Study of Creatine Kinase Activity and Antioxidants in Serum and white blood cells in induced Diabetic Rabbits

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Abstract

This study was included 22 rabbits induced diabetes mellitus disease (DM) by injection the alloxan under skin, and then evaluation the activity of creatine kinase and antioxidants of blood serum and white blood cells (WBCs),(glutathione and, uric acid) with lipid peroxidants (malondialdehyde).

Before starting the evaluation, it was prepared the (WBCs) and serum from the rabbits 22 control.

The results of the study showed that there are significantly increased in level of MDA (µmol/L) in serum of rabbits with DM 1.68 ± 0.42, 1.54± 0.43 for (WBCs) compared with controls 0.83 ±0.29, 0.77 ± 0.26 in both serum and (WBCs), respectively and significantly decreased in activity of creatine kinase in patients for serum 80.6±22 IU/L compared with controls 105.5±38 IU/L ($P \leq 0.01$) in both serum and, (WBCs) also significantly decreased in the level of the glutathione and uric acid in patients 16.3 ± 6.4, 144.6 ± 42 µmol/L compared with controls 26.7± 10.3, 240.3 ± 41 µmol/L , ($P \leq 0.01$) in both serum and, (WBCs).

Finally, this study was not found significant differences between the levels of serum and (WBCs) in each groups of rabbits for all the parameters studied (creatine kinase, glutathione, uric acid and malondialdehyde.

الخلاصة

اجريت هذه الدراسة على 22 أرنب أستحدث فيها مرض السكري بواسطة حقنها بالالوكسان تحت الجلد لمعرفة اهم التغيرات التي تحدث في فعالية انزيم الكرياتين كـاينيز و مـضادات الاكـسدة (حـامض اليوريك والكلوتوثايون) وناتج اكسدة الدهون (المالون ثنائي الالدهايد) في كل من المـصل وكريـات الـدم البيض ومقارنتها مع مجموعة السيطرة (22 أرنب طبيعي).

أظهرت نتيجة البحث أن مرض السكري يعطي زيادة معنوية الحاصلة في المالون ثنائي الالدهايـد (µmol/L 0.42 ± 1.68) للمصـــــــــل و(0.43 ± 1.54) لكريات الدم البيض مقارنة بقيمها فـي مجموعة السيطرة 0.29 ± 0.83 للمصل و0.26 ± 0.77 لكريـات الـدم البـيض ، والانخفـاض فـي

المتغيــرات CK و GSH و UA حيث بلغت قيمها في المصل 22 U/L ± 80.6 و6.4 µmol/L ± 16.3و µmol/L 42 ± 144.6 عند مقارنة قيمها مــع مجموعــة الــسيطــرة U/L 38± 105.5و µmol/L 10.3± 26.7 و µmol/L 41± 240.3 على التوالي.

وأخيرا" أظهرت الدراسة أنه لا يوجد فرق معنوي في نتائج مستوى فعالية أنزيم الكريــاتين كــاينيز وتركيز كل من (حامض اليوريك ، كلوتوثايون، المالون ثنائي الالدهايد)(P ≤ 0.01) في المصل عن تلك النتائج بكريات الدم البيض للأرانب المختبرية المستحدث فيها مرض السكري عند مقارنتهــا مــع مجموعــة السيطرة.

Introduction

Creatine Kinase (CK) (E C 2.7.3.2) is one of transferases enzymes which is transfer reversibly phosphate group from adenosine triphosphate (ATP) , to creatine (ADP, which is have a nitrogen group as acceptor group in the form of enzyme.[1]

Addition to this function it acts as one of the important antioxidants in the body , which is defines as a molecule quenching free radicals and converted into a saturated state, at this state, the free radicals becomes unable to oxidize another molecules in special important molecules (proteins , DNA , cells membrane of arteries , hormones). [2]

One of the most important antioxidant is glutathione (GSH) , (tripeptide , consists of glycine , cysteine and glutamate). Glutathione is an active antioxidant widely distributed in plants and animals tissues. [3]

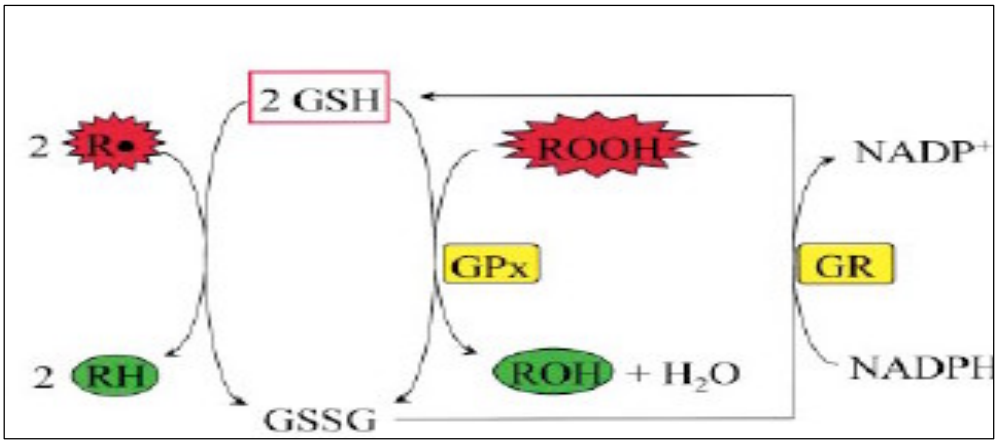


Figure (1) Function of GSH as an antioxidant.

Another important antioxidant in the human body is Uric acid , which is one of the final metabolic product of purins.[4]

Uric acid acts as scavenger of free radical because have a conjugated system of electrons which can disappear the free radical with along time in structure of molecule , that is preventing the another molecules from the influences of attaching of free radical. [5]

One of the types of effect of free radical, it's attachment to the molecules of Lipid leading to the Lipidperoxide, the rearrangement

process of this stabled molecule leaded to generation of complex products as a second lipidperoxide which is called malonyldialdehydee (MDA) [6] ,
the important molecule to assessment of level of oxidation of Lipids in the blood.

Aim of the study

1.Investigate the levels of important antioxidant such as uric acid , GSH and MAD and CK in the sera and white blood cells (WBC) in the rabbits with induced diabetic.
2. Study the relation ship between CK activity and (uric acid ,GSH, and MDA) in serum and WBC in the rabbits with and without induced diabetes.

Material and methods

44 Experimental rabbits was grouped into two groups, control with 22 rabbits and the others was induced diabetes group.

All the rabbits in the present study was males and with 4 – 9 month ages with weight intervial between 750 to 1750 gm.

The test group was fasted for 18 hrs, then injected under the skin with dose of alloxan (freshly prepared)150 mg/kg body weight, (this process was repeated for 3 days to reach 450 mg /kg as a total dose for body weight.

The experimental rabbits were feeding with 50% glucose solution ,and the rabbits was drinking water in the first day only after alloxan injection , the animal with alloxan was lete to rest , and lete to eat and drinking to enough state .

Apparent of indication for occurrence of diabetes after 7 day of injection rabbits with alloxan by giving glucose positive test in the urea , the level of glucose in the blood reached to 300 mg/ 100 ml and showing to urinate with duration of tired rabbits with injection alloxan with comparison with rabbits control group.

The experimental animals was an anesthetized by smelling chloroform for drawn (10-17)ml blood by fine needle (the animals was living at the same animals house for 10 – 15 day with the same plant eating).

Determination of CK activity in serum and WBC:

The activity of CK enzyme was determined indirectly , by using kit Cromatst , which is depending on , detection of ATP released from the reaction of creatine phosphate and ATP under the catalyzing of CK enzyme and the glucose in the series of these reaction was mediated to converted into glucose 6-phosphate , and the later was oxidized by $NADP^+$,which was converted into NADPH which was detected by the absorption of maximum wave length at 340 nm , which was proportional to the activity of CK . (Kit Cromatst)

$$CP + ADP \xrightarrow[PH=6.5]{CK(AMP,NAC)} Creatine + ATP$$

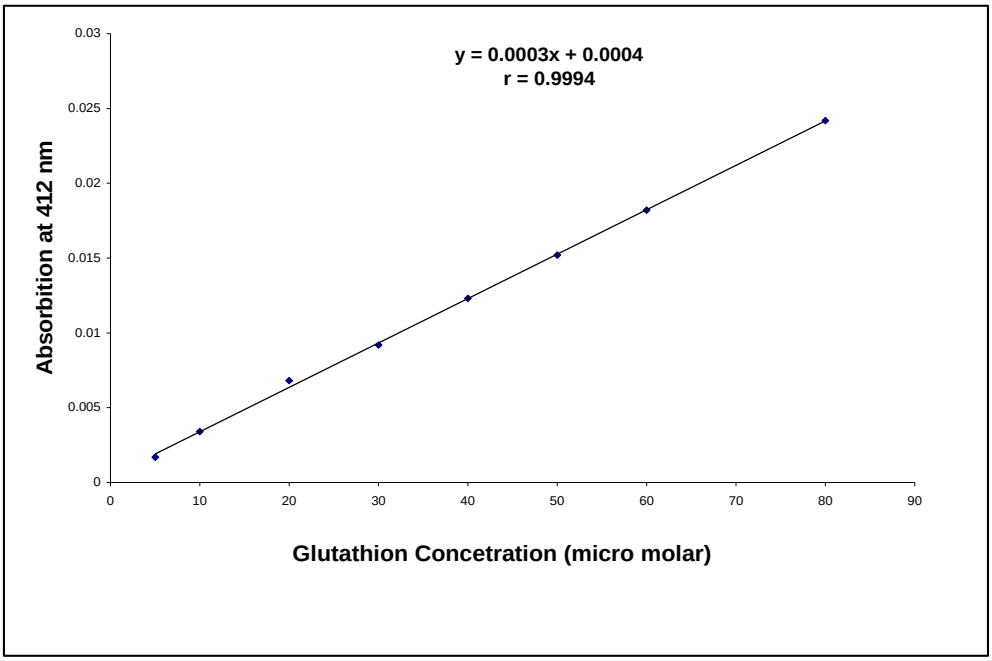
$$Glucose + ATP \xrightarrow{HK} G6P + ADP$$

$$G6P + NADP^+ + H_2O \xrightarrow{G6P\text{-}DH} Gluconate\ 6\ p + NADPH + H^+$$

Detection of GSH in serum and WBC:

Serum GSH reacted with 5,5-dithio bis (2-nitrobenzoic acid) (DTMB) which is called Elman's reagent, it is disulfide chromogen which is very sensitive to reduced while that is freshly prepared , of GSH to format GSSG with distinct yellow color ,detected the absorption at 412 nm , the maximum wave length , the standard carve was used to detected the concentration of GSH .[7]

Calculation

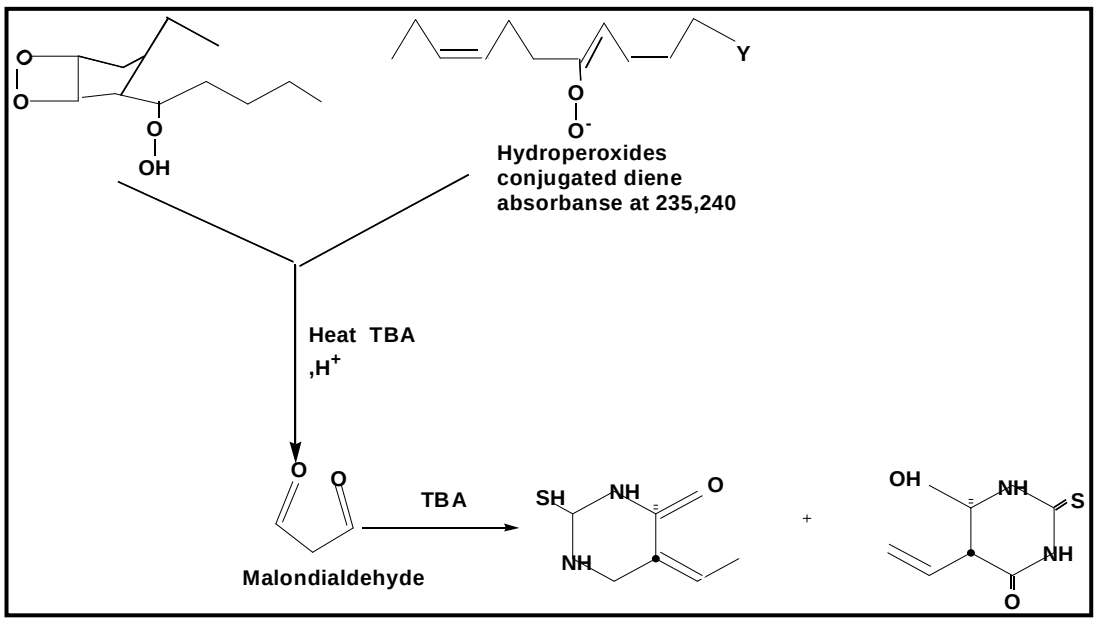


Figure(2):Glutathion concentration

Determination of Malonyldialdehyde in the serum and WBC:

The principle idea was depended on the changing of the color of thioparptiuric acid after reaction with MDA into pink , which was absorbed at 532 nm , the maximum wave length. [8]

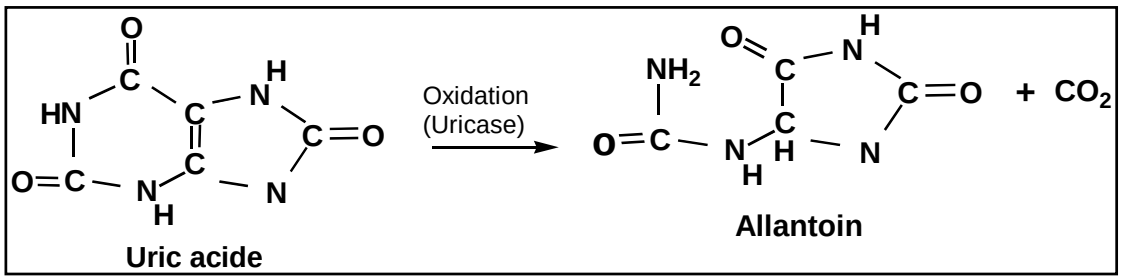
The reaction was occur according to the following scheme :



Figure(3):Schematic diagram for assessment lipid peroxidation via determination the by product ; Malodialdehyde.

Determination of uric acid levels in the serum and WBC:

Was determined the level of uric acid in serum and WBC according to the reaction (Biolabo kit) as following .



The absorption was detected at 520 nm (the maximum wave length) , it is proportionally with uric acid in the sample

Result and Discussion

The activity of CK decreased in sera and WBC of rabbits with diabetic when compared with control rabbits group. As shown in table 1 and

Table (1) represented the activity of CK (U /L)in sera and WBC of diabetic rabbits and control

		Mean	SD	CK U/L		SE	95% C.I.		P	Sign
				Upper	Lower		Upper	Lower		
Control (22)	Serum	105.5	38	169	54	8.1	------	------	------	------
	WBC	100	36.7	176.4	66	7.8	------	------	------	------
Rabbit with DM (22)	Serum	80.6	22	120	54	4.7	43.7	6.2	0.001	Sign
	WBC	79	12	107.7	66	2.7	36	4.7	0.001	Sign

Glutathion plays an important role in the defence system against oxidant species, by detoxifing the action of relative oxygen species , H_2O_2 and lipid peroxide directly or by the action of glutathione peroxidase (GSH-P_X).[9]

Glutathion also play several roles in the defence against deferent types of diseases ,these role are leaded to detoxify against the carcinogens against , free radicals peroxides and regulation the immune function and keeping the normal structures of proteins .[10]

The glutathion concentration in sera and WBC in the diabetic rabbits was decreased compared with control group. As shown in table (2)

Table (2): GSH concentration (µmol/L)in sera and WBC of diabetic rabbits and control

		Mean	SD	GSH µmol/L		SE	95% C.I.		P	Sign
				Upper	Lower		Upper	Lower		
Control (22)	Serum	26.7	10.3	37.1	7	2.2	------	------	------	------
	WBC	21.7	9.3	36	6	1.97	------	------	------	------
Rabbit with DM (22)	Serum	16.3	6.4	25	7	1.4	14.98	5.76	0.001	Sign
	WBC	12.8	7.1	35	5	1.5	14.58	3.1	0.001	Sign

significant decrease of GSH concentration in several type of diseases such as renal failure , liver failure, disease of depletion of vitamin B_{12} and in diabetic mellitus have beer reported .[11]

The reason of GSH depletion is a result of:

1.Increase in free radical generation in DM , where GSH act as free radical scavenger that is leaded to decrease in the levels of GSH , (for example, alloxan destroyed the β-cell via free radical generation therefore the single dose of GSH inhibit alloxan action).[12;13]

2. Intracellular GSH was play a defense role which is lead to oxidation of GSH under the action of GSH-peroxidase

$$H_2O_2 + 2GSH \longrightarrow GSSG + 2H_2O$$

3. it is found a balance between GSH/GSSG the important of this balance lead to the ability of accuration the other reaction between GSH and protein disulfide produced non- protein disulfide compounds [14]

$$GSH + Protein\text{-}s\text{-}s\text{-}Protein \longrightarrow Protein\text{-}S\text{-}H + G\text{-}S\text{-}S\text{-}Protein$$

The consuming of GSH levels in the present study in diabetic rabbits may consider as a protection factor against DM development.

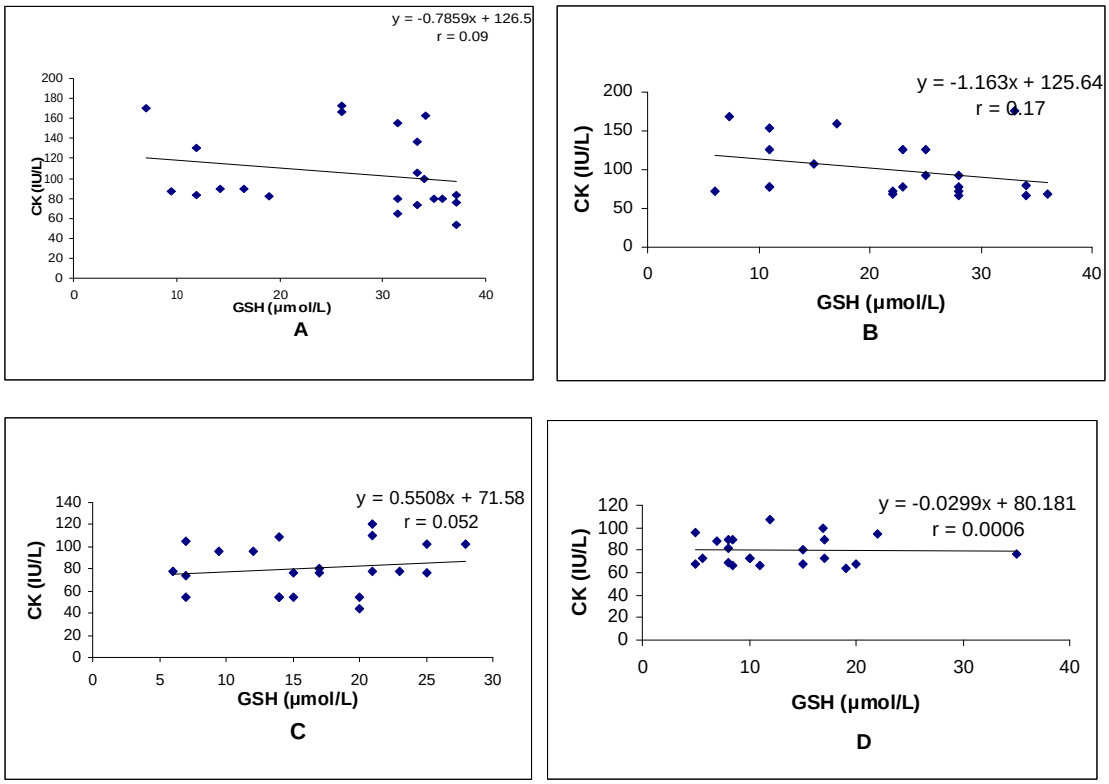


Figure (4): A.The relationship between CK and GSH in serum for control group. B.The relationship between CK and GSH in WBC for control group. C. The relationship between CK and GSH in serum for DM group. D.The relationship between CK and GSH in WBC for DM group.

In the present study ,the correlation between GSH and MDA was found to be not significant, and it might explain as following:
1. The consuming of GSH , result from accumulation of intracellular homocysteine in blood ,which is destroyed the epithelium cell by reduction and give rise to probability of heart diseases.(15)
2. Glutathion is played an important role in the protection of cells from bleeding due to peroxidation and it 's end product MDA which is occur in the DM patients. (16 ; 17)
3.The decrease in GSH levels was affected in the reversible way in CK activity decrease through S-glutathionylation mechanism, where, GSH associates in CK activity increase via bonding by GS group of S-glutathionylation of CK and formation of GSSG which attributed to formation GSH (reduced form) as the following reaction(18)

$$\text{CK-SG} + \text{GSH} \rightleftharpoons \text{CK} + \text{GSSG}$$

The equation was pointed to decrease in GSH levels and increase in CK levels.

Uric acid was produced from oxidation of purines.

Uric acid have property of scavenging free radical which is leading to

increase the capacity of antioxidants presents in serum and WBC . [19]

In the past studies were showed a reverse correlation between UA and oxidative stress, uric acid was oxidized in the skeletal muscle, when it's reacted with oxygen free radical or superoxid anion . [20]

Another studies were explained .

Table (3) UA concentration (µmol/L)in sera and WBC of diabetic rabbits and control

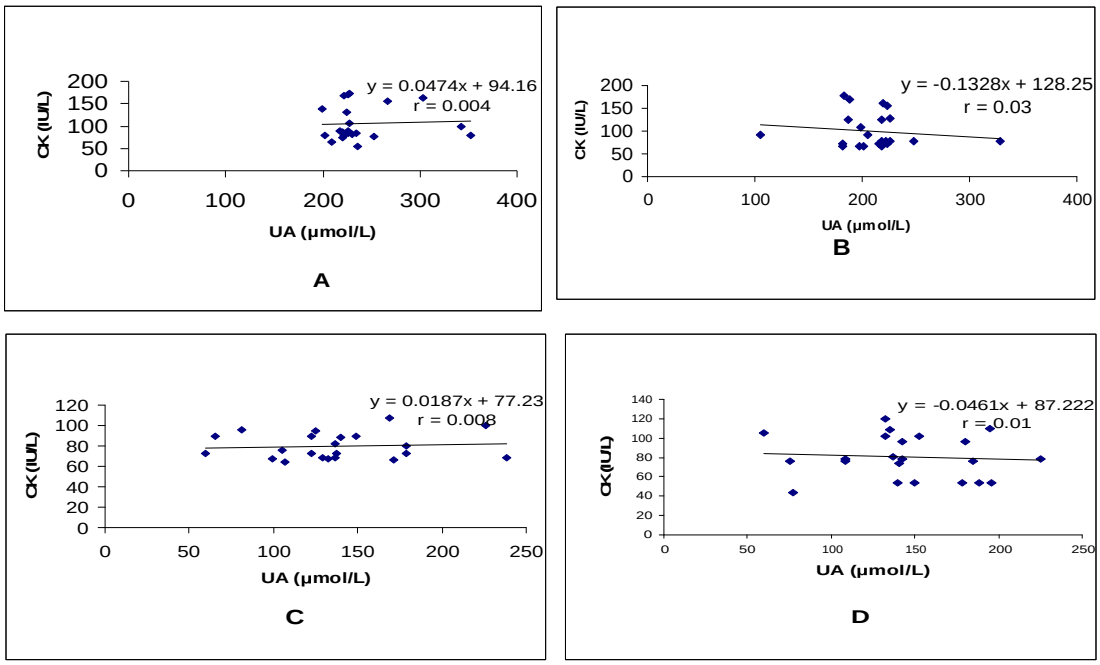
		Mean	SD	UA(µmol/L)		SE	95% C.I.		P	Sign
				Upper	Lower		Upper	Lower		
Control (22)	Serum	240.3	41	352	199.6	8.7	------	------	------	------
	WBC	209.9	38.6	328	105.5	8.2	------	------	------	------
Rabbit with DM (22)	Serum	144.6	42	225.6	75.3	8.99	122.1	69.3	0.001	Sign
	WBC	136.8	44.8	238	59.5	9.6	99.8	46.4	0.001	Sign

1. Uric acid acts as peroxnitrite scavenger (21) and this fact lead to increase free radical generation in DM. (12,13) (21)

2. Uric acid consumed NO_2 wich is occur in excess in diabetic patients.(12-13),(22)

The following figure represent the correlation between CK activity and UA in sera and WBC in patients and control.

The results of this study was agreed with previous studies which were considered uric acid as a marker of dysfunction of kidney and heart disease. [23]



Figure(5) (A)The relationship between CK and UA in serum for control group. (B) The relationship between CK and UA to WBC for control group. (C) The

relationship between CK and UA to serum for DM group. (D) The relationship between CK and UA to WBC for DM group.

The following figure represented the correlation between GSH concentration and MDA in sera and WBC in both DM rabbits and control group.

In this study, MDA level is found to be significantly increased in sera and WBC in the rabbits with DM , group, as shown in Table 4 and figure 9.

Table (4) MDA concentration (µmol/L)in sera and WBC of diabetic rabbits and control

		Mean	SD	MDA(µ mol/L)		SE	95% C.I.		P	Sign
				Upper	Lower		Upper	Lower		
Control (22)	Serum	0.83	0.29	1.5	0.47	0.06	------	------	------	------
	WBC	0.77	0.26	1.5	0.38	0.05	------	------	------	------
Rabbit with DM (22)	Serum	1.68	0.42	2.53	0.94	0.09	1.02	0.66	0.001	Sign
	WBC	1.54	0.43	2.4	0.85	0.09	0.96	0.59	0.001	Sign

Figure 6 represented the correlation between CK activity and MDA in sera and WBC in the rabbits with DM and control. The result was agreement with another studies which were considered the MDA as a marker of dysfunction of kidney and heart disease when it's appear with high levels in these diseases . [12]

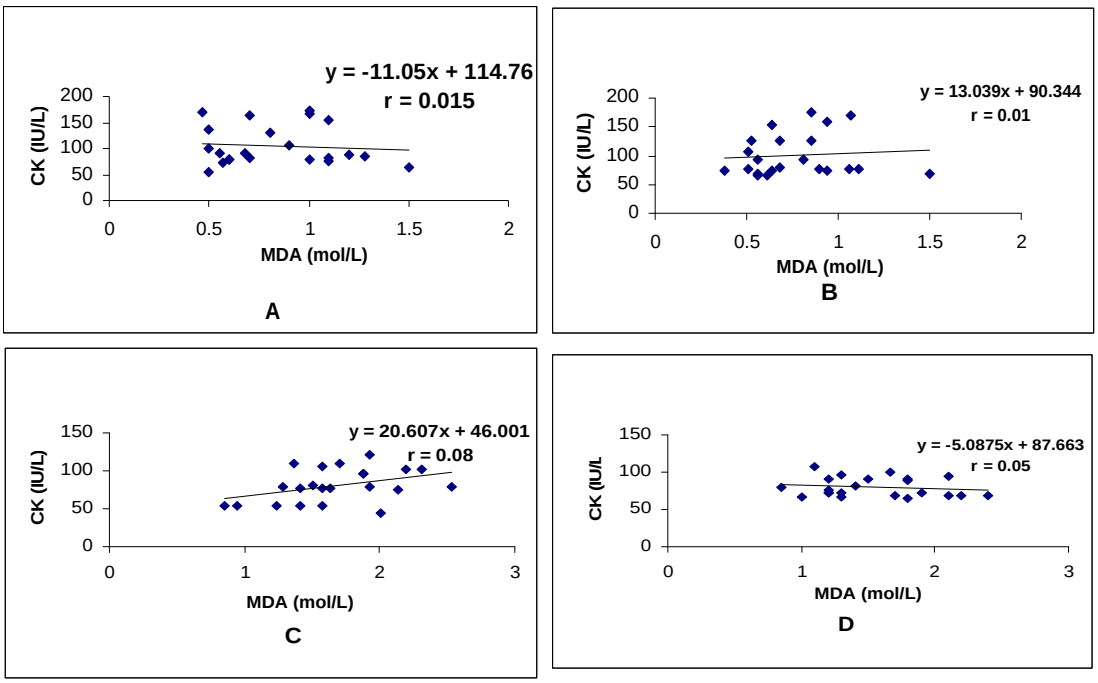


Figure (6) (A) The relationship between CK and MDA of serum of control group. (B) The relationship between CK and MDA of WBC of control group. (C) The relationship between CK and MDA of serum of DM group. (D) The relationship between CK and MDA of WBC of DM group.

This study is showing an increased level of MDA , which occurs after consuming of multiform of scavengers , which were prevented the oxidation of lipids.

MDA was one of the final products of lipid oxidation. The oxidation of lipid was passed through three steps of mechanism : [24 ; 25]

1.The initial step was begin by abstract of hydrogen atom (adjacent to the double bond of unsaturated fatty acid) by free radical species to form lipid radical(unsaturated fatty acid radical).

2. Progress steps were occurred by reaction the lipid with oxygen molecule to produce lipid peroxide radical, which is structure converted to the another shape by radical rearrangement with unsaturated bonds.

3.The termination step was formed lipid hydroperoxid by abstraction of hydrogen from allyl bond.

The increased in the lipid hydroperoxide in the patients , it was signed that increased of generation of free radical species and decrease of antioxidant levels.

One of the evidence of attachment of free radicals was digredation of cell membrane , and leaded to the tissue injury. [26]

The important free radical species which was abstract the hydrogen atom in the initial step , is hydroxyl radical , which was produced from fenton reaction with precursor molecule hydrogen peroxide in present ferrous ion or cupric ion cited in [27] as following reaction

$$H_2O_2 + Fe^{+2} \longrightarrow 2\dot{O}H + Fe^{+3}$$

It is also occur according to hyperweis reaction , in that reaction ferric ion is reduced ferrous ion by superoxide anion and formationof hydroxyl radical , as follow

$$H_2O_2 + Fe^{+2} \longrightarrow {}^{-}OH + Fe^{+3} + \dot{O}H$$

Conclusions

1.This study was shown no significant difference in the levels of CK, UA , GSH and MDA in the sera or in WBC in diabetic rabbits when compared with control group.

2. Diabetic Melluts was increased the generation of free radical species in rabbits when induced DM in it by increased MDA levels and decrease (CK activity , GSH, and UA) levels.

3.The changes in CK activity , UA, GSH and MDA levels can be used to investigate the amount of impact of DM on the rabbits .

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