Role of dietary antioxidant (ascorbic acid, vit.A and alfa tocopherole) in correction of liver dysfunction resulted from oxidative stress in rabbits affected experimentally with diabetes mellitus

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Summary

Determination of reliable biochemical parameters of experimental diabetic rabbit by alloxane monohydrates on liver function and role of diatery antioxidants (ascorbic acid, vit. A, and α-tocopherole) supplementation were investigated. Body weight gain, blood (plasma) chemistries, antioxidant enzymes and histopathological lesions were determined over 10 week in newzealand white rabbit group each contain 10 animals: control G1 animals group, diabetic only G2 animals group, diabetic and ascorbic acid G3 animals group, diabetic and Vit. A G4 animals group, diabetic and α-tocopherole G5 animals group . each of dietary ascorbic acid, vit. A and α-tocopherole that given to animals of G3, G4 and G5 respectively were significantly ($P \leq 0.01$) improve body weight gain and glucose level in blood than G2 diabetic group G3 and G5 significantly did not differ but these two groups significantly more best than G4 group, also these antioxidants improve levels of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and total protien. Enzymatic activity of liver glutathione peroxidase and superoxide dismutase were increased significantly more than of diabetic animals G2. histopathological structures of liver and pancrease confirm these results, as generally oxidative stress that affect liver function may diminished by administration of antioxidant ascorbic acid, Vit. A and α-tocopherole supplementation, and α-tocopherole group was the best group.

Introduction

Diabetes Mellitus is a complex metabolic disorder in which the pancreas produces insufficient amounts of insulin, or in which individual's system fail to respond appropriately to insulin. In people with diabetes, glucose levels build up in the blood and urine, causing excessive urination, thirst, hunger, and problems with fat and protein metabolism [1]. The disease is ranked seventh among the leading causes of death and third in terms of its complications and is a major health problem in developed and developing countries, the number of diabetic patients is increasing globally because of

diverse changes in diets in all cultures, It has been predicted that the number of diabetic patients will double from 143 million in 1997 to about 300 million by 2025 largely because of dietary intake and other lifestyle factors [2].

Liver act as one of the most important tissue in the body, studied found that liver disease is associated with diabetes, patients who have diabetes are twice as likely to have a liver disease as well, one of the biggest problems is that liver disease associated with diabetes, often goes undetected until it is too late, excessive glycogen may accumulate in liver due to defective activation of glycogen synthase[3]. Diabetes also associated with fat accumulation and cause fatty liver steatohepatitis, In addition to it's association with other liver disease like liver cirrhosis, cholelithiasis, and cholecystitis[4].

Antioxidant defense mechanisms involve both enzymatic and non enzymatic strategies. Common antioxidants include the vitamins A, C, and E, glutathione, and the enzymes superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase. Vitamins A, C, and E are diet-derived and detoxify free radicals directly. They also interact in recycling processes to generate reduced forms of the vitamins[5]. Tocopherol is reconstituted when ascorbic acid recycles the tocopherol radical; dihydroascorbic acid, which is generated, is recycled by glutathione, Vitamin E, a component of the total peroxyl radical-trapping antioxidant system [6], reacts directly with peroxyl and superoxide radicals and singlet oxygen and protects membranes from lipid peroxidation, The deficiency of vitamin E is concurrent with increased peroxides and aldehydes in many tissues.

There have been conflicting reports about vitamin E levels in diabetic animals and human subjects. Plasma and/or tissue levels of vitamin E are reported to be unaltered [7], increased [8], or decreased[9] by diabetes. Discrepancies among studies in terms of preventive or deleterious effects of vitamin E on diabetes induced vascular aberrations may arise from the variety of examined blood vessels or the administered dose of vitamin E.

Aim of study:

Type 1 diabetic rabbits were used to investigate various assays/liver tissue to identify and compare the role of oxidative stress markers in correction oxidative stress associated with diabetes mellites

Materials and methods

Animals:

Following 7 days acclimation period, 50 juvenile female, clinically healthy newzealand white rabbits were fastened for about 12 hrs then injected with alloxane monohydrate (BHD-England) (100mg/kg body weight) via marginal ear vein in order to induce type 1 diabetes mellitus, blood glucose measurement occur after 3 days, if it more than 200 mg/dl indicate diabetes onset.

Rabbits were randomly separated in to five groups each contain ten animals (G1) control normal, (G2) diabetes group treated with alloxane monohydrate 100 mg/kg only, (G3) diabetes and vitamin C group treated with alloxane monohydrate then supplemented orally with two doses of ascorbic acid 21 mg/kg between these two dose 10 hrs, (G4) diabetes and vitamin A group treated with alloxane monohydrate then ditary supplemented with 2000 IU/kg vit A. (G5) diabetes and α-tocopherole group treated with alloxane monohydrate then ditary supplemented with 500mg /day α-tocopherole.

Sample collection:

Initial body weight was obtained prior to induction of diabetes or antioxident supplementation. Subsequent body weight gain and samples measurements were taken at the end of experiment (10 weeks), whole blood was collected via cardiac puncture from anasthesized (ketamine 50mg/kg-xylazine 10mg/kg), rabbit were then euthanized with a single cardiac injection fatal plus (concentrated pentobarbital, 360 mg/kg).

Liver and pancreas tissues were collected in For histopathological studies, while some liver tissues homogenized on ice for 20 second then centrifuged at 30000 cc for 30 minutes at 4˚c and the supernatant collected for laboratory studies.

Blood chemistries

Blood glucose, ALT, AST, alkaline phosphatase and total protien concentration in plasma were determined using commercially available kit (sigma), glutathione peroxidase determined as in paglia and valentine method 1967[10], while total superoxide dismutase (SOD) activity was determined in plasma and liver according to a modified assay described by Pence and Naylor 1990[11].

Statistical analysis

Mean±SE was carried. Data were analyzed by Duncan's multiple range test [12] to determine if the treatment were significantly ($P \leq 0.01$) different or not.

Results

Alloxane treated rabbits were indeed diabetic after three days as indicated by plasma glucose level greater than 200 mg/dl and remain

hyperglycemic throughout the last week of experiment. Table (1) showed that body weight gain and glucose level in G3, G4 and G5 were significantly ($P \leq 0.01, 0.05$) best than that G2 group but at the same time glucose level of these groups was significantly higher than G1 group. There is no significance variation between G3 and G5 neither between G3 and G4 groups, while glucose level of G3 and T5 groups were significantly best than G4 group.

Table (1) showed that Alanine aminotranspherase (ALT), alkaline phosphatase levels in G2 were significantly higher than all groups, G5 level of these parameters were significantly more best than G3 and G4 groups, on the other hands G3 level for these parameters were significantly more best than G4 group.

Aspartate aminotranspherase (AST) level in G2 were significantly more than all groups, G5 group was significantly more best than G3 and G4, but there were no significant variation between G3 and G4 groups.

Total protein level for G1 as present in table 1 was significantly more best than all groups, G5 group was significantly higher than G4 and G3 groups, on the other hand G3 level was significantly more than G4 group, but there was no significant variation between G4 and G2 groups

Glutathione peroxidased and superoxide dismutase activity in G1 were significantly higher than all treatment groups as present in table (1), at the same time glutathione peroxidase level in G5 was significantly higher than G2, G3 and G4, there is no significant differences between T3 and G4 and between G2 and G4. Superoxide dismutase level in G3 and G5 groups significantly did not differ but these two groups were significantly higher then G4 as explaining.

Table 1 explain role of dietary ascorbic acid, Vit.A and α-tocopherole on different biochemical parameters of diabetic rabbit

biochemical parameters	G1 normal group	G2 diabetic only	G3 Vit. C + diabetic	G4 Vit. A + diabetic	G5 α-tocopherole+ diabetic
Body weight gain	1.5±0.091 d	0.225±0.03 a	0.63±0.02 bc	0.51±0.03 b	0.875±0.067 c
Glucose	172.4±1.359 a	389.4±8.668 d	265.8±2.303 b	342±8.666 c	247±2.38 b
ALT	42.4±0.702 a	68±0.447 e	58.6±0.221 c	62.4±0.266 d	51.4±0.426 b
AST	50±0.365 a	85.5±0.619 d	62±0.365 c	62.4± 0.6 c	54±0.21 b
Alkaline phosphatase	15±0.21 a	38±0.365 e	25.1±0.378 c	29±0.699 d	23±0.36 b
Superoxide dismutase	119.5±2.733 d	53±1.527 a	95±3.496 c	75.1±1.1 b	101.8±2.164 c
Glutathione peroxidase	460.5±45.273 d	142.5±3.67 a	223.9±1.206 b	170.8±2.878 ab	345.3±8.811 c
Total protien	6.7±0.213 e	3.5±0.129 a	4.5±0.138 c	3.8±0.133 a	5.19±0.246 d

Results are expressed as mean ± standard error.
Different letters between groups refer to significant variation under ($p<0.01$).
Degree of freedom :4, 36

These result were supported by histopathological examination of liver and pancreas.

Results indicate marked impreovement in diabetic quality of ascorbic acid, Vit.A and α-tocopherole treated groups, the biochemical finding of present study are confirmed with histopathological alteration observed in the animals organs who treated with dietary ascorbate, vit A and α-tocopherole groups, the degenerative signs that present in liver fig 1 (B, C and D respectivly) and pancreas fig 2(B, C and D respectivly) of these two animal groups are mild or less sever than diabetic T2 group.

Oral exposure to alloxane caused significant decrease in population of pancreatic islet cells fig 2 (A), atypical histological feature which may be compared with development of insulin dependant diabetes mellitus caused by beta cell injury from alloxane substance, oxidative stress has been

recognized as a major component in the chain of pathological events that express degenerative signs in liver fig 1(A) for T2 animals group by production of free radicals, glucose oxidation is the main source of free radicals.

Fig 1 (A)

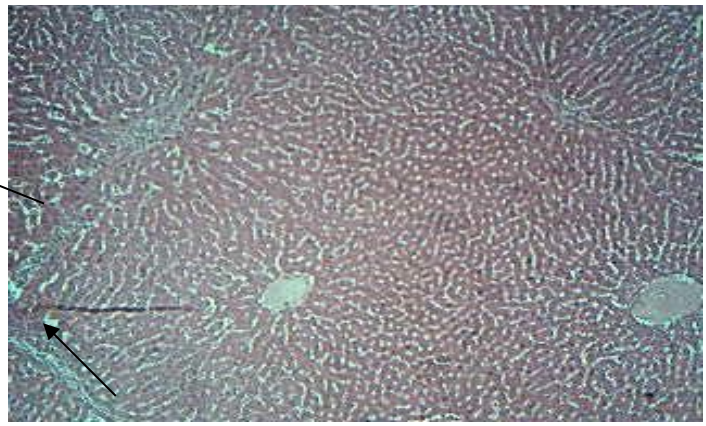
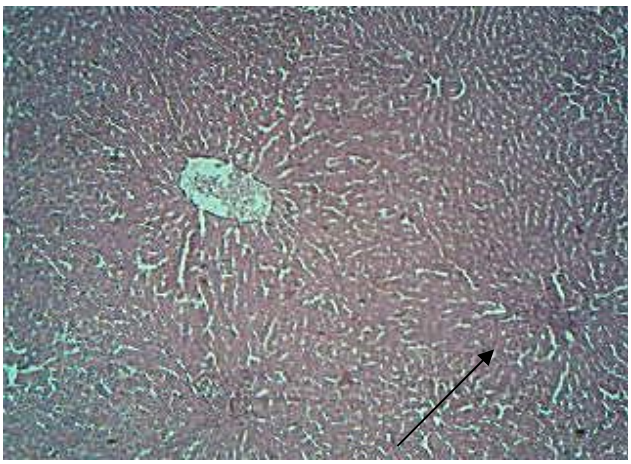


Fig 1 (**B**)

Fig 1 (C)



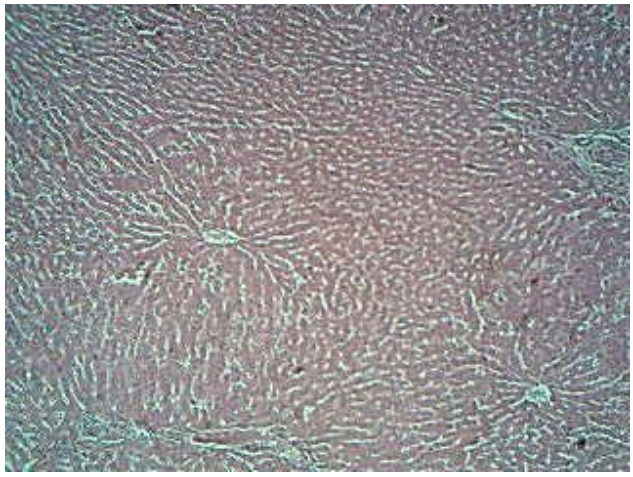


Fig 1 (D)

Photomicrographs of haematoxylin and eosin stained sections of rabbit liver; **(A)** Group represented a severe congestion of the hepatic tissue with dilation of central vein, nicrosis and fibrosis also present **(B)**, **(C)** and **(D)** Groups indicated a moderate congestion and dilation of central vein with less degenerative signs in hepatocytes. (H&E, 10×).

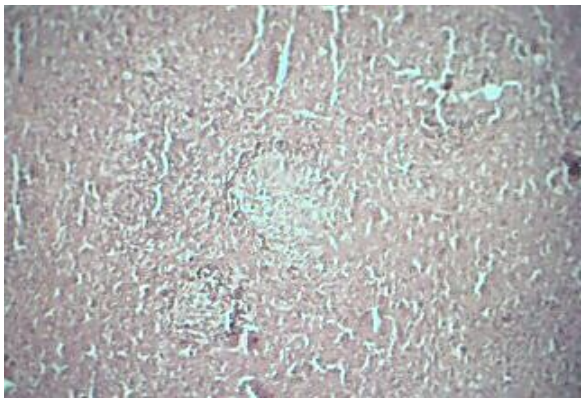


Fig 2 (A)

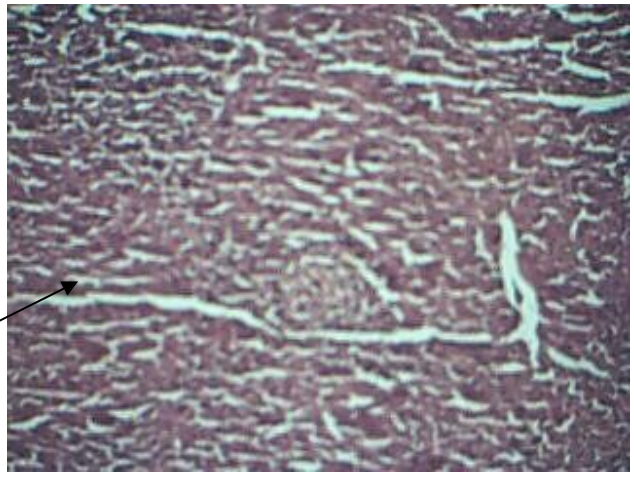


Fig 2 (B)

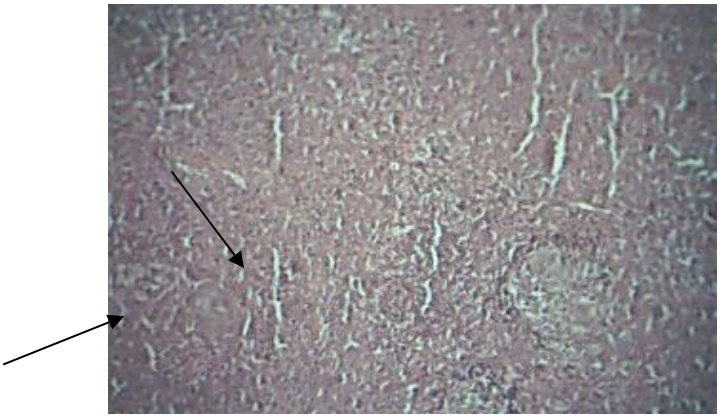


Fig 2 (C)

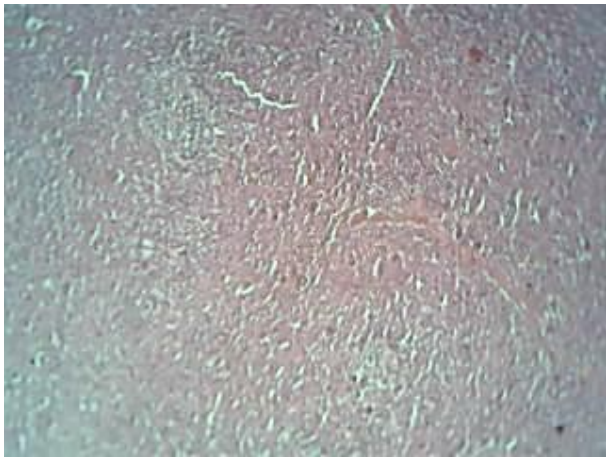


Fig 2 (D)

Photomicrographs of haematoxylin and eosin stained sections of rabbit pancrease; **(A)** Group represented degeneration of langerhans islets by alloxane monohydrate, **(B)**, (C) and (D) fig. showed less sever or mild degeneration , (H&E, 10×).

Discussion

Oxidative stress depicts the existence of products called free radicals and physiological conditions but become deleterious when not being quenched by the antioxidant systems[13] There are convincing experimental and clinical evidences that the generation of reactive oxygen species is increased in both type of diabetes and that the onsets of diabetes is closely associated with oxidative stress[14] Free radicals are formed disproportionately in diabetes by glucose autoxidation, polyol pathway and non-enzymatic glycation of protein[15] Abnormally high levels of free radicals and stimultaneous decline of antioxidant defense systems can lead to the damage of cellular organells and enzymes, increased lipid peroxidation and development of complications of diabetes mellitus [16].

In the present study, we examined oxidative stress pathway markers in the diabetic rabbits as compared to normoglycemic animals, from the results obtained it is evident that the diabetic rabbits had much higher glucose levels when compared with normoglycemic, the increase in blood glucose level and decreased insulin level depend upon the degree of β-cell destruction [17].

The increased level of glycosylated hemoglobin was observed in the diabetic G2 animals and this increase is directly proportional to the blood glucose level [18]. This suggest the increase in oxidative stress due to hyperglycemia, alloxane exert it's effect through it's reduction by glutathione to dialuric acid, in which redox recycling process generates ROS that damaged β cell, furthermore transition metals such as iron and copper which are potentially involved in the generation of hydroxyl free radicals are also involved in alloxane mediated killing of beta cells[19].

Our results refer to improvement in glucose concentration of antioxidants treated animals, as it present in G3 animals group, since vitamine C is an important antioxidant capable of scavenging oxygen free radicals, vit. C is structurally similar to glucose and can replace it in many chemical reaction and thus is effective in prevention of non enzymatic glycosylation of protein, Results also refer to improvement in G4 and G5 animals group, since vit. A and E share the same mechanism of free radical scavenging which may be have the same similar effect, Vit. A supplementation also may have an effect on chemical induced diabetes mellitus[20] .

Results refer to significant increase in the level of ALT and AST for G2 group as compared with all groups, these enzymes which involved in intermediary metabolism and stored in hepatocytes are released when hepatocytes are actually damaged, increased in serum concentration of these enzymes provide important clues to involvement of hepatocytes, ALT and AST are sensitive test of hepatocytes injury although often referred to as liver function test they don't measure hepatocytes but instead hepatocytes damage [21] decrease values of these enzymes for G3,G4 and G5 may be due to antioxidant activity for these antioxient in reduction of oxidative damage resulted from diabetes.

Results also refer to significant increament in alkaline phosphatase for G2 group as compared with antioxidant groups G3, G4 and G5, alkaline phosphatase is related with liver dysfunction this may be due oxidative damage resulted from diabetes mellitus, antioxidant activity for G3, G4 and G5 play important role in improvement of liver integrity[22].

Glutathione peroxidase and superoxide dismutase are two antioxidant enzymes act as substrates for free radicals hydrogen peroxide and superoxide respectively, these enzymes are indicator for oxidative stress status in body and their concentration in tissues are more reliable indicator than concentration in blood, their significant decrease in liver of diabetic G2 rabbits may be indicative of exhaustion of these enzymes as a consequence

of increased oxidative stress[23], While G3, G4 and G5 groups present more improvement in antioxidant activity.

Results refer to improvement in the level of total protein for G3, G4 and G5 groups as compared with G2 group, oxidative stress increase protein catabolim in addition to formation large quantity of urea and uric acids [24] while antioxidant activity for antioxidant treatment group play important role in remove free radicals and increase amino acids metabolism thus play important role in elevation the rate of protein synthesis[25].

Both fig 1(A) and fig 2(A) showed degenerative signs in liver and pancrease of diabetic rabbits, the increment of oxygen free radicals production is associated with low intracellular magnesium concentration and prior magnesium deplession make cell more sensitive to oxidative damage[26] that magnesium posses antioxidant properties by scavenging free radicals and affecting the rate of dismutation of superoxide ion [27]. Fig 1 (B, C, and D) and fig 2 (B,C and D) showed improvement in histopathological lesion of liver and pancreas, Vit E has been demonstrated to protect against magnesium deficiency-induced myocardial injury [28], on the other hand the effect of vitamin E on intracellular magnesium content lead to reduction in intracellular calcium thus resulting in improved smooth vascular cell relaxation [29]. Vitamin C has also been studied in diabetes, It plays a major role in regenerating vitamin E from the α-tocopheroxyl radical[30].

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دور مضادات التاكسد في تصحيح قصور الكبد الناتج من الاجهاد التاكسدي في الارانب المصابة تجريبياً بداء السكري

من قبل

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اجري التعرف على المعايير الكيموحيوية في الأرانب المصابة تجريبيا بداء السكري بفعل مادة alloxane monohydrate مع مقارنة مدى تأثير مضادات الاكسدة حامض الاسكوربيك, فيتامين A والفا توكوفيرول في تصحيح قصور الكبد المستحث بفعل الاجهاد التاكسدي الناتج عن داء السكري. إذ تم قياس مقدار الزيادة الوزنية ومستوى الكلوكوز في الدم وانزيمات الكبد (النين امينو ترانسفريز, اسبارتيت امينو ترانسفريز والكالين فوسفاتيز) في البلازما إضافة إلى التعرف على فعالية الإنزيمات المضادة للأكسدة والتي تعتبر قواعد للجذور الحرة في أجسام الأرانب بعد الأسبوع العاشر من التجربة. تم تقسيم هذه الحيوانات الى خمسة مجاميع ضمت كل مجموعة عشرة حيوانات مجموعة سيطرة G1 ومجموعة معاملة ثانية G2 أصيبت تجريبيا بداء السكري, مجموعة معاملة ثالثة G3 تم إحداث داء السكري فيها واعطيت جرعتين من حامض الاسكوربيك, مجموعة معاملة رابعة 4G تم إحداث داء السكري فيها واعطيت فيتامين A ومجموعة معاملة خامسة 5G تم إحداث داء السكري فيها واعطيت ألفا توكوفيرول. بينت النتائج وجود فروقات معنوية عند مستوى أحتمال ($P\leq0.01$) في مقدار الزيادة الوزنية ومستوى كلوكوز الدم في المجاميع الثالثة 3G والرابعة 4G والخامسة G5 مقارنة بالمجموعة الثانية 2G كما تبين وجود انخفاض معنوي ($P\leq0.01$) في مستوى انزيمات الكبد في مجاميع المعاملة الثالثة ل G والرابعة 4G والخامسة G5 مقارنة بمجموعة المعاملة الثانية 2G بينما تبين النتائج ارتفاع معنوي في مستوى الإنزيمات المضادة للاكسدة في مجاميع المعاملة الثالثة G3 والرابعة G4 والخامسة G5 مقارنة بمجموعة المعاملة الثانية 2G. كما تم دراسة التغيرات النسيجية في الكبد والبنكرياس وجاءت نتائج التقطيع النسيجي موافقة للنتائج الكيموحيوية والإنزيمية والتي تبين وجود تحسن في مجاميع المعاملة الثالثة G3 والرابعة G4والخامسة G5. بصورة عامة اشارت الدراسة إلى انخفاض نسبة تدمير خلايا الكبد بفعل الإجهاد التأكسدي الناتج من داء السكري من خلال العوامل المضادة للاكسدة حامض الاسكوربيك ,فيتامين A وألفاتوكوفيرول, أفضل المجموعات كانت مجموعة المعاملة الخامسة.