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Comparative effects of zinc, selenium and vitamin E or their combination on carbohydrate metabolizing enzymes and oxidative stress in streptozotocin induced-diabetic rats

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Abstract. – *Objective:* **It is well documented that oxidative stress is a basic mechanism behind the development of diabetic state. The current study was undertaken to elucidate the hypoglycemic role of zinc, selenium and vitamin E and their mixture in comparison with the antidiabetic drug glibenclamide.**

Materials and Methods: **Male Wistar rats weighing 250 ± 50 g were made diabetic by injection with a single i.p. dose of streptozotocin (STZ) (65 mg/kg b. wt). Diabetic groups were simultaneously i.p. injected either with zinc chloride ($ZnCl_2$) (5 mg/kg) or with selenium and vitamin E (1.5 mg/kg as sodium selenite and vitamin E 1000 mg/kg) or with zinc, selenium and vitamin E each element i.p. injected according to its corresponding therapeutic dose daily for one month. Another group was orally treated daily with glibenclamide drug (5 mg/kg) for one month.**

Results: **Blood and tissue samples were collected at day 3 post STZ injection (from one group serum glucose level significantly elevated ≤ 300, $p \leq 0.05$) and at day 30 post-treatment in other groups. Liver function, nitric oxide (NO), malondialdehyde (MDA) and phosphoenol pyruvate carboxykinase (PEPCK) were significantly increased, while superoxide dismutase (SOD), reduced glutathione (GSH), total protein, lactate dehydrogenase (LDH), pyruvate kinase (PK) and hexokinase (HK) were inhibited after STZ treatment. Histological examination of diabetic liver showed necrosis and degenerative changes of hepatocytes. Treatment of diabetic rats with $ZnCl_2$, selenium and vitamin E or their combination blunted the increment in serum glucose induced by STZ, preserved liver architecture and ameliorated all the previous mentioned biochemical parameters.**

Conclusions: **It was found that, the combined administration of zinc, selenium and vitamin E exhibited a more remarkable effect than either zinc or selenium and vitamin E. So, the results clearly indicate the beneficial effects of micronutrients combination in controlling hyperglycemia.**

Key Words:

Diabetes, Zinc, Selenium, Vitamin E, Glycolytic enzymes, Liver function, Antioxidant.

Introduction

In recent years, various agents have been utilized in an attempt to prevent or delay the onset of type 1 diabetes in prediabetic patients or animals. In addition to immunosuppressive therapy, early prophylactic treatments of diabetic rats with insulin has been shown to delay the onset of diabetes while preserving the structure and function of the pancreas[1]. The mechanism by which exogenous insulin exerts its beneficial effect in preventing the onset of diabetes is believed to be due to a feedback inhibition of pancreatic insulin secretion. This ''resting'' of the pancreatic beta cells may slow their destruction and help preserve their function. Investigation of the pathophysiology of the secondary complications of diabetes is focusing increasingly on the role of oxidative stress in their initiation and progression. Micronutrients may exert protective or scavenging effects, as well as being essential components of several key enzymes in intracellular antioxidant defense. Their deficiency, or excess, may contribute to derangement of the pro-oxidant/anti-oxidant balance, and hence to the progressive appearance of secondary complications as the disease advances[2]. A cyclic process wherein glycation of proteins causes oxidation of associated lipids, which in turn stimulates autoxidative reactions of sugars in a contin-

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uous positive feedback loop may be in operation[3]. Micronutrients are involved in the complex processes of development of the secondary complications of diabetes mellitus in a number of different areas. They may be integral components of antioxidant enzymes (e.g., Cu, Zn and Mn in the case of the superoxide dismutases, and Se for glutathione dehydrogenase), cofactors in a variety of enzymatic processes of importance in glucose and lipid metabolism (e.g., Zn, Mn, Cu), or potential pro-oxidant catalysts (e.g., Cu, Fe). Other micronutrients which may influence the progression of diabetes are themselves recognized antioxidants, especially ascorbic acid (vitamin C) and α-tocopherol (vitamin E). Still other micronutrients are relevant to diabetes since it has been shown that glucose intolerance developed when these elements were deficient, either experimentally or due to nutritional inadequacy (e.g. chromium, copper, manganese, and possibly selenium). Zinc, an essential nutrient, has been shown to have a number of insulin coadjuvant properties. Zinc has been reported to enhance glucose uptake and transport in a number of tissues[4] while inhibiting pancreatic insulin secretion and increasing pancreatic insulin content[5]. Zinc deficiency and altered zinc metabolism have been reported in both human and animal diabetes and may be associated with decreased insulin sensitivity and impaired glucose tolerance[6]. In addition, to its insulin-like actions, zinc also plays an important role in immune system function and has recently been studied as a possible protective agent against free radicals injury[7], two factors implicated in the pathogenesis of diabetes. The biochemical functions of selenium and vitamin E are interrelated, in that both are essential components of the antioxidant defense system, and they appear to have synergistic and compensatory effects in induced deficiency states of one or the other[8]. Animals rendered deficient in either vitamin E or selenium show a marked increase in susceptibility to the diabetogenic effects of streptozotocin (STZ) with a combined deficiency of both having additive effects. By contrast, vitamin E supplementation protected against STZ diabetes induction[9]. Therefore, it could be hypothesized that increasing micronutrient, zinc selenium and vitamin E supply could ameliorate or reduce the severity of the disease in rodents subjected to diabetic condition. The objective of this study was to test this hypothesis in Wistar rats with type 1 diabetes induced by STZ.

Materials and Methods

Chemicals

All chemicals in the present study were of analytical grade, products of Sigma, Merck and Aldrich. All kits were the products of Biosystems (Alcobendas, Madrid, Spain), Sigma Chemical Company (St. Louis, MO, USA), Biodiagnostic (Cairo, Egypt).

Animals

Male Wister albino rats (250±50 g) were obtained from animal house of National Research Centre, Dokki, Giza, Egypt. Rats were fed on a standard diet and free access to tap water. They were kept for one week to be acclimatized to the environmental conditions.

Experimental Design

Sixty male albino rats were selected for this study and divided to 6 groups (ten rats in each group) as follows:

Group 1: normal healthy control rats.

Groups 2: considered as diabetic groups; where type 1 diabetes was induced by STZ. Each rat was injected intraperitoneally with a single dose of STZ (65 mg/kg body weight dissolved in 0.01 M citrate buffer immediately before use[11]. After injection, animals had free access to food and water and were given 5% glucose solution to drink overnight to encounter hypoglycemic shock[12]. Animals were checked daily for the presence of glycosuria.Animals were considered to be diabetic if glycosuria was present for 3 consecutive days. After 3 days of STZ injection fasting blood samples were obtained and fasting blood sugar was determined (>300 mg/dl). Hyperglycemic rats were used for the experiment and classified as follows:

Group 3: diabetic animals i.p. injected with zinc chloride ($ZnCl_2$, 5 mg/kg b.w; for 30 days[13].

Group 4: diabetic animals i.p. injected with selenium (1.5 mg/kg b.w. as sodium selenite) and a high dose of vitamin E (1000 mg/kg b.w.) for 30 days[14,15].

Group 5: diabetic rats i.p. injected with selenium (1.5 mg/kg BW as sodium selenite) and a high dose of vitamin E (1000 mg/kg b.wt.) in combination with 5 mg /kg $ZnCl_2$ for 30 days.

Group 6: diabetic rats administered orally antidiabetic glibenclamide drug 5 mg /kg body weight daily for one month.

Sample Preparations

After 30 days of treatments, rats were fasted overnight (12-14 hours), anesthetized by diethyl ether and blood collected by puncture of the subtongual vein in clean and dry test tube, left 10 minutes to clot and centrifuged at 3000 rpm for serum separation. The separated serum was used for biochemical analysis of liver function and serum total protein. After blood collection, rats of each group were sacrificed, the livers were removed immediately (a part was fixed in 10% formalin for histopathological examination), weighed and homogenized in 5-10 volumes of appropriate medium using electrical homogenizer, centrifuged at 4000 rpm for 15 min, the supernatants were collected and placed in Eppendorff tubes and stored at –20°C and used for determination of oxidative stress markers (nitric oxide: NO and malondialdehyde: MDA), antioxidant (reduced glutathione: GSH and superoxide dismutase: SOD) and carbohydrate metabolizing enzymes (hexokinase: HK, lactate dehydrogenase: LDH, pyruvate kinase: PK and phosphoenol pyruvate carboxykinase: PEPCK). The homogenization was carried out as described by Newsholme et al[16].

Histopathology

Liver specimens were fixed in 10% formalin, processed to paraffin blocks, sectioned (4 μm thick) and stained with hematoxyline and eosin. They were examined using light microscopy for demonstration of hepatic pathological changes including degeneration, atrophy, cell destruction and necrosis and the efficiency of micronutrients to ameliorate these pathological features[17].

Blood Biochemical Analysis

- Glucose was determined in serum by colorimetric assay according to Trinder[18];
- Alkaline phosphatase enzyme activity was measured by the method of Belfield and Goldberg[19];
- AST and ALT were measured by the method of Reitman and Frankel[20];
- Total protein was assayed in serum according to Bradford[21].

Liver Tissue Biochemical Analysis

HK was assayed in liver tissue homogenate according to Abrahao-Neto et al[22].

LDH enzyme activity was determined in liver tissue homogenate according to the method of Bergmeyer et al[23].

PK enzyme activity was determined in liver tissue homogenate according to Bucher and Pfleiderer[24].

PEPCK was determined in liver tissue homogenate according to the method of Suarez et al[25].

Lipid peroxidation was determined in tissue liver homogenate according to Ruizlarre et al[26].

NO was determined in liver tissue homogenate according to Moshage et al[27].

GSH was assayed in liver tissue homogenate according to Beutler et al[28].

SOD was assayed in tissue liver homogenate according to Paoletti et al[29].

Statistical Analysis

Data were analyzed by comparing values for different treatment groups with the values for individual controls. Results are expressed as mean ± SD. The significant differences among values were analyzed using analysis of variance (one-way Anova) coupled with post-hoc least significance difference (LSD) at $p \leq 0.05$.

Results

The present results demonstrate the biochemical effects of zinc with or without selenium and vitamin E as well as selenium and vit. E in comparison with the current available antidiabetic glibenclamide reference drug against liver disorders induced by reactive oxygen species in diabetic rats.

Table I and Figure 1 demonstrate the blood glucose and tissue levels of NO, MDA, GSH, SOD in normal, diabetic, different diabetic-treated groups. Diabetic group shows significant increase in blood glucose levels post injection of STZ: 360.00 ± 5.20 mg/dL, with percentage increase +225.79%. Significant amelioration is noticed in blood glucose levels in all diabetic-treated groups. The most pronounced synergistic effect for the micronutrients Zn +Se and Vit. E with percentage increase amounting +49.52 as compared to normal control, while glucose level reached to 151.50 ± 2.10 mg/dl in case of glibenclamide treatment with percentages increase +37.10%. Regarding to Zn and Se + vit. E-treated diabetic rats the percentages of increase in blood glucose level recorded +54.93 and +65.70% respectively as compared to normal control. Considering antioxidant markers, significant increase was noticed in NO and

Table I. Effect of different micronutrient treatments on nitric oxide, lipid peroxidaton and antioxidant enzyme activities in diabetic rate liver.

Parameters Groups	Normal control (1)	STZ (2)	STZ + Zn (3)	STZ + Se + Vit. E (4)	STZ + Zn + Se + Vit. E (5)	Glibenclamide (6)
Blood glucose	110.50 ± 3.21	360.00 ± 5.20	171.20 ± 2.30	183.10 ± 1.50	165.22 ± 3.21	151.50 ± 2.10
LSD	(2-6)	(1, 3-6)	(1, 2, 4, 5, 6)	(1, 2, 3, 5, 6)	(1, 2, 3, 4)	(1-5)
ANOVA	0.000	0.000	0.000	0.000	0.000	0.000
NO	43.68 ± 0.71	77.13 ± 1.00	60.21 ± 1.88	64.45 ± 1.96	57.60 ± 2.179	55.10 ± 1.0
LSD	(2-6)	(1, 3, 4-6)	(1, 2, 4-6)	(1, 2, 3, 5, 6)	(1, 2, 4)	(1, 2, 3, 4)
ANOVA	0.000	0.000	0.004	0.000	0.000	0.001
MDA	18.53 ± 1.19	120.51 ± 1.47	74.13 ± 2.66	85.60 ± 3.72	68.10 ± 2.57	43.17 ± 2.63
LSD	(2-6)	(1, 3, 4-6)	(1, 2, 4-6)	(1, 2, 3, 5, 6)	(1-4, 6)	(1-5)
ANOVA	0.000	0.000	0.007	0.000	0.007	0.000
GSH	3.40 ± 0.1	1.21 ± 0.03	2.07 ± 0.12	1.92 ± 0.049	2.57 ± 0.30	3.06 ± 0.06
LSD	(2-6)	(1, 3)	(1, 2, 5, 6)	(1,2,5,6)	(1-4, 6)	(1-5)
ANOVA	0.000	0.000	0.000	0.000	0.000	0.000
SOD	9.88 ± 0.67	1.41 ± 0.11	3.76 ± 0.21	3.21 ± 0.28	4.53 ± 0.18	6.87 ± 0.15
LSD	(2-6)	(1, 3-6)	(1, 2, 5, 6)	(1, 2, 5, 6)	(1-4, 6)	(1-5)
ANOVA	0.000	0.000	0.000	0.000	0.01	0.000

Data are means ± SD of ten rat in each group. Blood glucose level is expressed in mg/dl. MDA is expressed in µmole/min/g tissue. GSH and SOD are expressed in µ mole/mg protein/min. NO is expressed in µg/g tissue. Statistics is carried out using ANOVA test and the difference between groups (LSD) is analyzed by post-HOC (SPSS computer program), where $p < 0.05$.

MDA post STZ injection amounting to 77.13±1.00 and 120.51±1.47 µg/g tissue as compared to normal control, with percent of elevation amounting to +76.58 and +550.35% respectively. Although, a significant reduction was recorded for GSH and SOD with percentages amounting to –64.41 and –85.73% respectively. It has been easily noticed a significant amelioration in NO, MDA, GSH and SOD levels post treatment of diabetic rats with zinc amounting to 60.21±1.88, 74.13±2.66, 2.07±0.12 and 3.76±0.21 µg/g tissue respectively with percentages of increase recording + 37.84 and +300.05% for NO and MAD respectively and with percentages of inhibition –39.12 and –61.94% for GSH and SOD respectively as compared to normal control level. Less significant improvement has been also noticed in hepatic NO, MDA, GSH and SOD levels post Se + Vit. E-treatment with percentage increase +47.55, +361.95 for NO and MAD and percentage decrease –43.53 and –67.51% for GSH and SOD respectively. On the other hand, significant synergistic enhancement in antioxidants parameters was recorded in Zinc-supplemented with Se+Vit. E as compared to normal control, where NO and MAD percentage recorded +31.87and +267.51% over control rates while GSH and SOD amounted –24.41 and + Vit. E –54.25% respectively. Considering, glibenclamide, the antidiabetic drug, the percentage of NO and MDA reached to +26.14 and +132.97%, while GSH and SOD showed values of –10 and –30.47% respectively. With respect to glycolytic and gluconeogenic enzymes, significant inhibition in HK, PK, LDH, with significant increase in PEPCK were recorded in STZ induced diabetic rats (Table II and Figure 2). Significant amelioration was noticed in all treated-diabetic groups, with the most dramatic effect for the combined micronutrients Zn + Se + Vit. E

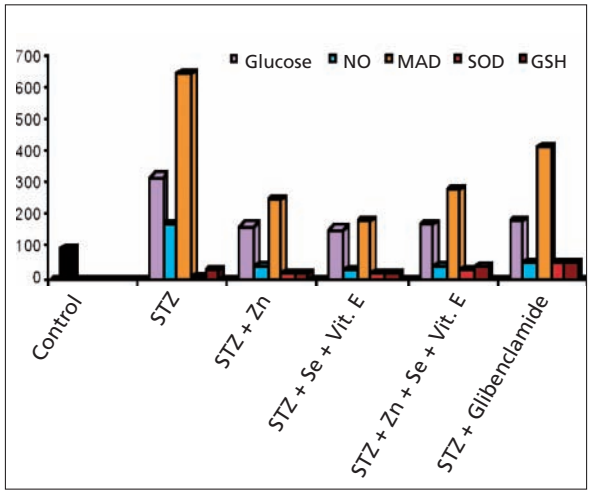

Figure 1. Improvement percent of blood glucose level and antixodant markers.

Table II. Effect of different micronutrient treatments on some glycolytic and gluconeogenic enzymes in diabetic rats.

Parameters Groups	Normal control (1)	STZ (2)	STZ + Zn (3)	STZ + Se + Vit. E (4)	STZ + Zn + Se + Vit. E (5)	Glibenclamide (6)
HK	105.12 ± 1.67	21.19 ± 1.69	49.62 ± 0.48	43.84 ± 3.18	54.18 ± 0.95	86.62 ± 3.89
LSD	(2-6)	(1, 3)	(1, 2, 4, 5, 6)	(1, 2, 3, 5, 6)	(1-4, 6)	(1-5)
ANOVA	0.000	0.000	0.000	0.000		0.000
PK	62.60 ± 2.45	20.43 ± 1.17	40.60 ± 1.73	34.63 ± 1.26	47.47 ± 2.03	54.10 ± 1.15
LSD	(2, 3, 4, 5, 6)	(1, 3)	(1,2,4-6)	(1,2,3,5,6)	(1-4, 6)	(1-5)
ANOVA	0.000	0.000	0.000	0.000	0.000	0.000
LDH	39.27 ± 0.80	14.41 ± 0.62	26.81 ± 0.24	26.12 ± 0.95	29.52 ± 0.90	33.47 ± 1.48
LSD	(2-6)	(1, 3-6)	(1, 2, 5, 6)	(1, 2, 5, 6)	(1-4, 6)	(1-5)
ANOVA	0.000		0.000	0.001	0.000	0.000
PEPCK	11.07 ± 0.60	19.16 ± 0.37	15.18 ± 0.38	15.46 ± 0.46	14.12 ± 0.11	12.97 ± 0.33
LSD	(2-6)	(1, 3-6)	(1, 2, 5, 6)	(1, 2, 5, 6)	(1-4, 6)	(1-5)
ANOVA	0.000	0.000	0.007	0.002	0.002	0.004

*Data are mean ± SD of ten rats in each group. *PK, HK, LDH and PEPCK are expressed in µ mole/mg protein min.

which recorded significant reduction amounting to –48.46, –24.17 and –24.83% for HK, PK and LDH respectively, while PEPCK recorded percentage increase of +27% with respect to glibenclamide. The glycolytic enzymes HK, PK and LDH reached to –17.59, –13.58 and –14.77%, while gluconeogenic enzyme PEPCK amounting to a percentage increase +17.16%. Treatment with Zn only again provided additional significant enhancement better than Se mixed with Vit. E, where the percentage of inhibition of glycolytic enzymes HK, PK and LDH reached to –52.79, –35.14, –31.73 respectively, while PEPCK recorded percentage increase amounting to +37.13%. In addition, treatment of diabetic rats with Se + Vit. E exhibited percentage decrease of –58.29, –44.68 and –33.49% for HK, PK and LDH respectively, while PECPK showed a value of +39.11% as compared to normal control. Table III and Figure 3 manipulated liver function enzyme activities and total protein content in different treated-groups. In STZ group a significant increase were observed in liver function enzyme activities: AST, ALT and ALP amounting to 3.85±0.05, 2.62 ±0.04 and 6.08± 0.15 µmole/mg protein/min as compared to normal control with percentage increase +52.17, +61.73 and +78.29%. However, significant decreases was obtained in total protein content amounting to 80.42±0.43 mg/ml (–31.62%). Significant normalization was noticed in liver enzymes with more or less simultaneous results for both Zn associated with Se and Vit. E and glibenclamide as compared to normal control rats where the percentage changes in AST, ALT, ALP and total protein content recorded +18.58, +16.05, +25.81 and –14.31% respectively in combined micronutrients, while the percentage changes reached to +12.65, +8.64, +18.18 and –9.01% in glibenclamide-treated diabetic rats. In addition, treatments with Zn and Se + Vit. E exhibited more or less parallel results where AST, ALT, ALP and total protein content recorded +28.06, +25.93,+39.58 and –20.64% respectively in Zn treatment as compared to control while in Se + Vit. E, the percentages amounting to +28.46, +36.42, +38.12 and –20.64% respectively.

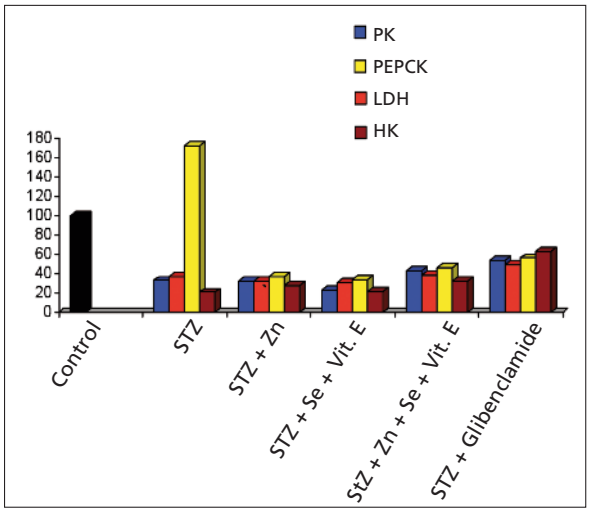

Figure 2. Glycolytic and gluconeogenic enzymes.

Table III. Effect of different micronutrient treatments on liver function enzyme activities and total protein in diabetic rats.

Parameters Groups	Normal control (1)	STZ (2)	STZ + Zn (3)	STZ + Se + Vit. E (4)	STZ + Zn + Se + Vit. E (5)	Glibenclamide (6)
AST	2.53 ± 0.16	3.85 ± 0.05	3.24 ± 0.15	3.25 ± 0.22	3.00 ± 0.01	2.85 ± 0.05
LSD	(2-6)	(1, 3-6)	(1, 2, 5, 6)	(1, 2, 5,6)	(1-4)	(1-4)
ANOVA	0.000	0.000	0.000	0.002	0.001	0.002
ALT	1.62 ± 0.038	2.62 ± 0.04	2.04 ± 0.06	2.21 ± 0.11	1.88 ± 0.26	1.76 ± 0.06
LSD	(2-6)	(1, 3-6)	(1, 2, 4-6)	(1, 2, 3, 5, 6)	(1-4, 6)	(2-5)
ANOVA	0.000	0.000	0.002	0.000	0.000	0.000
ALP	3.41 ± 0.08	6.08 ± 0.15	4.76 ± 0.23	4.71 ± 0.24	4.29 ± 0.04	4.03 ± 0.058
LSD	(2-6)	(1, 3-6)	(1, 2, 5,6)	(1, 2, 5, 6)	(1-4, 6)	(1-5)
ANOVA	0.000	0.000	0.000	0.003	0.003	0.000
T. protein	117.60 ± 2.0	80.42 ± 0.43	93.37 ± 4.12	93.33 ± 5.89	100.77 ± 2.01	107.00 ± 2.64
LSD	(2-6)	(1, 3-6)	(1, 2, 5, 6)	(1, 2, 5, 6)	(1-4, 6)	(1-5)
ANOVA	0.000	0.000	0.000	0.000	0.001	0.001

*Data are means ± SD of ten rats in each group.*Liver function enzyme activities are expressed in µ mole/mg protein/min. total protein is expressed in mg/ml. *Statistics is carried out using, ANOVA test and the difference between groups is analyzed by post hoc (SPSS Computer Program), where $p < 0.05$.

Histopathological examination showed that, diabetic liver exhibited foci of inflammatory cells in between hepatocytes and surrounding a central vein, necrosis and degenerative changes of hepatocytes (Figure 4). Treatment with zinc, selenium and vitamin E or their combination showing ordinary hepatic strands radiating from central vein, normal hepatocytes (some hepatocytes are slightly vacuolated) with normal nuclei and sinusoids (Figures 5-7).

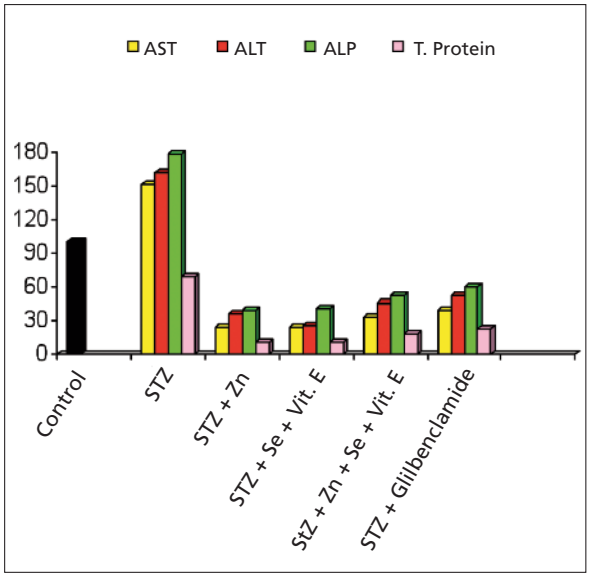

Figure 3. Liver function enzymes and total protein.

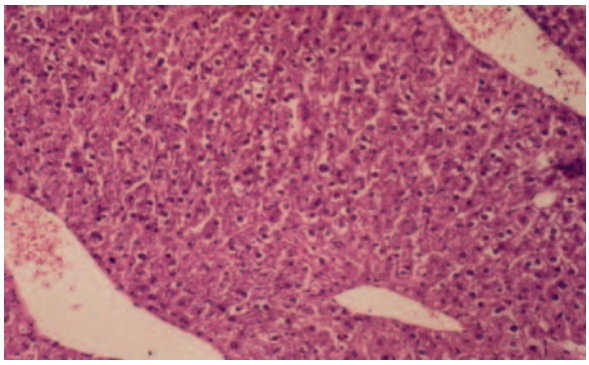

Figure 4. Liver section from a normal control, showing normal hepatocytes, no inflammatory cells in between, normal hepatic lobules and bile ducts. Numbers of inflammatory cells surrounding the central vein (H & E × 200).

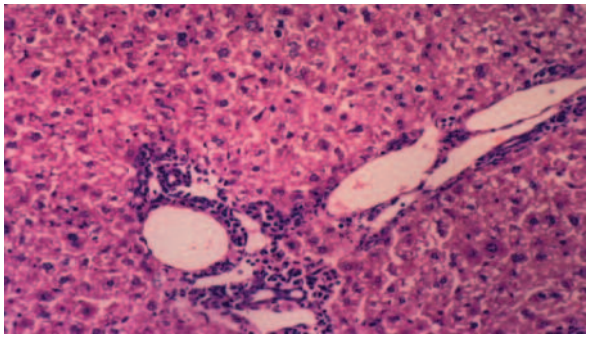

Figure 5. Liver section of diabetic rats showing foci of inflammatory cells in between hepatocytes and surrounding a central vein, necrosis and degenerative changes of hepatocytes (H & E × 200).

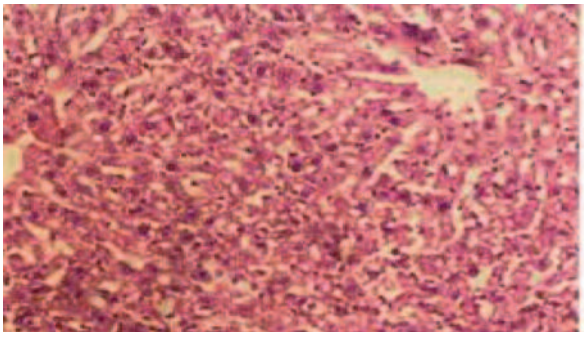

Figure 6. Photomicrograph of a liver section of diabetic rats treated with zinc. (H & E × 200).

Discussion

Oxidative stress has been found to play an important role in the pathogenesis of diabetes[30]. In turn, the generation of reactive oxygen species (ROS) has been shown to play an integral and possibly a causative part in the pathogenesis of diabetic retinopathy[31]. This hypothesis is supported by evidence that many biochemical pathways strictly associated with hyperglycemia (glucose autoxidation, polyol pathway, protein glycation) are initiated and augmented under oxidative stress. Furthermore, exposure of endothelial cells to high glucose (as indicated in the present results), leads to augmented production of superoxide anions, which may quench nitric oxide, a potent endothelium-derived vasodilator that participates in the general homeostasis of the vasculature. In further support of the consequential injurious role of oxidative stress, is the finding that many of the adverse effects of high glucose on endothelial functions are reversed by antioxidants[32]. Moreover, antioxidant therapy may be a suitable approach for halting the intrinsic changes within liver and retinal capillary bed that lead to the development of diabetic liver fibrosis and retinopathy. The present histological examinations at the cellular level reveal foci of inflammatory cells in between hepatocytes and surrounding a central vein, necrosis and degenerative changes in hepatocytes of rats indicating establishment of diabetic state (Figure 5). Holemans et al[33] stated that, as a result of the streptozotocin action, beta cells undergo destruction by necrosis. STZ is widely used for inducing type 1 diabetes in a variety of animals. It selectively induces degenerative alterations and necrosis of pancreatic β-cells resulting in, insulin deficiency and impairment in glucose oxidation. The use of lower dose of streptozotocin (40 mg/kg b.w.) produced an incomplete destruction of pancreatic beta cells even though rats became permanently diabetic[34]. In accordance to the present study, Mitra et al[35] earlier reported that the diabetic liver showed degeneration and congestion after injection of STZ. Hyperglycemia is observed with a concomitant drop in blood insulin followed by hypoglycemia about six hours due to decrease in insulin levels. Akbarzadeh et al[36] confirmed the destruction of islet cells in pancreatic biopsy of diabetic rats due to the effect of streptozotocin and added that 60 mg/kg dose of STZ ensured induction of diabetes in rats and hyperglycemia. The present results also indicate a significant elevation in oxidative stress markers, NO and lipid peroxidation products (MDA) in liver of diabetic rats. These elevated levels may be due to oxidative stress which is considered one of the necessary causative factors that link diabetes with the pathogenic complications of several tissues[37]. It was reported that NO over production has been

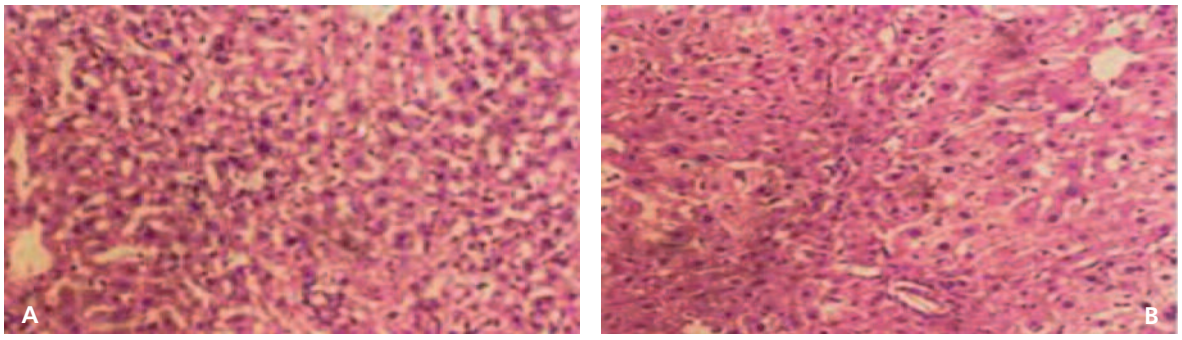

Figure 7. Selenium and vitamin E ***(A)*** or zinc associated with selenium and vitamin E ***(B)*** showing ordinary hepatic strands radiating from a central vein. Some hepatocytes are slightly vacuolated. Kupffer cells are hypertrophied. Normal nuclei and sinusoids. (H & E × 200).

linked to a variety of clinical inflammatory diseases[38]. Experimental studies suggested that NO may be responsible for the increased liver injury[39]. The direct toxicity of NO is enhanced by reacting with superoxide radicals to give powerful secondary toxic oxidizing species, such as peroxynitrite (ONOO) which is capable of oxidizing cellular structure and causes lipid peroxidation[40], a process leading to membrane damage and considered the proximal cause of cell death. Lipid peroxidation can damage protein, lipid, carbohydrates and nucleic acids, and is one of the risk factor of protein glycation.

Oxygen free radicals are implicated as mediators of tissue injury in cardiovascular pathology. Cytotoxic effect of ROS is related to lipid peroxidation and subsequent membrane destruction[41]. Oxygen free radicals, in addition to the myocardial damaging effect, may also be responsible for the release of lysosomal or hydrolytic enzymes such as elastase[42]. The study of Moustafa[43] reported elevated rates of liver lipid peroxidation accompanied with the deterioration in glucose tolerance in GSH-depleted rats. It has been suggested that in free radicals initiating systems, the deterioration in glucose tolerance is attributed to impaired insulin action[44]. Initiating lipid peroxidation by free radicals, in the lipid moiety of the cell membrane was supposed to result in distortion of the structural and functional integrity of the cell membrane or internal cellular components. This would interfere with the ability of insulin to initiate and propagate its normal sequence of actions which may account, at least in part, for STZ-induced hyperglycemia[45].

Moreover, the current data show also that, STZ caused a reduction in GSH and SOD levels in the liver of diabetic rats. The decline in the activity of free radical scavenging enzyme SOD may be due to its inactivation caused by excess ROS production. SOD neutralizes superoxide as it cannot cross lipid membrane producing hydrogen peroxide. Hydrogen peroxide can cross biological membranes. Catalase detoxifies hydrogen peroxide which has the principle role in tissue damage. So, reduction in SOD may damage the first line of enzymatic defense against superoxide anion and hydrogen peroxide[46]. The significant depletion of GSH in liver of diabetic rats, indicates damage to the second line of antioxidant defense. This probably further exacerbates oxidative damage by adversely affecting critical GSH-related processes such as free-radical scavenging, detoxification of electrophilic compounds, modulation of cellular redox status and thiol-disulphide status of proteins and regulation of cell signaling and repair pathways[47].

Concerning glycolytic (LDH, PK and HK) and gluconeogenic (PEPCK) enzymes, significant decrease in glycolytic enzymes was noticed, while significant increase was recorded in gluconeogenic enzymes in diabetic group as compared to normal control. In accordance to the present results Sherlock and Dooley[48] found that in diabetic state, degradation of liver glycogen and gluconeogensis are increased and glycolysis is decreased while glucose utilization is inhibited. Glucose-6-phosphatase increases in the liver, facilitating glucose release into the blood. The opposing enzyme which phosphorylates glucose, i.e hexokinase, is unaffected by insulin and decreases in diabetes. As a result, the liver continues to produce glucose even with severe hyperglycemia. Under this circumstance the normal liver would shut off and deposit glycogen. As the liver plays a central and crucial role in the regulation of carbohydrate metabolism, its normal function is essential for the maintenance of blood glucose levels and of a continued supply to organs that require a glucose energy source. This central role for the liver in glucose homeostasis offers a clue to the pathogenesis of glucose intolerance in liver diseases which is attributed mainly to an impaired insulin action.

The present results demonstrate significant elevation in liver function enzyme markers associated with significant reduction in total protein content as compared to normal control group. The high serum levels of these enzymes post STZ treatment are associated with inflammation and/or injury to liver cells, a condition known as hepatocellular liver injury and apoptosis. In parallel with the present work, previous reports revealed significant increased activities of serum enzymes relative to their normal levels[49,50]. Supporting our findings, it has been found that hyperglycemia resulted in hepatolysis reflected by increased blood serum aminotransferases as one of the consequences of diabetic complication. The increment of such serum markers may be due to the leakage of these enzymes from the liver cytosol into the blood stream as a result of hepatomegaly (fatty liver)[51].

The significant reduction in total protein content in diabetic rats is in concomitant with the results of Otsuki and Williams[52], who found significant reduction in serum total protein concentrations in diabetic rats and this may be due to re-

duction in the three major phases in protein secretion, intracellular transport and discharge. Alderson et al[53] explained the reduction in total protein due to significant increase in protein excretion. Mendez et al[54] reported that non-enzymatic glycation of albumin was the potential to alter its biological structure and function. It is mainly due to the formation of a Schiff base between amino-group of lysine (and sometimes arginine) residues and excess glucose molecules in blood to form glycoalbumin. Hypoalbuminemia is one of the factors responsible for the onset of ascites related to liver fibrosis[55].

Treatment of diabetic rats with micronutrients or antioxidants strengthens the endogenous antioxidant defenses from ROS and restores the optimal balance by neutralizing the reactive species. They are gaining a great importance by virtue of their critical role in disease prevention. Many studies have addressed the importance of antioxidants for the control of the abnormalities in diabetic tissue[56-58]. The present results reveal the ability of zinc with or without selenium and Vit. E, both to minimize the perturbations in serum glucose, AST, ALT, ALP and total protein in STZ-diabetic rats and its tendency to ameliorate the deteriorative changes in the levels MDA reactants, GSH, NO, SOD, LDH, PK, HK and PEPCK content in the liver tissue. Our data agree with Tobia et al[59] who found that administration of zinc to streptozotocin- induced diabetic rats was able to restore normoglycemia by enhancing glucose oxidation and stimulating glucose uptake by tissues. Zinc similarly exerts insulin-suppressive action by attenuating the hypersecretion of insulin associated with the prediabetic pancreas, thus ''rest-'' beta cells and preserving their structure and function[60]. Zinc, also acts as a protective agent in free radical injury in heart and kidney, while reducing lipid peroxidation in a number of tissues[61]. The mechanism by which zinc may protect against free-radical injury is believed to lie in the ability of zinc to displace and compete for binding sites with transition elements such as copper and iron in a number of biological systems and, thus, prevent transition metal generation of free radicals. The protective effects of zinc against increased rates of lipid peroxidation could be also due to its ability to bind and stabilize cellular membranes against lipid peroxidation and disintegration[62]. An alternative protective mechanism of zinc may be its ability to induce metallothioneins (MT) synthesis. The high sulfhydryl content enables MT to efficiently scavenge oxyradicals[63]. Another possible protective mechanism of MT is its ability to release Zn for binding at sites on membrane surfaces, displacing adventitious iron thereby inhibiting lipid peroxidation[62]. Moreover, the suggested effect of zinc in inducing the SH-rich MT synthesis may preserve the SH-residue in many functional proteins. Therefore, Zn may preserve the structural and functional integrity of the SH-dependent enzymes including those regulating glucose: LDH, PK, HK and PEPCK metabolism. Recently, St Croix et al[64] hypothesized that MT which is cysteine-rich, plays a role in nitric oxide (NO) signaling events via sequestration or release of Zn^{+2} by the unique thiolate clusters of the protein. Considerable evidence indicates that maintenance of protein redox status is of fundamental importance for cell function, whereas structural changes in proteins are considered to be among the molecular mechanisms leading to diabetic complications[65]. Additionally, maintenance of the redox potential of the cell was reported to be an essential condition for maintaining its synthetic capacity[66]. The present study as well as earlier ones such as that of Agardh et al[67] indicate that GSH depletion in diabetic liver, may disturb the redox status inside the tissue. Accordingly, STZ-induced GSH depletion in the tissue of diabetic-rats may impair metallothionein synthesis. Actually, significantly lower content of sulphydryl proteins was found in the liver and eye lens and vitreous of diabetic patients than in those of non-diabetic and control subjects[65]. In turn, the suggested ability of Zn to stabilize biological membranes may extend to involve the microsomal membranes, which would naturally result in the enhancement of the synthetic capacity of the microcosms. Indeed, the study of Chavapil et al[68] has shown that Zn in adequate amounts was found, both *in vivo* and *in vitro*, to stabilize microcosms and reduce the oxidative damage. Consecutively, the machinery of metallothioneins synthesis could be protected by zinc. The observed increase in GSH concentration induced by Zn in the pancreas[58] may not represent a complete protective mechanism on pancreatic cells that would restore the synthetic capacity of these cells or completely inhibit other phases of oxidative stress damage caused to these cells. Moreover, one type of superoxide dismutase (SOD), a major antioxidant enzyme is zinc-dependent (Cu/Zn-SOD). Cu/Zn-SOD is a potent antioxidant enzyme, which has recently been proposed to have a tumor-suppression effect[69]. Therefore,

the importance of zinc as a protective antioxidant against diabetic complications may lie in its ability to initially exert a good hypoglycemic control, thus, inhibiting the development of the deleterious consequences of hyperglycemia. Second, it could be an important factor in halting the progression of the intrinsic changes in diabetic tissue that eventually lead to the development of degenerations[59,70].

The treatment of diabetic rats with selenium associated with Vit. E was found to produce an improvement in all parameters under investigations but with less pronounced significant effect than zinc supplementation. Ezaki[71], implicated selenium in the regulation of specific beta-cell target genes and suggested that selenium potentially promotes an overall improvement in islet function and hence normalizing glucose homeostasis, glucose metabolizing enzymes and insulin secretion. Therefore,it has a biological function similar to that of insulin. In addition, Se can inhibit high glucose- and high insulin-induced expression of adhesion molecules. So, Se may be considered as a potential preventive intervention for diabetes-accelerated atherosclerosis[69]. The correlation between selenium and gestational hyperglycemia might be due to the antioxidant and insulin-mimetic function of this element[72], so, Se and Vit E provided protective effects against, pathogenic factors induced alterations in islet β cells[73]. Therefore, patients with an increased lipid peroxidation and reduced antioxidant status may contribute to the development of complications in diabetes. Our findings are in parallel with Kimura[74] who found that Se and Vit. E induce a sustained improvement of glucose homeostasis in diabetic individuals by an insulin-like action and a significant increase in the glutathione concentration(GSH). Vitamin E (α-tocopherol) regulates the production of ROS in mitochondria, modulating the expression and activation of signal transduction pathways and other redox-sensitive biological modifiers, thereby delaying or preventing degenerative tissue changes[75]. It has been reported that α-tocopherol concentration remained relatively unchanged during oxidizing conditions in heart mitochondria from rats fed diets supplemented with vitamin E, which also was associated with very low formation of thiobarbituric acid reactive substance (TBARS)[76].

The drop in the concentration of GSH in STZ diabetic rats could be due to: an inhibition of synthesis; oxidation to glutathione disulfide; a deficit in GSH salvage pathway; or an increased rate of GSH consumption. Previous results support the hypothesis that decreased levels of GSH were not due to a defective activity of glutathione reductase, which was increased in these animals in both mitochondria and cytosol. The increased glutathione reductase/glutathione peroxidase ratio is indicative that, the tissues were able to recycle GSH. One role for α-tocopherol in maintaining protein thiols during lipid peroxidation has been suggested. It has been proposed that α-tocopherol competes with protein thiols for trapping free radicals, and that oxidation of intrinsic buried thiols may commence after α-tocopherol has been consumed[76]. It was found that, supplemental vitamin E decreased plasma lipid peroxidation, triglycerides, platelet lipid biosynthesis and urine ketone bodies but did not affect platelet reactivity in the STZ-diabetic animal model[77]. In type I human diabetics 400 mg α-tocopherol daily for 4 weeks reduces oxidative stress likewise to type II diabetics where Ceriello et al[78] showed that glycosylated hemoglobin and other proteins were significantly decreased after 2 months' treatment with either 600 or 1200 mg/d vitamin E. The potential for vitamin E in pharmacological doses to be used as a means to delay or prevent secondary complications of diabetes seems well established but requires further testing for long term efficacy. The biochemical functions of selenium and vitamin E are interrelated, in that both are essential components of the antioxidant defense system and they appear to have synergistic and compensatory effects in induced deficiency states of one or the other[9,79]. Significant normalization was recorded in all parameters under investigation as a result of synergistic treatment of diabetic rats with zinc associated with vitamin E and selenium better than zinc or selenium and vitamin E given separately as compared to both antidiabetic glibenclamide drug and normal control rats.

In conclusion, the present study demonstrates the antiglycemic, antioxidant effects of micronutrient: zinc with or without selenium and vitamin E and selenium and vitamin E in comparison with glibenclamide antidiabetic drug. The present data reveal that zinc-treatment to STZ-diabetic rats produce a remarkable amelioration better than selenium and vitamin E. However, the combination of zinc with Se and vitamin E exhibit a synergistic powerful beneficial hypoglycemic control, scavenging free radical, normalizing liver function as well as they have principle role in treatment and amelioration liver damage at the cellular level. Thus, the safely

promising therapeutic dose used in the current study, can be effective in treatment and enhanced liver tissue from the damage induced by diabetes and may used as candidate antidiabetic drugs.

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