Comparative study for some antioxidants in correction of oxidative stress markers for experimental induced diabetic rabbits

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Abstract

Determination of reliable biochemical parameters of experimental diabetic rabbit by alloxane monohydrates 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 lesion were determined over 10 week in new Zealand white rabbits: control T1 group, diabetic T2 group, diabetic and ascorbic acid T3 group, diabetic and Vit. A T4 group, diabetic and α-tocopherole T5 animals group. each of dietary ascorbic acid, vit. A and α-tocopherole that given to animals of T3, T4 and T5 respectively were significantly (P≤0.01) reduced glucose level in blood than T2 diabetic group, also these antioxidants improve levels of cholesterol and triglyceride. Enzymatic activity of liver glutathione peroxidase and superoxide dismutase were significantly increased more than of diabetic animals T2. histopathological structures of liver and pancrease confirm these results which indicate mild congestion and less degenerative signs in hepatocytes with mild degeneration of langerhans islets, generally oxidative stress resulted from diabetes and 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, a common metabolic disorder resulting from defects in insulin secretion or action or both, is characterized by hyperglycemia often accompanied by glycosuria, polydipsia, and polyuria (1). During diabetes, persistent hyperglycemia causes increased production of free radicals especially reactive oxygen species (ROS), for all tissue from glucose auto-oxidation and protein glycosylation (2). Free radicals are generated as by-products of normal cellular metabolism, however, several conditions are known to disturb the balance between ROS production and cellular defence mechanism. This imbalance can result in cell dysfunction and destruction resulting in tissue injury, the increase in the level of ROS in diabetes could be due to their increased production and/or decrease destruction by nonenzymatic and enzymatic catalase (CAT), glutathione peroxidase (GSH-px) and superoxide dismutase (SOD) antioxidants the level of these antioxidant enzymes critically influences the susceptibility of various tissues to oxidative stress and is associated with the development of complication in diabetes. Also this is particularly relevant and dangerous for the beta islet, which is among those tissues that have the lowest levels of intrinsic antioxidant defenses (3). On the one hand hyperglycemia engenders free radicals, on the other hand it also impairs the endogenous antioxidant defense system in many ways during diabetes (4). Antioxidant defense mechanisms involve both enzymatic and nonenzymatic 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. These vitamins also foster toxicity by producing prooxidants under some conditions.
Vitamin E, a component of the total peroxyl radical-trapping antioxidant system, 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, increased, or decreased 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:**
This study was conducted to identify the role of oxidative stress markers in oxidative stress correction associated with type 1 diabetes mellitus in rabbits.

**Materials and methods**

**Animals:**
Following a 7 days acclimation period, 50 juvenile female and male, new zealand (NZW) 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 hyperglycemia was measured after 3 days for checking the occurrence of diabetes onset. Rabbits were randomly separated into five treated groups each include ten animals (T1) control normal, (T2) diabetes group treated only with alloxane monohydrate 100 mg/kg, (T3) diabetes and vitamin C group treated with alloxane monohydrate then supplemented orally with two doses of ascorate 21 mg/kg with 10 hrs intervals between each dose, (T4) diabetes and vitamin A group treated with alloxane monohydrate then dietary supplemented with 2000 IU/kg vit A. (T5) diabetes and α-tocopherole group treated with alloxane monohydrate then dietary supplemented with 500mg /kg α-tocopherole.

**Sample collection:**
Initially body weight was obtained prior to induction of diabetes or antioxidant supplementation. Subsequently body weight gain and samples measurements were taken at 10 weeks, whole blood was collected via cardiac puncture from anesthetized (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 for histological studies, while some liver tissues homogenized on ice for 20 second then centrifuged at 30000 cc for 30 minutes at 4ºc and the supernatant collected for laboratory studies (oxidative stress enzymes).

**Blood chemistries**
Glucose in blood, cholesterol and triglyceride concentration in plasma were determined using commercially available kit (sigma), glutathione peroxidase determined as in paglia and valentine method, while total superoxide dismutase (SOD) activity was determined in plasma and liver according to a modified assay described by Pence and Naylor 1990.

**Statistical analysis**
Mean±SE was carried. All data are analyzed using Duncan's multiple range test to determine if the treatment were significantly different or not.

**Results**
After three days alloxane treated rabbits were indeed diabetic 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 T3, T4 and T5 were significantly (P≤0.01) different or not.
between T3 and T4 groups, while glucose level of T3 and T5 groups were significantly lower (P≤0.01) than T4 group. Table (1) also showed that cholesterol and triglyceride levels in T2 were significantly higher (P≤0.01) than T3, T4 and T5, on the other hand there is no significant variation in the level of cholesterol between all antioxidant treatment groups, while triglyceride in T5 group was significantly reduced than T3 and T4 groups, there is no significant variation between triglyceride level of T3 and T4 groups. Glutathione peroxidase and superoxide dismutase activity in T1 were significantly higher (P≤0.01) than all treatment groups as present in table (1), at the same time glutathione peroxidase level in T5 was significantly higher than T2, T3 and T4, there is no significant differences between T3 and T4 and between T2 and T4. Superoxide dismutase level in T3 and T5 groups significantly did not differ but these two groups were significantly higher (P≤0.01) then T4 as explaining.

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

<table>
<thead>
<tr>
<th>biochemical parameters</th>
<th>T1 normal group</th>
<th>T2 diabetic only</th>
<th>T3 Vit. C + diabetic</th>
<th>T4 Vit. A + diabetic</th>
<th>T5 α-tocopherole + diabetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight gain</td>
<td>1.5±0.091 d</td>
<td>0.225±0.03 a</td>
<td>0.63±0.02 bc</td>
<td>0.51±0.03 b</td>
<td>0.875±0.067 c</td>
</tr>
<tr>
<td>Glucose</td>
<td>172.4±1.359 a</td>
<td>389.4±8.66 8 d</td>
<td>265.8±2.303 b</td>
<td>342±8.666 c</td>
<td>247±2.38 b</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>72.3±1.961 a</td>
<td>149.9±2.90 7 c</td>
<td>91.1±1.642 b</td>
<td>97.2±4.855 c</td>
<td>93.4±0.541 b</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>76.2±1.942 a</td>
<td>238±3.666 d</td>
<td>98±2.211 c</td>
<td>176.5±2.362 c</td>
<td>101.2±5.401 b</td>
</tr>
<tr>
<td>Glutathione peroxidase</td>
<td>460.5±45.27 3 d</td>
<td>142.5±3.67 a</td>
<td>223.9±1.206 b</td>
<td>170.8±2.878 ab</td>
<td>345.3±8.811 c</td>
</tr>
<tr>
<td>Superoxide dismutase</td>
<td>119.5±2.733 d</td>
<td>53±1.527 a</td>
<td>95±3.496 c</td>
<td>75.1±1.1 b</td>
<td>101.8±2.164 c</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± standard error.

a: no significant variation

Different letters between groups refer to significant variation under probability (p<0.01).

Degree of freedom: 4, 36

These results were supported by histopathological examination of liver and pancreas. Results indicate marked improvement in T3, T4, and T5 groups as compared with T2 group, the biochemical finding of 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 respectively) and pancreas fig 2(B, C and D respectively) of these two animal groups are less sever than diabetic T2 group.
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 arrow, necrosis and fibrosis also present (B). (C) and (D) groups showed a moderate congestion and dilation of central vein with less degenerative signs in hepatocytes. (H&E, 10×).
Photomicrographs of haematoxylin and eosin stained sections of rabbit pancreas; (A) Group represented degeneration of langerhans islets by alloxane monohydrate, (B), (C) and (D) fig. showed mild degeneration, (H&E, 10×).
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 simultaneous decline of antioxidant defense systems can lead to the damage of cellular organelles 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 \( ^{(17)} \), regression in body weight gain and increment the level of glycosylated hemoglobin was observed in the diabetic T2 animals and these conditions were directly proportional to the blood glucose level \( ^{(18)} \). This suggest the increase in oxidative stress due to hyperglycaemia and subsequent protein glycation as presented in bad result of body weight gain and glucose level of T2 group. Alloxane exert it's effect through it's reduction by glutathione to dialauric 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 \( ^{(19)} \). Increment the activity of enzyme fatty acyleoxygenase A oxidase which resulted from hypoinsulnemia incites beta oxidation of fatty acids, resulting in lipid peroxidation \( ^{(20)} \) that impairs membrane function by decreasing membrane fluidity and changing the activity of membrane-bound enzymes and receptors. The products of lipid peroxidation are harmful to most cells in the body and are associated with a variety of diseases, such as atherosclerosis and brain damaged \( ^{(21)} \). In our study significant increase \( (p<0.01) \) of cholesterol and triglyceride observed in the plasma of diabetic T2 rabbits. Our result refer to improvement in body weight gain and glucose concentration of antioxidants treated animals, as it present in T3 animals group, since vitamin 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 that improve body weight gain, in addition, vit. C act as regular of catabolism of cholesterol to bile acid this effect the reduction of cholesterol and triglyceride level in T3 group \( ^{(22)} \). Our results also refer to improvement in T4 and T5 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, furthermore it is of interest that all trans β-carotene, reduced lesion formation in hypercholesterolemic rabbits, β-carotene is metabolized to retinoids which exert powerful effects on growth and differentiation via their interaction with two families of nuclear transcription factors, the retinoic acid receptors and retinoid X receptors, The retinoic acid receptor is activated by both all-trans retinoic acid and 9- cis retinoic acid, whereas the retinoid X receptor is selectively activated by 9-cis retinoic acid raising the possibility that genes specifically controlled by the retinoic acid receptor may inhibit atherosclerosis. Retinoic acid regulates the expression in cultured cells of several proteins implicated in atherogenesis, including
thrombomodulin and monocyte chemoattractant protein-1 \(^{(23)}\). Also in view of its antioxidant property, it may as well delay complication arising from diabetes disease \(^{(24)}\). Vit. E also improve glucose concentration in T5 animals group, the excess glucose present in blood react with hemoglobin to form HBa1c (glycosylated hemoglobin) HBa1c is used as a marker for estimation the degree of protein glycation in diabetes mellitus \(^{(25)}\), administration of \(\alpha\)-tocopherole to diabetic rabbit reduced the glycation of hemoglobin and thus decrease the level of glycosylated Hb, thus normalize glycosylated Hb, subsequently reduced glycated protein and improve body weight gain \(^{(20)}\). Vitamin E also improve lipid peroxidation of histological cell membrane by potent scavenging super oxide and other free radicals \(^{(27)}\). In addition vit E increase the bioavailability of nitric oxide that play important role in restore endothelial function this had significant role in cholesterol and triglyceride reduction \(^{(28)}\). 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 T2 rabbits may be indicative of exhaustion of these enzymes as a consequence of increased oxidative stress \(^{(29)}\). Liver glutathione peroxidase and superoxide dismutase were negatively correlated with plasma glucose, cholesterol and triglyceride levels \(^{(30)}\). The results of histopathological changes in diabetic group showed degenerative signs in liver and pancreas of diabetic rabbits, the increment of oxygen free radicals production is associated with low intracellular magnesium concentration and prior magnesium depletion make cell more sensitive to oxidative damage \(^{(31)}\) that magnesium posses antioxidant properties by scavenging free radicals and affecting the rate of dismutation of superoxide ion \(^{(32)}\). While, the histopathological changes in T3, T4 and T5 groups revealed improvement in tissue lesion of liver and pancreas, Vit E has been demonstrated to protect against magnesium deficiency-induced myocardial injury \(^{(33)}\), 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 \(^{(34)}\). Vitamin C has also been studied in diabetes, It plays a major role in regenerating vitamin E from the \(\alpha\)-tocopheroxyl radical \(^{(35)}\).

References

1. ADA (American Diabetes Association), (2005) Diagnosis and classification of diabetes mellitus. Diabetes Care 28 (suppl. 1), S37-S43.


myeloid cell lines. Blood. 84:2776-2782.


دراسة مقارنة لبعض مضادات الأكسة في تصحيح معلومات الإجهاد التاكسدي للإربات المقصبة تجريبياً بداء السكري

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الخلاصة

الاريخ على المعايير الحيوية في الأربات المقصبة تجريبياً بداء السكري بعمال مادة alloxane monohydrate مع مقارنة مدى تأثير مضادات الأكسة حمض الأسكوربيك، فيتامين A والفا توكوفيروول في تقليل الإجهاد التاكسدي الناتج عن داء السكري. إذ تم قياس مقدار زيادة الوزنية والكليه والكليه المتبقيات ثلاثة في البلازما إضافة إلى التعرف على فعالية مضادات الأكسة التي تعتبر قواعد للذكور في جسم الأرنك بعد الأسابيع الباشر من التجربة. تم تقسيم هذه الحيوانات إلى خمسة مجموعات ضمت كل مجموعة عشرة حيوانات مجموعات T1 ومجموعة معالمة ثانية T2. A أصيبت تجربياً بداء السكري، مجموعة معالمة ثانية T3 تم إحداث داء السكري فيها وأعطيت جرعتين من حمض الأسكوربيك، مجموعة معالمة ثانية T4 تم إحداث داء السكري فيها وأعطيت جرعة فيتامين A ومجموعة معالمة ثانية T5 تم إحداث داء السكري فيها وأعطيت الفا توكوفيروول. أوضحت النتائج إلى وجود فروقات معنوية (P<0.01) في مقدار زيادة الوزنية ومستوى كلووز الدم في المجاميع الثلاثة T1 والرابعة T4 والثانية T3 والرابعة T4 بالمقارنة بالمجموعة الثانية T2 كما تم قبول انخفاض معنوي T5 والعنت T3 بالمعالمة الثلاثة في مجموعات T3 والرابعة T4 والثانية T1 وتباين النتائج ارتفاع معنوي في مستوى الإتزامات مضادات الأكسة في T2 بينما تبين النتائج انخفاض معنوي في مستوى الإتزامات مضادات الأكسة في T2. كما تم دراسة التغيرات في الكبد والكليه واجت التطور النسيجي للكبد والكليه. ونحتاج إلى التأكد من النتائج الإيجابية والانكسجية التي تبين وجود خلاصة الكبد وجزر لونكيرات. بصورة عامة، تم التأكد من النتائج من داء السكري بفعل العوامل المضادة للأكسة حمض الأسكوربيك فيتامين A والفا توكوفيروول، أفضل المجموعات كانت مجموعة المعالمة الخامسة.