant compared with other used in this study. Rabbits used were divided into the induced diabetic rabbits (n=5) , rabbits control (n=5), treatment groups with following antioxidants aspirin (n=5) ,GSH(n=5) ,Vitamin C (n=5) and with preparation compound (3A) (n=5) which believed to be as antioxidants, The results show that levels of serum glucose and MDA in diabetic rabbits were significantly increased ($p<0.000$) for both as compared with control group and significantly decreased in treatment groups with aspirin, GSH, Vit C, and (3 A) while the level of membrane protein has been significantly decreased ($p<0.000$) as compared with control group and significantly decreased in treatment groups with aspirin, GSH, Vit C, and (3 A), these results verified that compound (3A) considered as antioxidant, but another study needs to detect the ability to used as drug.
Keyword: Diabetes Mellitus, Malondialdehyde(MDA), Aspirin, Reduced glutathione (GSH), 3A.

1.Introduction

Diabetes Mellitus(DM) is not only a disease but also a heterogeneous group of syndrome characterized by elevation of fasting blood glucose caused by inadequate release in insulin, It is aggravated by an excess of glucose (Jasim et al.,2011). Type 1 diabetes is the consequence of an autoimmune-mediated destruction of pancreatic β-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 by diet alone or by 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. These cases dreaded complications which increase 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) as products of natural oxygen metabolized oxygen or oxygen radical in the biological system (Ayas et al.,2011) .The balance between production and disposal oxidant molecules is essential for tissue homeostasis, that increased rate of free radical production or decreased rate of removal lead to free radical accumulation and cellular damage (Ahmed et al.,2010) , however stress may be amplified and propagated by autocatalytic cycle of metabolic stress, tissue damage and cell death, leading to further increase in free radical production and depletion of antioxidant (Baynes,1991). In addition ,oxygen free radicals exert their cytoplasm effect by peroxidation of membrane phospholipids ,which leads to changes in the permeability and loss of membrane integrity by attack to the poly unsaturated fatty acids in membranes to produce lipid peroxides leading to change in membrane permeability. Erythrocytes are 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 (Hussein et al.,2010).

The aim of this work to prove that [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) act as antioxidants by comparison with known antioxidants for experimental animals induced with diabetes.

2. Material and Method

2.1.Experiment Animals:

2.1.1.Thirteen rabbits (male & female) with average weight of 1.5 Kg were selected for the study.

2.1.2. Induced Diabetes Mellitus : The rabbits were injected with alloxane ((2,4,5,6-tetraoxyhexa hydro pyrimidine)) into the vein to induce 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 then 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 , latter the rabbits had diabetes indicated by the positive glucose test in blood glucose more than 200 mg/dL.

2.2. Experiment Strategy :

The rabbits were divided into six groups after injection with alloxane.

2.2.1. Control group G1 (n=5) was fed with the control diet consist of alfalfa and concentrate pullet (Crude protein 10%, ground soybean 20%, wheat flour 35%, corn 35%, mineral & vitamins 1 gm/Kg). Total energy was 13.6 Kj/Kg protein.

2.2.2. Untreatment group G2 (n=5) , with induced diabetic .

2.2.3. Treatment group G3 (n=5) with aspirin (300mg/kg).

2.2.4. Treatment group G4 : (n=5) with GSH (50mg/kg), the active concentration was get after the several attempts.

2.2.5. Treatment group G5 : (n=5) with vitamin C(50mg/kg), the active concentration was get after the several attempts.

2.2.6. Treatment group G6 : (n=5) 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) Fig.(1) (Fairs,2010) , , (50mg/kg), the active concentration was get after the several attempts.

Blood samples were drained after treatment with lasting 4 weeks ,and fasting condition.

3. Biological Measurements:

3.1. Determination of glucose(Kit glucose GOD-POD Lipid).

3.2. Determination of Protein Membrane by (Bradford Protein Assay Kit)

3.3. Determination of Serum Malondialdehyde(MDA) (Burtis and Ashwood ,1999)

4. Results and Discussion:

The proposal antioxidants systems used in this study as follows :

4.1 Aspirin as scavenger antioxidants system(Giovanni et al.,2008) because it has highly conjugated of π electrons bonds between the aromatic ring and with hydroxyl and acetate groups, which leads to its scavenger the free radicals.

4.2 GSH acts as termination the growth chain reaction of free radical by formation GSSG as the following equation (Dale and Henry,2002).

$$H_2O_2 + 2GSH \xrightarrow[\text{peroxidase}]{\text{glutathione}} 2H_2O + GSSG$$

$$ROOH + 2GSH \xrightarrow[\text{peroxidase}]{\text{glutathione}} 2ROH + GSSG$$

4.3 Vitamin C act, as equilibrated the free radicals by addition an electron to free radical such as hydroxyl radicals which convert to ascorbate free radical and dehydroascorabte, then ascorbate radical reduced to ascorbate by enzyme dependent NADH + H^+ (Mono-dehydro-L-ascorbate oxidoreductase), thus also recycled it(Khalid et al.,2004).

4.4 Compound [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) ,by comparison the results with other antioxidants.

This compound(3A) improves the level of glucose after treatment (100 mg/dl) compared with aspirin (113.36 mg/dl), GSH(191.66 mg/dl), Vit C(146.66 mg/dl) as show in the table(1), and the compound(3A) also improves the level of MDA in the serum of rabbits diabetes after treatment with it (2.67mg/dl), as compared with aspirin(1.724 mg/dl), GSH(2.48 mg/dl),Vit C(3 mg/dl) as show in the table(2).The compound(3A) also improve the level of membrane proteins after treatment (38.44 µg/ml), as compared with aspirin (40.39µg/ml), GSH(34.36 µg/ml), Vit C(35.9 µg/ml) as show in the table(3).

From the above, results which were obtained from the compound (3A), as compare with the natural antioxidants aspirin, GSH, and Vit C, the compound (3A) shows the ability to scavenger and quencher of the free radicals because of their conjugated system and several functional groups of (NH, OH, S, C=O) as shown in figure below that acts as antioxidant.

Conclusion:

In this study, it is proved that preparation of compound [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) acts as antioxidant in the treatment of diabetic rabbits groups, depending on the results in the tables (1,2,3) compared with the results which given by other

antioxidants (aspirin, GSH and Vit C.), the compound (3A) acts as scavenger and quencher the free radicals as in aspirin and Vit C. From this activity it becomes a good antioxidant, because it has two antioxidant properties (scavenger as aspirin) and (quencher as Vit C.).

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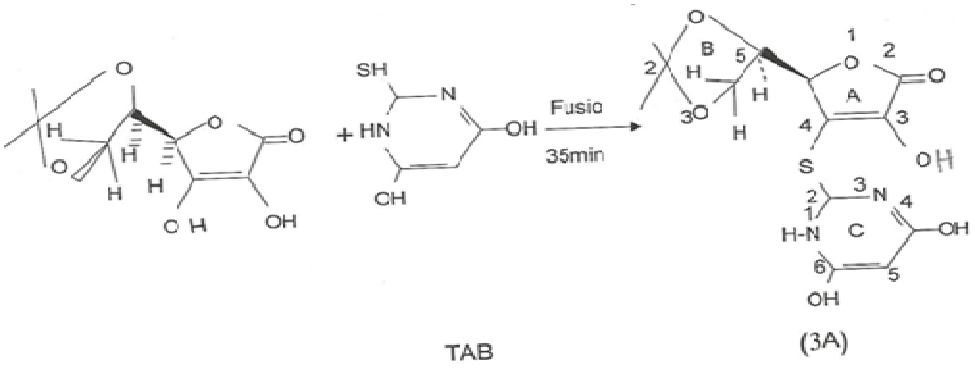

Fig.(1): represented the preparation compound(3A) 4(1,2-di hydro -4,6-dihydroxy pyrimidine -2- yH thio)-3-hydroxy-5-(2,2-dimethyl-1,3-dioxolan-4-yl) furan-2(5H)one(Fairs,2010).

Table 1:The levels of glucose(mg/dl) in serum rabbits with induced diabetic as compare with control and treatment rabbits.

Groups	Mean ±SD	SE	P-value
Control group G1*	96.8±20.19	9.0	0.000
Untreatment group induced diabetic mellitus with alloxane G2**	320±64	28	0.000
			0.000
			0.000
			0.000
Treatment group3 with aspirin	113.36±7.5	3.7	
Treatment group 4 with GSH	191.66±25	11.4	
Treatment group5 with vit .C	146.66±19	8.7	
Treatment group 6 with4(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.	100± 15	6.8	

* : It means a significantly related group 2 with group 1.
** It means a significantly related group 3 or group 4 or group 5 or group 6 with group 2.

Table (2) The level of Malondialdehyde (µmole/l) in diabetic rabbits as compare with control and treatment rabbits.

Groups	Mean ±SD	SE	P-value
Control group G1*	1.66±0.3	0.14	0.000
Untreatment group induced diabetic mellitus with alloxan G2**	5.29±0.66	0.29	0.000
			0.000
			0.001
			0.000
Treatment group3 with aspirin	1.724±0.5	0.24	
Treatment group 4 with GSH	2.48±0.55	0.26	
Treatment group5 with vit .C	3.00±0.45	0.20	
Treatment group 6 with4(1,2-di hydro -4,6-dihydroxy pyrimidin -2- yH thio)-3-hydroxy-5-(2,2-dimethyl-1,3-dioxolan-4-y furan-2(5H)one(3A).	2.67±0.57	0.25	

* : It means a significantly related group 2 with group 1.
** It means a significantly related group 3 or group 4 or group 5 or group 6 with group 2.

Table(3) The level of membrane protein(µg/ml) for RBC rabbits induces diabetes as compare with control and treatment rabbits

Groups	Mean ±SD	SE	P-value
Control group G1*	43.36± 4.8	2.1	0.000 0.004 0.013
Untreatment group induced diabetic mellitus with alloxan G2**	24.7± 5.9	2.66	0.000 0.002 0.001 0.000
Treatment group3 with aspirin	40.39±2.1	0.96	
Treatment group 4 with GSH	34.36± 3.37	1.50	
Treatment group5 with vit .C	35.9±3.02	1.35	
Treatment group 6 with 4(1,2-di hydro -4,6-dihydrox pyrimidine -2- yH thio)-3-hydroxy-5-(2,2-dimethyl-1,3 dioxolan-4-yl) furan-2(5H)one(3A).	38.44± 5.76	2.57	

* : It means a significantly related group 2 or group 4 or group 5 with group 1.
** It means a significantly related group 3 or group 4 or group 5 or group 6 with group 2.

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