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Combined effect of vitamins C and E on zinc status, carbohydrate metabolism and antioxidant values in diabetic rats fed zinc-deficient diet

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Abstract. The aim of this study was to investigate the combined effect of vitamin C and vitamin E on carbohydrate metabolism, zinc status and antioxidant enzymes activities in diabetic rats fed low zinc diet. Female diabetic albino Wistar rats were randomly assigned into five groups. The first group received a diet containing a 54 mg zinc/kg diet (adequate zinc, AZ), the second group received a diet containing 1 mg zinc/kg diet (zinc deficient group, ZD), and the three other groups received ZD diet and treated orally with vitamin E (500 mg/kg body wt) (ZD+VitE), vitamin C (500 mg/kg body wt) (ZD+VitC), and combined vitamins C and E (250 + 250 mg/kg body wt) (ZD+VitC+VitE) respectively. Body weight and food intake were recorded regularly. After four weeks of dietary manipulation. Serum and tissues zinc concentration, alkaline phosphatase activity of low-zinc diabetic animals were significantly lower than those of the control diabetic animals. Dietary zinc deficiency also increased significantly blood glucose, HbA1c, cholesterol and triglycerides concentrations, glutamate oxaloacetate transaminase, glutamate-pyruvate transaminase activities, and malondialdehyde level of low zinc diabetic rats. In contrast, catalase and glutathione peroxidase activities and liver glutathione level were reduced. Vit C or vit E may have partial ameliorative effects on these disturbances, whereas vit C and vit E together assured a more efficient protection. In conclusion vitamin E and C act as beneficial and potent antioxidants protect against lipid peroxidation and oxidative damage due to zinc deficiency associated with diabetes mellitus.

Keywords: Vitamin E, vitamin C, zinc deficiency, diabetic rats, carbohydrate metabolism

1. Introduction

Diabetes mellitus is a chronic disease characterized by inappropriate hyperglycemia due to deficiency or resistance to insulin [1]. During diabetes, persistent hyperglycemia causes increased production of free radicals especially reactive oxygen species (ROS), for all tissues 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 defense mechanisms. This imbalance can result in cell dysfunction and destruction resulting in tissue injury. Free radicals may play an important role in the causation and complications of diabetes mellitus [3].

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Zinc also is involved in diabetes, plays a clear role in the regulation of insulin production by pancreatic tissues and glucose utilization by muscles and fat cells [4]. The abilities to synthesize and secrete insulin and use glucose are impaired in the zinc deficient state [5]. There are several reasons for suspecting that an abnormal zinc metabolism could play a role in the pathogenesis of diabetes mellitus, which is accompanied by sever oxidative stress (especially lipid peroxidation) it lead to an accelerate the development of cellular and vascular damage complication.

Vitamins C and E can be used as antioxidants separately or in combination. Both vitamins act synergistically [6] to defend against the damaging effects of high oxidative stress in diabetes. Vitamin C serves as an antioxidant, directly by scavenging aqueous peroxyl radicals, and indirectly by regenerating reduced vitamin E [7]. Vitamin E is a highly effective antioxidant and able to reduce the oxidative stress in many pathological conditions including diabetes mellitus. The protective effect of vitamin E on oxidative stress in diabetes mellitus may be mediated through inhibition of free radical formation [8].

So antioxidant treatment using vitamin C and vitamin E is necessary for prevention against lipids damage by oxygen free radicals in other words can preserve pancreatic beta-cell function [9]. Furthermore vitamin E is well known, in other words the V is minuscule not majuscule as an important antioxidant in biological system; it acts synergistically with other antioxidant -like vitamin C- in cells to protect them from damage and lyses induced by oxidative stress [10], for this it has been proposed as a prophylaxis against syndromes caused by oxygen toxicity and/or membrane instability such as diabetes mellitus. Therefore, this study was carried out to examine the combined effect of vitamin E and vitamin C supplementation on prevention of diabetic development in zinc deficient state by evaluating zinc status, carbohydrate metabolism, lipid peroxidation and antioxidant enzymes activities.

2. Materials and methods

2.1. Chemicals

Alloxan, 5, 5′dithiobis-(2-nitrobenzoic acid (DTNB), vitamin E (α-tocopheroll),vitamin C (ascorbic acid) were purchased from Sigma Chemical Co (St Louis, France). All other chemicals used in the experiment were of analytical grade.

2.2. Animals

Female albino (Wistar) rats of 10 weeks of age, weighing 200–250 g, were obtained from Pasteur Institute (Algiers, Algeria). Animals were acclimated for one week under the same laboratory conditions of photoperiod (12 h light/12h dark) with a relative humidity of 40% and room temperature of $22 \pm 2°C$. Standard rat food and deionized water were available *ad-libitum.*

2.3. Induction of diabetes and diet

Diabetes was induced with fresh alloxan monohydrate solution using a previously described method [11]. Alloxan was intraperitoneally administered at a dose of 150 mg/kg body weight dissolved in citrate buffer (0.01 M, pH 4.5). Blood glucose was measured 7 days after induction of diabetes on samples taken from tail vein. The diabetic state was confirmed by a glucose-meter (ACCU-CHEK, Roche Diagnostics, Paris, France) when the glucose concentration exceeded 14 mmol/l.

The diet for rats consisted of (g/kg diet): cornstarch 326, sucrose 326, protein 168 (egg white solids), lipids 80 (corn oil), fiber 40 (cellulose), vitamin mix (sigma) and mineral mix 40. The latter was formulated to contain either adequate (54 mg/kg) or deficient (1.2 mg/kg) quantities of Zn, as determined by atomic absorption spectroscopy. The mineral mix supplied (g/kg diet) calcium hydrogen orthophosphate 13; disodium hydrogen orthophosphate 7.4; calcium carbonate 8.2; potassium chloride 7.03; magnesium sulphate 4; ferrous sulphate 0.144; copper sulphate 0.023; potassium iodide 0.001, manganese sulphate 0.180 and zinc carbonate 0.1. The low Zn diet contained no additional zinc carbonate.

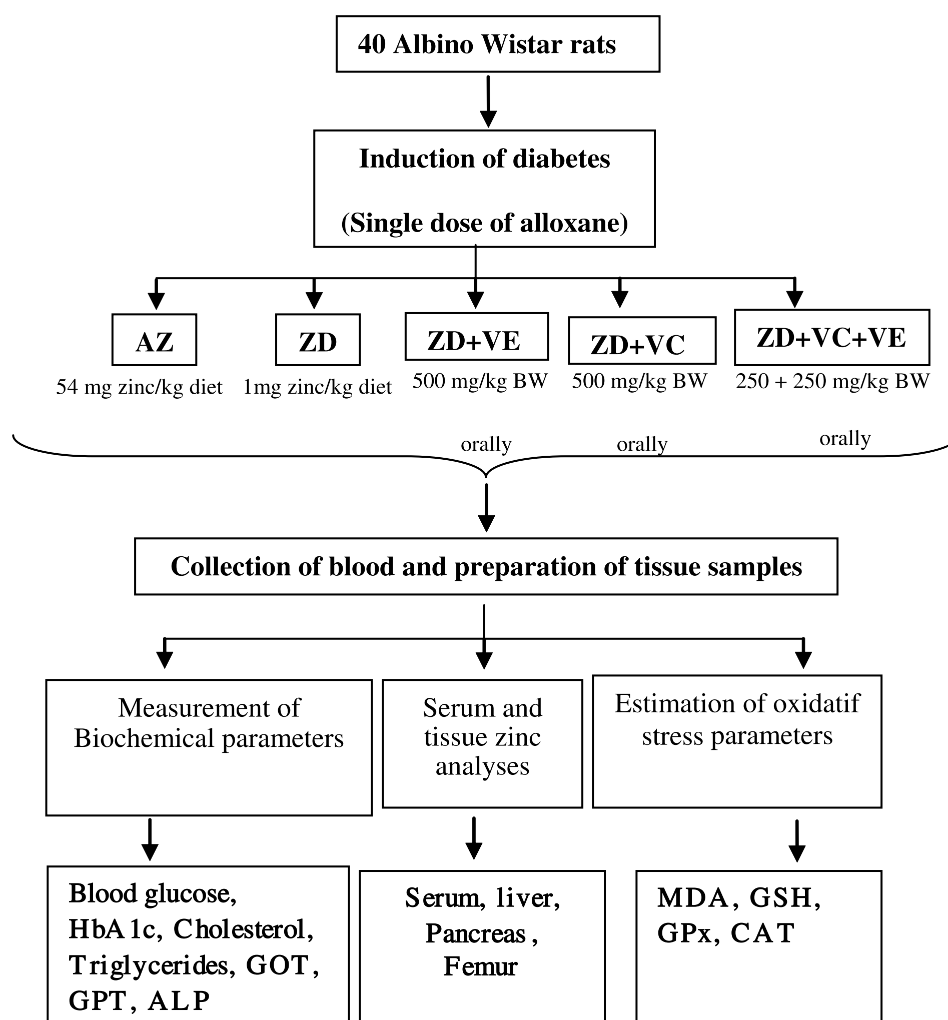

Fig. 1. Show the experimental design of the study.

2.4. Groups

The rats were randomly assigned into five groups of 08 animals each (Fig. 1). The first group received a diet containing a 54 mg zinc/kg diet (adequate zinc, AZ) [12], the second group received a diet containing 1 mg zinc/kg diet (zinc deficient group, ZD) for 28 days. The third and the fourth groups received ZD diet and treated orally with vitamin E (500 mg/kg) (ZD+vitE) and vitamin C (500 mg/kg) (ZD+vitC) respectively. The fifth group received ZD diet and in combination vitamins C and E (250 + 250 mg/kg body weight) (ZD+vitC+vitE) [10].

2.5. Blood collection and preparation of tissue samples

At the end of the experimental period (28 days) after overnight, fast rats were decapitated after being slightly anesthetized with diethyl ether and blood samples were transferred into ice cold centrifuge tubes, a portion was taken for HbA1c analysis. The serum was prepared by centrifugation for 10 min at 3000 revolutions/min and utilized for zinc, Cholesterol, triglycerides, transaminases and alkaline phosphatase assays. One fragment of liver were rapidly excised, weighed, freeze-clamped at −196°C, ground under liquid nitrogen and stored at −20°C for oxidative stress

parameters analysis. The pancreas and the second fragment of the liver were washed with isotonic saline (9 g sodium chloride/l distilled water) and blotted to dry. The right femur was taken and the connective tissues and muscle were removed. After that, the pancreas, the fragment of livers and femurs were weighed, dried at 80°C for 16 hours and zinc concentration in each tissue was determined.

2.6. Measurement of biochemical parameters

HbA1c % was measured by immunologic assay commercial kit (ref: AxSYM HbA1c 3L93-20) from whole blood. The activities of glutamate oxaloacetate transaminase (GOT), glutamate-pyruvate transaminase (GPT) and alkaline phosphatase (ALP) were determined with commercial kits from Spinreact, Spain (refs: GOT-1001161, GPT-1001171 and ALP-1001131). Cholesterol and triglycerides concentrations were also measured using commercial kits (Spinreact, refs: cholesterol-1001091 and triglycerides-1001311).

2.7. Serum and tissue zinc analyses

Dried liver, pancreas, and femur were heated in silica crucibles at 480°C for 48 hours and the ash taken up in hot hydrochloric acid (11.7 M) for Zn analysis by atomic absorption spectrophotometer (Pye Unicam SP 9000 Hitchin, UK). The accuracy of zinc recovery was checked using standard reference materials bovine liver and wheat flour. These standards were prepared and analyzed in similar conditions to the test items to assess recovery. Zinc in serum was analyzed after a twenty-fold dilution of the serum by Flame Atomic Absorption Spectrophotometer. In this case zinc standards were prepared from a 1 mg/ml zinc nitrate standard solution using 5% glycerol to approximate the viscosity characteristics, and to avoid zinc contamination from exogenous sources.

2.8. Antioxidant parameters estimations

2.8.1. Preparation of homogenates

About 1 gram of liver was homogenized in 2 ml of buffer solution of phosphate buffer saline 1:2(w/v; 1 g tissue 2 ml TBS, pH = 7,4), Homogenates were centrifuged at 10000× *g* for 15 minutes at 4°C, and the obtained supernatant was used for the determination of reduced glutathione and MDA levels, catalase and glutathione peroxidase activities.

2.8.2. Determination of malondialdehyde (MDA)

Liver homogenates were made prepared at 10% (w/v) in 0.1 mol/L Tris-HCl buffer, pH 7.4, and malondialdehyde (MDA) steady-state level was determined. MDA was measured according to the method described by Sastre [13]. Thiobarbituric acid 0.67% (w/v) was added to aliquots of the homogenate previously precipitated with 10% trichloroacetic acid (w/v). Then the mixture was centrifuged, and the supernatant was heated (100°C) for 15 min in a boiling water bath. After cooling, n-butanol was added to neutralize the mixture, and the absorbance was measured at 532 nm. The results were expressed as nmol of MDA/g tissue.

2.8.3. Estimation of reduced glutathione level (GSH)

The GSH content of liver homogenates was measured by the method of Ellman [14]. 1 g of liver was homogenized in 3 volumes of 5% TCA using Dounce homogenizer. The samples were centrifuged at 2000 rpm for 15 min. The supernatant (50 μl) was diluted in 10 ml phosphate buffer (0.1 M, *p*H 8). Consequently, 20 μl of DTNB 0.01 M was added to 3 ml of the dilution mixture. The measurement was performed at 412 nm against a control prepared in the same conditions using 5% TCA. The concentrations are expressed in mmoles of GSH/g of liver. They are calculated from a range of GSH, which was prepared with the same conditions as dosage did.

2.8.4. Determination of Glutathione peroxidase activity (GSH-Px)

Glutathione peroxidase (E.C. 1.1.1.1.9) activity was measured by the procedure of Floche and Gunzler [15]. Supernatant obtained after centrifuging 5% liver homogenate at 15000× *g* for 10 min followed by 10.000× *g* for 30 min at 4°C was used for GSH-Px assay. 1 ml of reaction mixture was prepared which contained 0.3 ml of phosphate buffer (0.1 M. pH7.4). 0.2 ml of GSH (2 mM), 0.1 ml of sodium azide (10 mM), 0.1 H_2O_2 (1 mM) and 0.3 ml of liver

supernatant. After incubation at 37°C for 15 min, the reaction was terminated by addition of 0.5 ml 5% TCA. Tubes were centrifuged at 1500× *g* for 5 min and the supernatant was collected. 0.2 ml of phosphate buffer (0.1 M pH 7.4) and 0.7 ml of DTNB (0.4 mg/ml) were added to 0.1 ml of reaction supernatant. After mixing, absorbance was recorded at 420 nm.

2.8.5. Assay of catalase activity (CAT)

The activity of catalase was estimated according to the method of Clairborne [16]. Determination of CAT activity depends on changes in absorbance result from the decomposition of H_2O_2 by CAT. This change is measured at 240 nm every min for 2 min. enzyme activity was expressed as unit per mg protein.

2.8.6. Protein determination

Protein concentration in the liver homogenates was determined by Bradford method, using bovine serum albumin (BSA) as a standard [17].

2.9. Statistical analysis

One-way analysis of variance (ANOVA) followed by *post hoc* Tukey–HSD test were used for data analysis. Results are presented as mean ± SEM. Values were considered statistically significant if $p < 0.05$.

3. Results

3.1. Daily body weight gain and food intake

Body weight gain and food intake are shown in Table 1. Body weight gain and food intake of diabetic zinc deficient animals (ZD) at the end of four week dietary manipulation were significantly lower than those of adequate zinc diabetic rats (AZ) ($p < 0.05$ and $p < 0.01$). Vitamin E, vitamin C supplementation, alone or in association increased the body weight gain and food consumption ($p < 0.05$ and $p < 0.001$).

Table 1

Mean Body weight gain, food intake, serum zinc, liver, pancreatic and femur zinc concentrations of AZ, ZD, ZD+VE, ZD+VC and ZD+VE+VC groups after four weeks of treatment

Parameters	Experimental groups				
Z	AZ ($n = 08$)	ZD ($n = 08$)	ZD+VE ($n = 08$)	ZD+VC ($n = 08$)	ZD+VE+VC ($n = 08$)
Body weight gain (g/day/rat)	3.53 ± 0.14	1.55^{*} ±0.65	$3.35^{a\mu}$ ± 0.12	2.40^{c} ± 0.82	2.20^{c} ± 0.57
Food Intake (g/day/rat)	15.48 ± 1.8	8.59^{**} ± 0.7	16.94^{c} ± 0.59	18.11^{c} ± 0.62	17.55^{c} ± 1.01
Serum zinc (µg/100 ml)	259.7 ± 37.1	115.1^{**} ± 33.3	$212.3^{b\beta}$ ± 7.5	241.9^{a} ± 46.1	283.8^{b} ± 22.2
Liver Zn (µg/g dry wt)	117.75 ± 9.37	56.09^{**} ± 11.21	106.35^{b} ± 13.56	127.3^{a} ± 46.1	123.81^{c} ± 5.69
Pancreatic Zn (µg/g dry wt)	198.69 ± 4.48	145.15^{**} ± 15.31	$204.36^{b\mu}$ ± 10.41	171.03^{a} ± 9.95	175.82^{a} ± 5.99
Femur Zn (µg/g dry wt)	225.5 ± 23.0	192.1^{*} ± 20.9	238.8^{a} ± 29.9	242.9^{a} ± 22.5	267.4^{c} ± 2.2

Statistically significant differences from AZ: $^{*}p < 0.05$, $^{**}p < 0.01$; from ZD: $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$; from ZD+VC+VE: $^{\mu}p < 0.05$, $^{\beta}p < 0.01$. Values are given as mean ± SEM, $n = 8$ number of animals.

Table 2
Mean blood glucose, HbA1c, serum cholesterol, and serum triglycerides concentrations of AZ, ZD, ZD+VE, ZD+VC and ZD+VE+VC groups after four weeks of treatment

Parameters	Experimental groups				
	AZ ($n = 08$)	ZD ($n = 08$)	ZD+VE ($n = 08$)	ZD+VC ($n = 08$)	ZD+VE+VC ($n = 08$)
Blood glucose (mg/100 ml)	343.6 ± 3.50	$399.0^{***} \pm 1.0$	$277.5^{c\mu} \pm 35.3$	$271.3^{c} \pm 41.0$	$243.0^{c} \pm 15.0$
HbA1c (%)	9.15 ± 1.16	$11.61^{**} \pm 0.62$	$7.66^{c} \pm 0.60$	$5.88^{c} \pm 0.11$	$6.17^{c} \pm 0.40$
Cholesterol (mg/dl)	0.94 ± 0.008	$1.79^{***} \pm 0.14$	$1.08^{*c\beta} \pm 0.08$	$0.70^{c\mu} \pm 0.09$	$0.83^{c} \pm 0.06$
Triglycerides (mg/dl)	2.47 ± 0.25	$3.03^{*} \pm 0.09$	$1.57^{b} \pm 0.32$	$1.37^{c} \pm 0.12$	$1.18^{c} \pm 0.12$

Statistically significant differences from AZ: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$; from ZD: $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$; from ZD+VE+VC: $^{\mu}p < 0.05$, $^{\beta}p < 0.01$. Values are given as mean ± SEM, $n = 8$ number of animals.

3.2. Serum, liver, pancreas and femur zinc concentration

The concentration of zinc in serum, liver, pancreas and femur are shown in Table 1 for all studied groups. These concentrations were significantly lower in the ZD group than in the AZ group ($p < 0.05$ and $p < 0.01$) and significantly higher in ZD+VE ($p < 0.05$ and $p < 0.01$), ZD+VC ($p < 0.05$) groups compared to ZD group, combined vitamin E and vitamin C treatments significantly elevated liver, femur ($p < 0.001$), serum ($p < 0.01$) and pancreatic ($p < 0.05$) zinc status compared to ZD group and a significant rise in serum ($p < 0.01$) and pancreatic ($p < 0.05$) zinc concentrations compared to ZD+VE group.

3.3. Blood biochemical values

Blood glucose, HbA1c, serum cholesterol, triglycerides, GOT, GPT, PAL values are shown in Table 2 and Fig. 2. Blood glucose ($p < 0.001$), HbA1c ($p < 0.01$), serum cholesterol ($p < 0.001$), triglycerides ($p < 0.05$), GOT ($p < 0.05$), GPT ($p < 0.05$) values were higher in ZD group than in AZ group. In contrast ALP activity ($p < 0.001$) of ZD was lower than those of AZ rats. The serum glucose ($p < 0.001$), HbA1c ($p < 0.001$), serum cholesterol ($p < 0.001$), triglycerides ($p < 0.01$ and $p < 0.001$), GOT ($p < 0.05$), and GPT ($p < 0.001$ and $p < 0.01$) values were significantly lower in ZD+VE and ZD+VC groups in comparison with ZD group. Meanwhile ALP activity ($p < 0.001$) was higher in these two groups ZD+VE and ZD+VC. Moreover combined vitamin E and vitamin C treatments significantly reduced blood glucose ($p < 0.001$), HbA1c ($p < 0.001$), serum cholesterol ($p < 0.001$), triglycerides ($p < 0.001$), GOT ($p < 0.001$), and GPT ($p < 0.01$) values and elevated ALP activity ($p < 0.001$) compared to ZD group and the administration of vitamin E and vitamin C in association improved blood glucose ($p < 0.05$), serum cholesterol ($p < 0.05$ and $p < 0.01$), GOT ($p < 0.05$), GPT ($p < 0.05$) and ALP ($p < 0.05$) activities in comparison with ZD+VE or ZD+VC.

3.4. Oxidative stress parameters

The mean level of MDA was significantly higher in diabetic animals fed low zinc diet as compared to the adequate zinc group ($p < 0.05$). MDA concentration markedly declined after vitamin E and Vitamin C administration ($p < 0.001$ and $p < 0.01$) compared to ZD group. Also there was a marked decrease in the GSH level ($p < 0.05$), GSH-Px ($p < 0.05$) and CAT ($p < 0.05$) activities in liver low zinc diabetic rats (ZD) as compared to AZ group. However, there was a significant rise of GSH level ($p < 0.05$ and $p < 0.001$), antioxidant enzymes activities: GSH-Px ($p < 0.05$), CAT ($p < 0.001$ and $p < 0.01$), in liver of diabetic rats fed zinc deficient diet after vitamin E and vitamin C supplementation (Figs. 3 and 4). In addition administration of vitamin E and vitamin C together improved in part MDA $p < 0.01$),

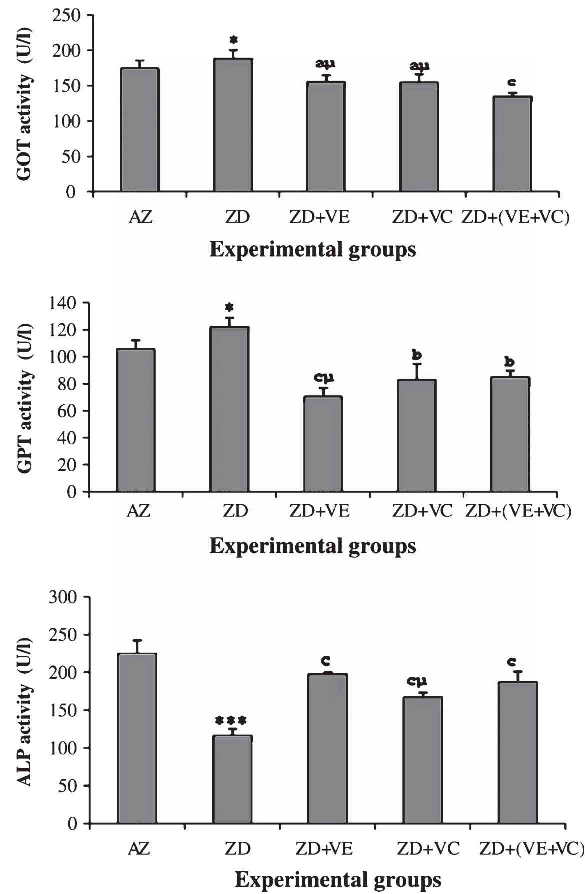

Fig. 2. Serum GOT, GPT and alkaline phosphatase activities of AZ, ZD, ZD+VE, ZD+VC and ZD+VE+VC groups after four weeks of treatment. Statistically significant differences from AZ: $*p<0.05$; $***p<0.001$; from ZD: $^{a}p<0.05$, $^{b}p<0.01$, $^{c}p<0.001$; from ZD+VE+VC: $^{\mu}p<0.05$. Values are given as mean ± SEM for group of 8 animals each.

GSH ($p<0.01$), GSH-Px ($p<0.01$) and CAT ($p<0.001$) compared to ZD group, and on the other part ameliorated MDA ($p<0.05$), GSH (p 0.05) and CAT ($p<0.001$) when compared either with (ZD+VE) or (ZD+VC) groups.

4. Discussion

Results of the present study showed that diabetic rats fed zinc deficient diet (ZD) had lower body weight gain compared with rats fed adequate zinc diet (AZ). This in agreement with some previously published reports [18]. This was due in part to decrease appetite, decrease food intake and impaired protein synthesis [19]. The body weight gain of diabetic rats fed low zinc diet treated with vitamin E (ZD+VE) or vitamin C (ZD+VC) was higher than that of zinc deficient diet (ZD) group, this in agreement with the results obtained by Stephens et al. [20] who noted an increase in body weight gain by older lambs supplemented with vitamin E. Similarly Danuel et al. reported that diabetic rats treated with vitamin C for four weeks showed a gain in body weight as compared with no treated groups [21].

Blood glucose and HbA1c have been affected by low zinc diet. The higher blood glucose level observed in low zinc fed animals might relate to altered glucose utilization by tissues or to the increased rate of endogenous glucose production [22], higher levels of HbA1c% and fasting glucose have been noted by other investigators [23]. Moreover, there are close relationships between zinc, insulin physiology and glucose metabolism, for that reason dietary zinc

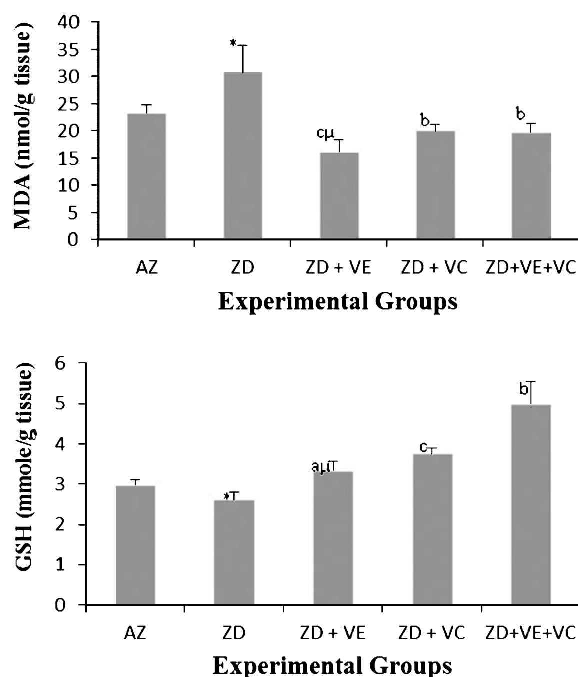

Fig. 3. Liver MDA and reduced glutathione levels in diabetic rats fed AZ, ZD, ZD+VE, ZD+VC and ZD+VC+VE after four weeks of treatment. Statistically significant differences from AZ: $^{*}p < 0.05$; from ZD: $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$; from ZD+VE+VC: $^{\mu}p < 0.05$. Values are given as mean ± SEM for group of 8 animals each.

may be involved in the maintenance of normal glucose homeostasis [24]. Blood glucose and HbA1c level was improved in all groups treated with vitamins compared to (ZD) group. In other words the findings showed markedly significant decrease serum glucose level, which indicates that both vitamin C and E exerted a hypoglycaemic effect probably through suppressing glycogenolysis rather than by enhancing peripheral tissue uptake of glucose. The hypoglycaemic action of combined vitamins C and E in diabetic animals was reported by various studies [25]. This might be due that vitamin E which alters insulin receptors in muscle and/or adipose tissues by increasing membrane motility and enhance glucose uptake by the diaphragm [26], or could be attributed to the competition of vitamin C with glucose for reaction with amino groups on the hemoglobin beta chain [27].

In the present study, results indicated also elevated serum cholesterol and triglycerides levels in (ZD) animals compared with (AZ) group, which are consistent with previous reports [28]. The treated groups: ZD+vitE, ZD+vitC and ZD+vitE+vitC have low cholesterol and triglycerides concentrations, these results are in line with that of Jain et al. [29], who reported that vitamin E supplementation can lower cholesterol and triglycerides levels in diabetic patients. Many reports accentuate that vitamin C improves lipid profile in diabetes, It was originally suggested that ascorbate is necessary for the transformation of cholesterol to bile acids by controlling the microsomal 7α-hydroxylation, as this reaction is the rate-limiting step of the cholesterol catabolism in liver, ascorbic acid deficiency induces a marked slowing down of this reaction, leading to cholesterol accumulation in liver and in blood. By contrast, ascorbate supplementation will accelerate the conversion of cholesterol into bile acids, decreasing cholesterol concentrations in liver and serum [30].

In this experiment there was a significant rise in serum GOT and GPT activities in diabetic rats, which could relate to excessive accumulation of amino acids (glutamate and alanine) in the serum of diabetic animals as a result of amino acids mobilization from protein stores [31]. These excessive amino acids are then converted to ketone bodies (a keto-glutaric and pyruvate) for which the enzyme GOT and GPT are needed, leading to increased enzyme activity. Supplementation of vitamin C and vitamin E reduce transaminase activities, it could be concluded that these vitamins is capable of ameliorating the impaired hepatocellular function [32].

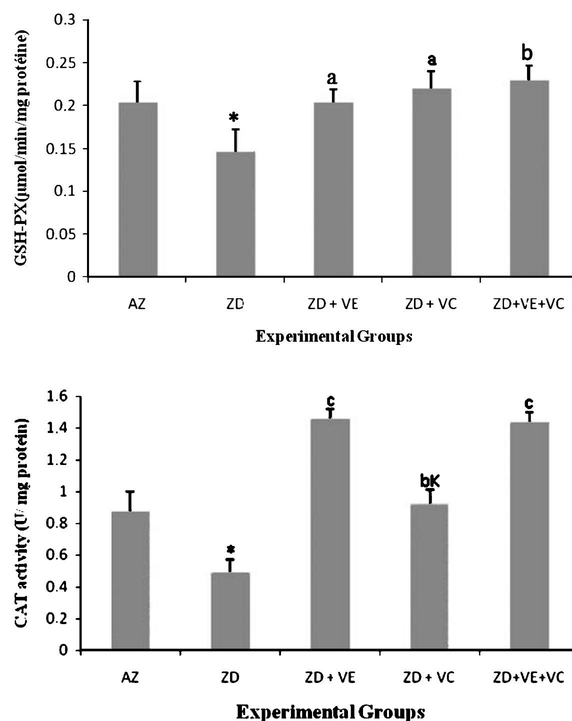

Fig. 4. GSH-PX and CAT activities in liver of diabetic rats AZ, ZD, ZD+VE, ZD+VC and ZD+VC+VE after four weeks of treatment. Statistically significant differences from AZ: $^{*}p < 0.05$; from ZD: $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$; from ZD+VE+VC: $^{K}p < 0.001$. Values are given as mean ± SEM for group of 8 animals each.

Serum alkaline phosphatase has been used as an indication of animal Zn status. Prasad [33] reported that many Zn-dependent enzymes activities have been shown to be affected adversely in Zn deficient tissues. Three enzymes, ALP, carboxypeptidase and thymidine kinase, appear to be most sensitive to Zn restriction in that their activities are affected adversely within 3–6 days of institution of a Zn-deficient diet to experimental animals. The present experiment demonstrated that zinc deficiency significantly decreases ALP activity, which is in agreement with the results of previously studies [34].

However, the increased activity of alkaline phosphatase in serum after vitamin C and vitamin E administration is probably a result of increased zinc concentration. Serum, femur, pancreatic and liver zinc concentrations in rats fed zinc deficient diet were lower than that of adequate zinc group. These findings, indicating the effect of low zinc diet on body zinc status, are in agreement with some investigations [35].

There was a significant increase in serum zinc concentration in the vitamin E and vitamin C supplemented groups. The reason for increased zinc concentration after vitamins supplementation is not clear. The existence of an interaction between zinc and vitamin E has been suggested. Hurley et al. [36] and Kechrid et al. (37) reported higher plasma zinc concentrations in rats supplemented with vitamin E.

Several studies demonstrated increased free radical production or increased oxidative damage in response to Zn deficiency *in vitro* or *in vivo* [38]. MDA levels are increased in ZD rats compared to AZ group. This data confirms the deleterious effect of zinc deficiency in increasing lipid peroxidation [39].

Zinc status has been shown to affect glutathione concentrations in tissues; in this investigation a reduction in GSH level, GSH-Px and catalase activities in liver of diabetic rats fed low zinc diet was noticed. Glutathione acts synergistically with zinc in protecting sulfhydryl groups, the reason for depletion of glutathione may have contributed to the higher consumption of glutathione and higher oxidative damage in zinc deficient rats [40]. In liver, the observed decline in GSH-Px and catalase activities might be due to modification of the sulfhydryl groups in this enzyme by oxygen free radicals [41]. However the administration of vitamins C and E decreased the degree of lipid peroxidation,

in other words reduced MDA concentration, improved liver GSH level and increased CAT and GSH-Px activities. The increased activity of antioxidant enzymes after supplementation of vitamin E indicates that vitamin E may restore the decreased overall antioxidant capacity in the diabetic animals. It is possible that vitamin E may have been adequate to metabolize the increased cellular peroxide to protect the enzyme activity [42]. Vitamin C is hydrophilic and is an important free radical scavenger in extracellular fluids, trapping radicals and protecting biomembranes from peroxidative damage. Naidoo and Lux [43] indicated that short-term intake of vitamin C (1 g/d) can significantly reduce plasma MDA levels.

5. Conclusion

In conclusion, the present study demonstrate that combined administration of vitamins C and E to diabetic rats fed low zinc diet attenuated hyperglycemia, improved lipid profile, ameliorated the depletion of serum and tissues zinc concentrations and protected against impaired oxidative damage. So preventive action of vitamins C and E may related to their antioxidant efficacy that inhibits lipid peroxidation and free radicals alterations due to the severity of zinc deficiency and diabetes mellitus.

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