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Original Article

Serum and pancreatic zinc levels in diabetic rats and role of zinc supplementation on glycemic control and possible mechanism of action.

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A B S T R A C T

Abstract: Zinc is one of the most important trace elements in biological systems. It has critical effect on oxidative stress which is implicated as an important cause of degenerative diseases including diabetes.

Objective: This study was set to determine if there is a difference in serum and pancreatic zinc concentrations between normoglycemic and diabetic rat groups. Another aim was to evaluate, whether the hyperglycemia and oxidative stress could be ameliorated by zinc therapy.

Materials and Methods: Forty eight male albino rats weighing 150-200 grams were randomly allocated to four groups each contained 12 animals. Group 1**:** control group received saline. Group 2**:** was given zinc sulfate orally 100 mg/kg/day. Group 3**:** diabetic control rats induced by alloxan (150 mg/kg, I.P.) as a single dose. Group 4**:** in which diabetes was induced by alloxan was given zinc sulfate orally. Six rats from each group were sacrificed by decapitation after 2 weeks and the other six rats after 4 weeks of treatments for estimation of blood glucose level, and blood and pancreatic zinc (Zn), superoxide dismutase (SOD), catalase (CAT), and malondaldhyde (MDA).

Results: There were significant increase in blood glucose as well as serum and pancreatic MDA levels. While serum and pancreatic Zn, SOD, CAT levels were significantly reduced after 2 and 4 weeks of single I.P. alloxan administration compared to control group. Treatment of diabetic rats with zinc sulfate led to significant decrease in blood glucose level and elevation in serum Zn levels only after 4 weeks of treatment. However, significant decrease in serum and pancreatic MDA levels, and increase in serum and pancreatic SOD and CAT and also in pancreatic Zn after 2 and 4 weeks of treatment compared to diabetic untreated groups.

Conclusion: Serum and pancreatic Zn concentrations in diabetic rat groups were significantly lower than control rats. Although zinc therapies can partially ameliorate the alloxan- induced changes in diabetic rat after 2 weeks of treatment, significant recovery occurred in the measured parameter after 4 weeks of treatment due to its antioxidant effect.

Key Words: Zinc sulfate, Oxidative stress, Alloxan, Diabetes, Serum zinc, pancreatic zinc.

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1. INTRODUCTION

The prevalence of diabetes is increasing worldwide because of population growth, aging, urbanization, increasing prevalence of obesity and physical inactivity (Ranasinghe et al., 2013). Diabetes is a leading cause of morbidity and mortality and Global estimates predict that the proportion of adult population with diabetes will increase 69% for the year 2030 (Shaw et al., 2010). Increasing evidence suggests that oxidative stress plays a role in the pathogenesis of diabetes mellitus and its complications (Brownlee, 2001). In addition, antioxidant mechanisms are diminished in diabetic patients, which may further augment oxidative stress (Maritim et al., 2003)

Zinc is one of the most important trace elements in biological systems (Jurowski et al., 2014). It has critical effect in homeostasis, in oxidative stress, and in aging. It is a structural component of key antioxidant enzymes such as superoxide dismutase, which is vital for intra- and extracellular antioxidant defense, and zinc deficiency may impair their synthesis (Zheng et al., 2008). Pancreatic β cells contain the highest amount of zinc among cells within the human body. It is essential in insulin action and carbohydrate metabolism (Chausmer, 1998), and hence, the

relationship between zinc and diabetes has been of great interest. So, the current study aimed to determine if there is a difference in serum and pancreatic zinc concentrations between normoglycemic and diabetic rat. Another aim was to evaluate, whether the hyperglycemia and oxidative stress could be ameliorated by zinc therapy or not.

2. MATERIALS AND METHODS

2.1. Materials

Zinc sulfate was purchased from (Sigma-Aldrich Chemical Company, USA); Alloxan monohydrate was obtained from (Pharco Company for pharmaceuticals. Cairo. Egypt); Kits for determination of oxidative stress parameters: (SOD, CAT, MDA) were obtained from Bio diagnostic company, Egypt; Kits for determination of blood glucose level were supplied by Egyptian Company for Biotechnology (Cairo-Egypt).

2.2. Animals

Forty eight male adult Wister rats weighing 150-200gm have been used. Animals were obtained from the animal house Faculty of Medicine, Assiut University, Egypt and fed on standard diet of commercial rat chow and tap water. Rats were left to acclimatize to the environment for one week prior to inclusion in the experiment at Faculty of Medicine, Sohag University. Rats were maintained under standard laboratory conditions at an ambient temperature of 25±2°C, with 12 hour light/12 hour dark cycles. Animals were given a free access for food and water up to 24 hours prior to their use. This study was approved by the Institutional Animal Care and Use Ethical Committee of Faculty of Medicine, Sohag University. No. 19/2013.

2.3. Experimental protocol

2.3.1. Induction of Diabetes

Overnight fasted animals were injected with single intra-peritoneal injection of alloxan monohydrate in a dose of 150 mg/kg body weight (Ojezele and Abatan 2011). Alloxan was freshly dissolved in 0.9% of sterile normal saline. Development of hyperglycemia in rats was confirmed by fasting blood glucose measurement in blood sample withdrawn from the retro-orbital venous plexus after 72 hours of alloxan treatment. Rats which showed blood glucose levels of 200 mg/dl and above were considered diabetic and included in the study (Stanley et al., 2001).

2.4. Treatment

Rats were randomly allocated to either control or three treated groups each containing twelve animals. Control animals received normal saline using the same volume and similar methods. The first treated group was given zinc sulfate orally 100 mg/kg/day dissolved in distilled water (Bolkent et al., 2006). The second treated group, diabetic control rats, was injected with normal saline; the third diabetic group was given zinc sulfate orally 100 mg/kg/day.

2.5. Sample collection

Six rats from each group were fasted for 16 hours and sacrificed by cervical dislocation after 2 weeks and then the other six animals after 4 weeks of treatments, and blood samples were collected. Serum was separated for estimation of blood glucose, serum Zn, SOD, CAT, and MDA levels. The serum was kept at -80ºC until the time of analysis.

Each pancreas was quickly removed from the sacrificed rats, placed in ice cold saline solution, trimmed of adipose tissues, divided into two halves and weighed. The first half of pancreases was finely minced and homogenized in 10 ml cold buffer (50 mM potassium phosphate, pH 7.5, 1mM EDTA) per gram tissue (v/w) using Glas-Col, LLC USA motor driven homogenizer to a final concentration of 10% homogenate, then centrifuged at 4000 rpm for 15 min at 4ºC. The supernatant was removed for assay the pancreatic oxidative parameters and kept at -80ºC until the time of analysis. The second half of the pancreas was dried at 110°C for 24 hours for determination of pancreatic zinc concentrations.

2.6. Biochemical analysis:

2.6.1. Determination of blood glucose:

Serum glucose was determined by GOD-POD enzymatic colorimetric method, as described by Caraway and Watts (1987); using commercially available kits, Egypt.

2.6.2. Determination of zinc levels:

Dried pancreases were weighted and heated in silica crucibles using oven furnace at 550ºC for 24 hours and the ash taken up in hot hydrochloric acid (3 N) for pancreatic Zn analysis by atomic absorption spectrophotometer (Perkin Elmer 2380 USA) (Kechrid et al., 2001). Serum Zn level was determined by atomic absorption spectrophotometer (Perkin Elmer 2380 USA) using an air-acetylene flame and hollow cathode lamps. Serum Zn level was determined by using wave length at 213.9 nm and lamp current is 10 mA. Zinc standards were prepared from a 1mg/ml zinc nitrate standard solution (BDH) using 5 % glycerol (Kechrid et al., 2007).

2.6.3. Determination of antioxidant enzymes activities:

2.6.3.1. Measurement of SOD activity: SOD activity in serum and pancreatic tissue homogenate was determined by colorimetric method using commercially available kits. The method was described by Nishikimi et al., (1972). This assay relies on the ability of the enzyme to inhibit the phenazine methosulphate-mediated reduction of nitroblue tetrazolium (NBT) dye. The change in the absorbance was measured at 560 nm for control and sample at 25ºC. SOD activity was expressed in U/ml in serum and U/g in pancreatic tissues.

2.6.3.2. Measurement of catalase (CAT) activity: CAT activity in serum and pancreatic tissue homogenate was determined by colorimetric method using commercially available kits, as described by Aebi (1984). This assay relies on the reaction of CAT with known quantity of H_2O_2. The reaction is stopped after exactly one minute with CAT inhibitor. In the presence of peroxidase, the remaining H_2O_2 reacts with 3, 5-Dichloro-2-hydroxybenzene sulfonic acid (DHBS) and 4-Amino-phenazone (AAP) to form a chromophore. The change in the absorbance was measured at 510 nm. CAT activity was expressed in U/L in serum and U/g in pancreatic tissues.

2.6.4. Determination of lipid peroxidation level: Measurement of serum and pancreatic MDA levels as an indicator to lipid peroxidation**.** MDA in serum and pancreatic tissue homogenate was determined by colorimetric method using commercially available kits, as described by Ohkawa et al., (1979). The principle of the method is based on spectrophotometric measurement of the color formed during the reaction of thiobarbituric acid with MDA. They react in acidic medium at temperature of 95ºC for 30 min to form thiobarbituric acid reactive product. The absorbance of resultant pink product can be measured at 534nm. MDA level was expressed in nmol/ml in serum and nmol/g in pancreatic tissues.

2.7. Statistical Analysis

In the present experimental work, differences between control and treatment groups were analyzed by one way analysis of variance (ANOVA) while multiple comparisons were performed using Tukey's test as post hoc using SPSS program (version 16). All values are given as mean± standard error (SE) of mean. Differences were considered to be significant at $p<0.05$.

3. RESULTS

3.1. Alloxan-induced changes in rats:

Treatment of rats with 150 mg/kg alloxan as a single I.P. dose led to a significant increase ($p<0.001$) in blood glucose (Table 1) and serum and pancreatic MDA levels ($p<0.001$) (Fig.5&6) compared to control group. On the other hand, there were significant reductions ($p<0.001$) in serum and pancreatic zinc (Table 1), serum and pancreatic SOD and CAT activities ($p<0.001$) (Fig.1, 2, 3 & 4) compared to control group after 2 and 4 weeks of alloxan administration, and the changes which occurred by alloxan after 4 weeks were significant compared to changes which occurred after 2 weeks.

3.2. Effects of zinc sulfate on alloxan- induced changes:

3.2.1 Blood glucose levels:

Zinc sulfate administration in a dose of 100 mg /kg/day orally to diabetic rats did not have any significant effect on mean blood glucose level following 2 weeks of treatment but it caused a significant decrease in blood glucose level after 4 weeks compared to the alloxan treated groups ($p<0.001$) (Table 1).

3.2.2. Zinc levels:

Serum zinc level: Although an increase was observed in the mean serum zinc level following administration of 100 mg /kg/day zinc sulfate orally to diabetic rats for 2 weeks but it was not significant in comparison to the alloxan treated group. However, administration of zinc sulfate orally in the same dose to diabetic rats for 4 weeks produced significant increase ($p<0.01$) in serum zinc level compared to alloxan treated group (Table 1).

Zinc therapy completely recovered deleterious effects of alloxan on serum zinc level after 4 weeks, so there was no significant difference between alloxan +zinc sulfate of 4 weeks group and the control group (Table 1).

Pancreatic zinc levels: Supplementation of zinc to diabetic rats produced a significant ($p< 0.01$) elevation in rats pancreatic zinc concentration after 2 weeks of oral 100 mg /kg zinc sulfate consumption compared to corresponding alloxan treated group. Moreover, a complete recovery was observed in pancreatic zinc concentration in zinc sulfate treated diabetic group after 4 weeks compared to corresponding alloxan treated group and control group ($p<0.001$) (Table 1).

3.2.3. Antioxidant enzymes activities

3.2.3.1. SOD activity: Significant increase ($p< 0.05$) was observed in the mean SOD activity following administration of 100 mg /kg/day zinc sulfate orally to diabetic rats for 2 weeks duration compared to corresponding alloxan treated group.

However, more significant recovery was observed after 4 weeks of zinc sulfate treatment compared to alloxan treated group ($p<0.001$) and control group ($p<0.01$) (Fig. 1).

On the other hand, the activity of pancreatic SOD showed a significant increase ($p <0.01$) in diabetic rats that had received zinc sulfate in the first 2 weeks but interestingly zinc impact on the activity of pancreatic SOD was very highly significant ($p<0.001$) after 4 weeks of treatment compared to alloxan treated group. There was no significant difference between alloxan + zinc sulfate after 4 weeks group and the control group (Fig. 2).

3.2.3.2. CAT activity:

Zinc therapy significantly elevates ($p<0.001$) the activity of serum catalase and recovered deleterious effects of alloxan in the first 2 weeks compared to alloxan treated group but this recovery was not complete, where the activity of serum catalase was significantly ($p<0.05$) lower than control group (Fig.3). However, Zinc therapy for 4 weeks significantly ($p<0.001$) elevated the activity of serum catalase compared to alloxan treated group and this recovery was complete, where the serum catalase activity was not significantly different from control group (Fig.3).

The activity of pancreatic catalase showed a significant ($p<0.05$) increase in the group that had received zinc sulfate for 2 weeks compared to the corresponding alloxan treated group, and this effect was highly significant ($p<0.001$) after 4 weeks compared to the corresponding alloxan treated group (Fig. 4), there was no significant difference between zinc sulfate+alloxan for 4 weeks group and the control group.

3.2.4. MDA levels:

Serum MDA: Zinc therapy completely recovered the deleterious effects of alloxan on MDA level, where, there is significant decrease ($p<0.001$) in serum MDA level after 2 and 4 weeks compared to corresponding alloxan treated group.

In addition, there was no significant difference between serum levels of MDA after 2 and 4 weeks of zinc therapy compared to control group and ($p=0.98$) respectively (Fig. 5).

Pancreatic MDA: The oral administration of 100 mg/kg/day of zinc sulfate showed statistically significant decrease ($p<0.05$) on pancreatic MDA level after 2 weeks when compared to the diabetic control group. In the group that was treated with zinc sulfate in the same dose for 4 weeks, there was also statistically significant decrease ($p<0.001$) on pancreatic MDA level compared to the diabetic control group (Fig. 6).

Table 1: Effect of oral administration of 100 mg/kg/day zinc sulfate on blood glucose, serum zinc and pancreatic zinc levels in alloxan-induced diabetes in rats after 2 and 4 weeks of treatment.

	Control	**Experimental groups**					
		2 weeks after treatment			**4 weeks after treatment**		
		zinc sulfate	Alloxan	Alloxan+ zinc sulfate	zinc sulfate	Alloxan	Alloxan + zinc sulfate
No. of rats	N=12	N=6	N=6	N=6	N=6	N=6	N=6
Blood glucose level (mg/dl)	88.50 ±1.00	85.83 ±2.30	205.17 ±0.60^{ab}	193.83 ±0.31^{a}	79.17 ±3.20	307.33 ±5.55$^{ab\ d}$	98.17 ±2.68^{ce}
Serum Zn (ug/dl)	115.08 ±2.04	117.33 ±1.78	75.17 ±1.54^{ab}	78.00 ±0.86^{ab}	123.33 ±3.03	44.6 7 ±3.29^{adb}	112.67 ±2.94^{bce}
Pancreatic Zn (µg/g dry wt)	60.45 ±0.57	62.10 ±1.11	51.92 ±0.71^{ab}	58.20 ±0.45^{c}	72.00 ±1.98^{a}	44.22 ±0.75^{abd}	68.83 ±2.51^{ace}

Each value represents the mean ±SEM. N= number of rats. Zn=zinc**.**

aSignificant at ($p<0.05$) vs. control group.

bSignificant at ($p<0.05$) vs. corresponding zinc treated group.

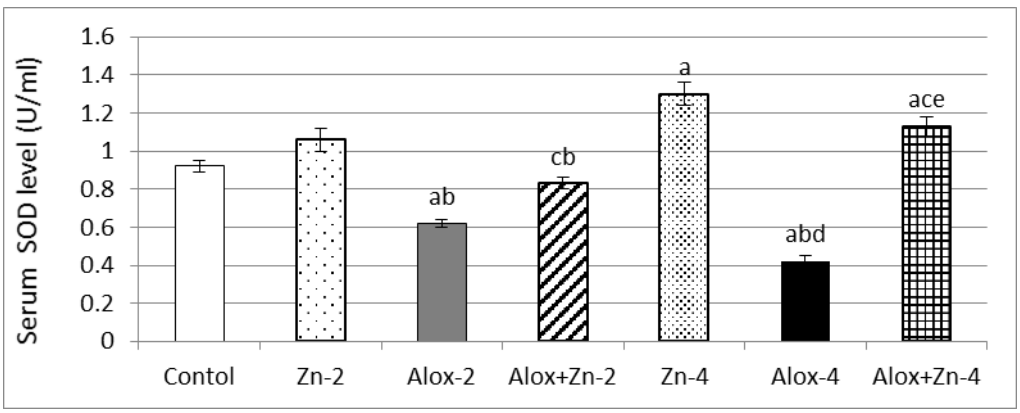
c Significant at ($p<0.05$) vs. corresponding alloxan treated group.

d Significant at ($p<0.05$) vs. alloxan -2 weeks group.

e Significant at ($p<0.05$) vs. alloxan+Zn-sulfate -2weeks group.

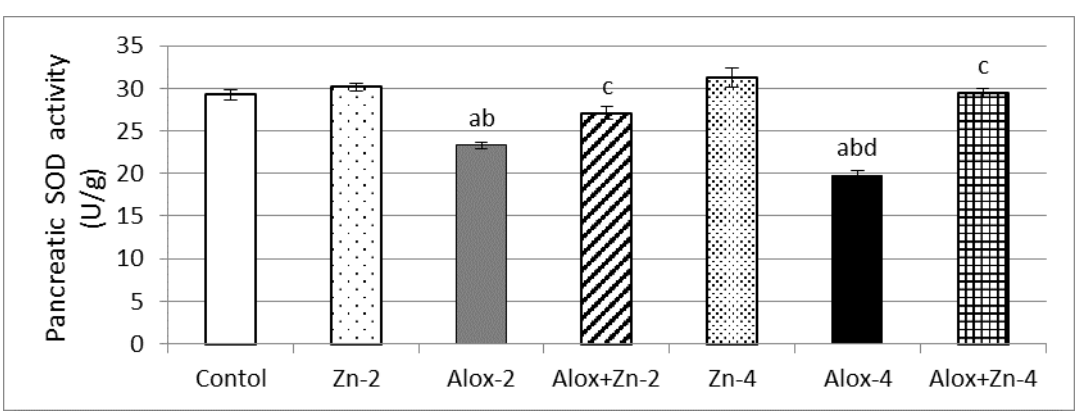
Data were analyzed by one way ANOVA followed by post hoc analysis with least significant difference (LSD).

Figure (1): Effect of oral administration of 100 mg/kg/day zinc sulfate on serum SOD activity in alloxan-induced diabetes in rats after 2 and 4 weeks of treatment.



Each value represents the mean ±SEM. (n=6), control n=12.
Alox=alloxan, Zn= zinc sulfate.
aSignificant at ($p<0.05$) vs. control group.
bSignificant at ($p<0.05$) vs. corresponding zinc treated group.
c Significant at ($p<0.05$) vs. corresponding alloxan treated group.
d Significant at ($p<0.05$) vs. alloxan -2 weeks group.
e Significant at ($p<0.05$) vs. alloxan+Zn-sulfate -2weeks group.
Data were analyzed by one way ANOVA followed by post hoc analysis with least significant difference (LSD).

Figure (2): Effect of oral administration of 100 mg/kg/day zinc sulfate on pancreatic SOD activity in alloxan-induced diabetes in rats after 2 and 4 weeks of treatment.



Each value represents the mean ±SEM. (n=6), control n=12.

Alox=alloxan, Zn= zinc sulfate.
aSignificant at ($p<0.05$) vs. control group.

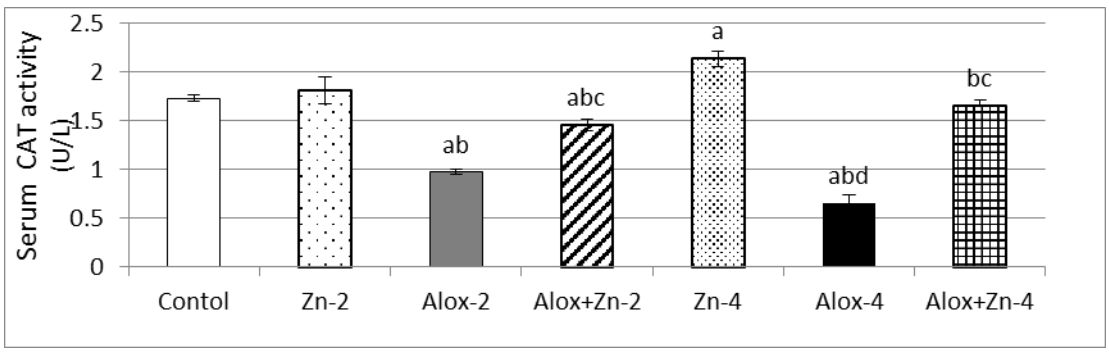
bSignificant at ($p<0.05$) vs. corresponding zinc treated group.

c Significant at ($p<0.05$) vs. corresponding alloxan treated group

d Significant at ($p<0.05$) vs. alloxan -2 weeks group.

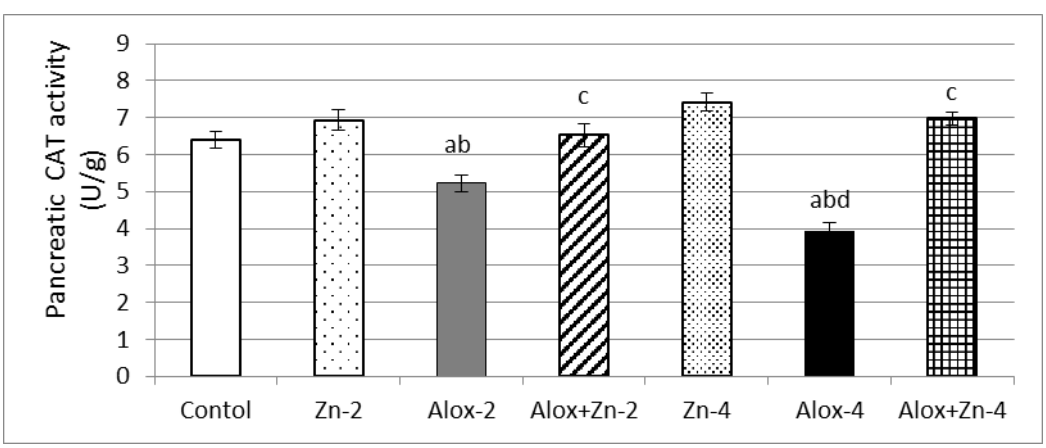
Data were analyzed by one way ANOVA followed by post hoc analysis with least significant difference (LSD).

Figure (3): Effect of oral administration of 100 mg/kg/day zinc sulfate on serum CAT activity in alloxan-induced diabetes in rats after 2 and 4 weeks of treatment.



Each value represents the mean ±SEM. (n=6), control n=12.
Alox=alloxan, Zn= zinc sulfate.
aSignificant at ($p<0.05$) vs. control group.
bSignificant at ($p<0.05$) vs. corresponding zinc treated group.
cSignificant at ($p<0.05$) vs. corresponding alloxan treated group.
dSignificant at ($p<0.05$) vs. alloxan -2 weeks group.
Data were analyzed by one way ANOVA followed by post hoc analysis with least significant difference (LSD).

Figure (4): Effect of oral administration of 100 mg/kg/day zinc sulfate on pancreatic CAT levels in alloxan-induced diabetes in rats after 2 and 4 weeks of treatment.



Each value represents the mean ±SEM. (n=6), control n=12.

Alox=alloxan, Zn= zinc sulfate.

aSignificant at ($p<0.05$) vs. control group.

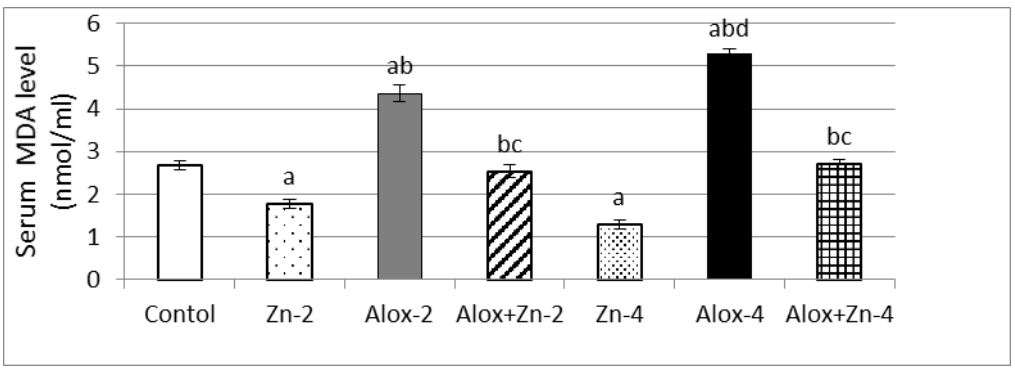
bSignificant at ($p<0.05$) vs. corresponding zinc tre34ated group.

c Significant at ($p<0.05$) vs. corresponding alloxan treated group.

d Significant at ($p<0.05$) vs. alloxan -2 weeks group.

Data were analyzed by one way ANOVA followed by post hoc analysis with least significant difference (LSD).

Figure (5): Effect of oral administration of 100 mg/kg/day zinc sulfate on serum MDA levels in alloxan-induced diabetes in rats after 2 and 4 weeks of treatment.



Each value represents the mean ±SEM. (n=6), control n=12.

Alox=alloxan, Zn= zinc sulfate.

aSignificant at ($p<0.05$) vs. control group.

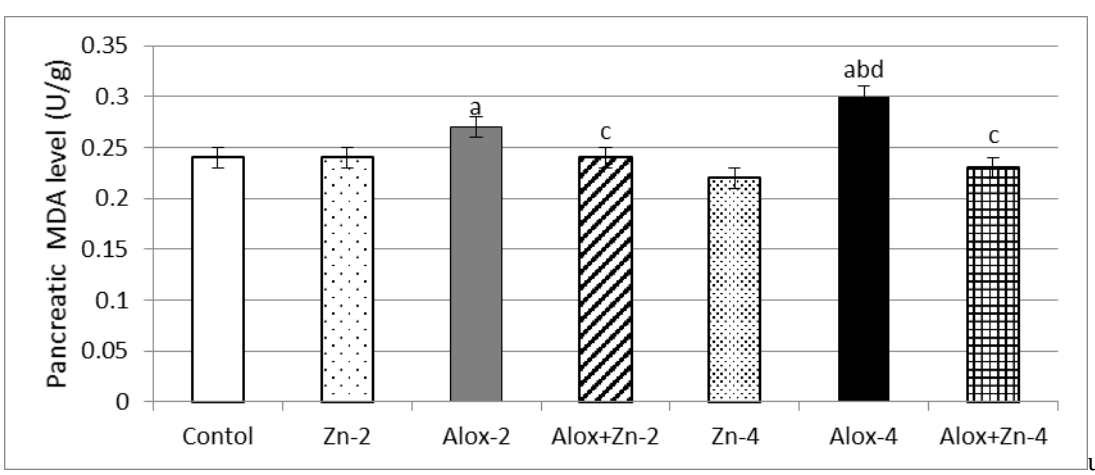
bSignificant at ($p<0.05$) vs. corresponding zinc treated group.

c Significant at ($p<0.05$) vs. corresponding alloxan treated group.

d Significant at ($p<0.05$) vs. alloxan -2 weeks group.

Data were analyzed by one way ANOVA followed by post hoc analysis with least significant difference (LSD)

Figure (6): Effect of oral administration of 100 mg/kg/day zinc sulfate on pancreatic MDA levels in alloxan-induced diabetes in rats after 2 and 4 weeks of treatment.



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Each value represents the mean ±SEM. (n=6), control n=12.

Alox=alloxan, Zn= zinc sulfate.

a Significant at ($p<0.05$) vs. control group.

bSignificant at ($p<0.05$) vs. corresponding zinc treated group.

c Significant at ($p<0.05$) vs. corresponding alloxan treated group.

d Significant at ($p<0.05$) vs. alloxan -2 weeks group.

Data were analyzed by one way ANOVA followed by post hoc analysis with least significant difference (LSD).

4. DISCUSSION

Diabetes mellitus is a metabolic disorder resulting from a defect in insulin secretion, insulin action, or both (Kumar and Clark, 2002). This dreadful disease is found in all parts of the world and is becoming a serious threat to mankind (Jarald et al., 2008).

The pancreas is both an endocrine and exocrine organ, contributing to the homeostasis of several aspects of digestion and the hormonal control of blood glucose concentration by insulin secretion (Kelleher et al., 2011). Alloxan is one of the usual substances used for the induction of diabetes mellitus. Alloxan has a destructive effect on the beta-cells of the pancreas. Therefore, alloxan-induced diabetes represents a good model for the study of type-1 diabetes mellitus (Prince and Menon, 2000).

Increased oxidative stress is a widely accepted participant in the development and progression of diabetes mellitus and its complications (Ceriello, 2000).

So, in this study, we demonstrated difference between normal and diabetic rats in blood glucose, serum and pancreatic zinc, SOD, CAT, and MDA levels, also, the role of zinc supplementation on these parameters in diabetic animals after 2 and 4 weeks duration of treatment.

The present study showed that blood glucose level was increased in alloxan-treated animals, This result are in harmony with the work of Prince and Menon, 2000; Jelodar et al., (2003); Kechrid et al., (2007); Dawud et al., (2012) and Derouiche et al., (2013), where they explain the hyperglycemia induced by alloxan by that it causes a massive reduction or complete absence of insulin release, by the destruction of beta-cells of Islet of Langerhans and hence, inducing hyperglycemia. Alloxan and the product of its reduction, dialuric acid, establish a redox cycle with the formation of superoxide radicals. These radicals undergo dismutation to hydrogen peroxide with simultaneous massive increase in cystolic calcium concentration, which causes rapid destruction of pancreatic beta-cells of Islets of Langerhans. In addition, one of the targets of the reactive oxygen species is DNA of pancreatic islets, where this leads to cell death by necrosis, thereby inducing hyperglycemia (Ravikumar et al., 2010).

On the other hand, we found that the mean serum and pancreatic zinc levels were significantly lower in diabetic rats as compared to controls. This result is consistent with finding reported by others as Blostein-Fujii et al., (1997); Al-maroof, and Al-Sharbatti, (2006) and Al-Timimi_and Bakir, (2009). This finding may be due to the ability of diabetic animals to excrete higher amounts of zinc in urine by frequent urination than normal ones (Brandao-Neto et al., 2000) or to the degranulation, cytolysis and to other pathological changes in the pancreatic tissues associated with progression of the condition (Chausmer, 1998).

Others explained this finding by that, one of the most important clinical features of diabetes is its harmful effects on the kidneys small blood vessels (microangiopathy) (Bakris, 2011). It is characterized by persistent albuminuria and decline in glomerular filtration rat (GFR) (Bahnam et al., 2010). During progression of microangiopathy, morphological and structural changes occur in the glomerular and basement membrane of the kidneys, hyperfiltration, thickening of glomerular basement membrane (GBM), mesangial expansion and podocyteloosing. All of these changes will cause leakage of albumin along with other enzymes and trace metals to the urine including zinc (Zargar et al., 2002).

Our result demonstrated that, there was an increase in the levels of serum and pancreatic MDA, also, decrease in activities of SOD and CAT in serum and pancreas of diabetic rats compared to the control group.

MDA is degradation product of peroxidation of polyunsaturated fatty acids (PUPA) in the cells membrane. Presence of higher MDA in the serum is an indication of induced lipid peroxidation and of oxidative stress of which has been reported as one of the underlying causes of diabetes mellitus (Ceriello, 2000); (Akinosun and Bolajoko, 2007). This result is consistent with finding reported by Tripathi, and Chandra (2010); Gwarzo et al., (2014) and Kanthlal et al., (2014) who studied the effect of alloxan on oxidative stress and they showed significant increase in MDA level and decrease in SOD and CAT activity after I.P. administration of alloxan . Concurrent with our changes, El-Missiry and El Gindy, (2000) reported that, there was an increase in the concentration of MDA and 4-hydroxynonenal in the liver. This oxidative stress was related to a decreased glutathione (GSH) content and SOD activity in the liver of alloxan-diabetic rats.

In addition to previous changes, we observed that there were progressive changes in levels of blood glucose, zinc and antioxidant status in blood and pancreas with progression of diabetes, where, the changes which occurred after 4 weeks were significant compared to those which occurred after 2 weeks of I.P. injection of alloxan. These results are in harmony

with the work of Kakkar et al., (1998); Al-Timimi_et al., (2014) where the explanation of the progressive increase in oxidative stress seems mainly to be due to increased production of free radicals and/or a sharp reduction of antioxidant defenses. Progressive free radical production caused by progressive hyperglycemia occurs at least via four different routes: auto-oxidation of glucose, increased glycolysis, intracellular activation of sorbitol (polyol) pathway and non-enzymatic protein glycation (Behrens and Madere 1991; Ceriello et al., 1992 and Maritim et al., 2003).

In the present study, oral zinc administration (100 mg/kg/day zinc sulfate) to diabetic rats resulted in non-significant changes in blood glucose levels after 2 weeks of treatment, while, significant decrease was seen after 4 weeks.

Zinc is one of the important micro elements needed for the beta-cells (Mahomed et al., 2007; Jarald et al., 2008). Several molecular mechanisms are believed to be involved in the regulation of blood glucose levels following zinc supplementation. The protein tyrosine phosphatase 1B (PTP 1B), a key regulator of the phosphorylation state of the insulin receptor is known to be a target of zinc ions (Haase and Maret, 2005). Studies have shown that Zinc may play a role in improving peripheral insulin sensitivity, as it can potentiate insulin-stimulated glucose transport (Tang and Shay, 2001). Zinc may also protect pancreatic β cells from glucolipotoxicity (Preciado-Puga et al., 2014). Some genome wide association studies have found the islet-restricted Zinc transporter ZnT8 (SLC30A8) as a potential controller of insulin secretion and hence may modulate the risks of developing type 2 diabetes (Rutter, 2010).

Our results are in agreement with Dawud et al. (2012) but in their study the decrease in blood glucose occurred after one week of treatment with 50 mg/kg body weight of zinc. The study of Derouiche et al. (2013) showed that oral administration of 231 mg zinc sulfate /kg/day produced significant decrease in blood glucose levels but these changes occurred after 3 weeks of treatment. These results are in contrast to the work done by Cunningham et al. (1994), where they reported that, although, supplementation with zinc reportedly improves immune function, but zinc supplementation of 50 mg/day for 4 weeks adversely affected control of blood glucose in type-1 diabetics. In another study, supplementation of type 2 diabetics with 30 mg/day of zinc for six months reduced a non-specific measure of oxidative stress (plasma MDA) without significantly affecting blood glucose control (Anderson et al., 2001).

There is a considerable heterogeneity amongst the included studies, which stems from; differences in zinc doses, formulae, study durations, type of diabetes and type of species used in the study ; human or rats (Jayawardena et al., 2012).

In the present study, zinc administration produced non-significant change in serum zinc levels after 2 weeks but this change became significant after 4 weeks of treatment. On the other hand, there was significant increase in pancreatic zinc after 2 and 4 weeks of zinc treatment. The explanation of this result is that pancreas attracts zinc faster than any other organ in the body. Thus, zinc is highly abundant in the entire pancreas but most concentrated within the islets (Zalewski et al., 1994). These results are in harmony with Mishima et al. (2014) who reported that zinc supplementation to rats produced significant increase in serum and pancreatic zinc levels after 2 weeks of administration of 1000 ppm in diet.

The present experiment clearly demonstrated that zinc administration has significant beneficial effect against deleterious effects of diabetes on serum and pancreatic SOD, CAT, MDA levels after 2 and 4 weeks of treatment. In agreement with our study, some authors have reported that the high levels of myocardial MDA and the reduced concentration and activities of GSH, SOD, GPx and CAT were significantly attenuated by oral pretreatment with zinc for 4 weeks duration, indicating the radical scavenging and antioxidant activities of this trace metal (Nouf et al., 2013). Other studies were also in harmony with our result; Faure et al., (1995); Anderson et al., (2001); Roussel et al., (2003). The other possible antioxidant actions of zinc are increase the production of metallothionein, an effective scavenger of hydroxyl radicals and it has been suggested that zinc-metallothionein complexes provide protection against free radical attack (Burke and Fenton, 1985), protection against vitamin E depletion (Bunk et al. 1989; Kim et al., 1998), stabilization of membrane structure, restriction of endogenous free radical production (Sakanashi et al. 1993) and contribution to the structure of the antioxidant enzyme extracellular superoxide dismutase (Davis et al. 1998). In the contrary with our result, Afkhami-Ardekani et al. (2008) reported that, zinc sulfate consumption didn't lead to significant decrease in blood glucose levels after six week of treatment with zinc sulfate in type 2 diabetic patients. More recently, a placebo-controlled study in 40 men with type 2 diabetes found that high-dose zinc supplementation (240 mg/day) for three months did not improve measures of oxidative stress or vascular function, but normalized zinc levels (Seet et al., 2011). A possible explanation involves the multiple nutrient problems that can be present in the diabetic patients studied, the difference in the type of diabetes that was studied, also our study was done on rats.

In conclusion, serum and pancreatic Zn concentration in diabetic rat groups were significantly lower than normal control rats. Although, zinc therapies can partially ameliorate the alloxan-induced changes in diabetic rat after 2 weeks of treatment, significant recovery occurred in the measured parameter after 4 weeks of treatment. So, zinc may be recommended to be used concurrently with insulin.

While, the reported activity of zinc is of interest, this work does not establish its mechanism of action. Further studies will be required to identify the exact mechanism of action.

5. REFERENCES

Abei H (1984): Catalase in vitro. Methods Enzymol 105,121-126.

Afkhami-Ardekani, M., Karimi, M., Mohammadi, S. (2008): Effect of zinc sulfate supplementation on lipid and glucose in type 2 diabetic patients. Pak. J. Nutr., 7:550-553.

Akinosun, O., Bolajoko, E. (2007): Total antioxidant status in type 2 diabetic patients: experience at University College Hospital (UCH) Ibadan, Nigeria. Niger. J. Clin. Pract. ; 10(2):126-129.

Al-Maroof, R., Al-Sharbatti, S. (2006): Serum zinc levels in diabetic patients and effect of zinc supplementation on glycemic control of type 2 diabetics. Saudi. Med. J. 27(3): 344-350.

Al-Timimi, D., Bakir, M. (2009): Evaluation of zinc status in patients with metabolic syndrome. J Arab Board Health Specialization 10(3): 23-28.

Al-Timimi, D., Sulieman, D., Hussen, K. (2014): Zinc Status in Type 2 Diabetic Patients: Relation to the Progression of Diabetic Nephropathy. J. Clin. Diagn Res 8(11): 43-36.

Anderson, R., Roussel, A., Zouari, N. (2001): Potential antioxidant effects of zinc and chromium supplementation in people with type 2 diabetes mellitus. J. Am. Coll. Nutr.; 20(3):212-218.

Bahnam, M., Ghayour, M., Ghayour, N. (2010): Microvascular complications of diabetes. J. Biol. Sci.; 10(5):411–423.

Bakris. G (2011): Recognition, pathogenesis and treatment of different stages of nephropathy in patients with type 2 diabetes mellitus. Mayo Clin. Proc.; 86(5):444–456.

Behrens, W., Madere, R. (1991): Vitamin C and vitamin E status in the spontaneous diabetic BB rat before the onset of diabetes. Metabolism, 40: 72-76.

Blostein-Fujii, A., DiSilvestro, R., Frid, D. (1997): Short-term zinc supplementation in women with non-insulin-dependent diabetes mellitus: effects on plasma 5'-nucleotidase activities, insulin-like growth factor I concentrations, and lipoprotein oxidation rates in vitro. Am. J. Clin. Nutr.; 66(3):639-642.

Bolkent, S., Bolkent, S., Yanardag, R. (2006): Alterations in Somatostatin Cells and Biochemical Parameters Following Zinc Supplementation in Gastrointestinal Tissue of St reptozotocin-Induced Diabetic Rats. Acta histochemica et cytochemica 39(1): 9.

Brandao-Neto, J., Silva, C., Figueiredo, N. (2000): Zinc kinetics in hyujikibn nb m-dependent diabetes mellitus patients. Biometals, 13: 141-145.

Brownlee, M. (2001). Biochemistry and molecular cell biology of diabetic complications. Nature, 414(6865), 813-820

Bunk, M., Dnistrain, A., Schwartz, M. (1989): Dietary zinc deficiency decreases plasma concentrations of vitamin E. Proc. Soc. Exp.Biol. Med. 190: 379 –384.

Burke, J., Fenton, M. (1985): Effect of a zinc deficient diet on lipid peroxidation in liver and tumor subcellular membranes. Proc Soc Exp Biol Med; 179: 187-191.

Caraway, W., Watts, M. (1987): Carbohydrates. In: Tietz NW, ed.Fundamentals of Clin. Chem. 3rd ed. Philadelphia: WB Saunders; 422-447.

Ceriello, A., Quatraro, A., Giugliano, D. (1992): New Insights on Non-enzymatic Glycosylation May Lead to Therapeutic Approaches for the Prevention of Diabetic Complications. Diabet. Med. 9(3): 297-299.

Ceriello, A. (2000): Oxidative stress and glycemic regulation. Metabolism, 49(2):27-29.

Chasapis, C., Loutsidou, A., Spiliopoulou, C. (2012): Zinc and human health: an update. Arch. Toxicol., 86(4): 521-534.

Chausmer, A. (1998): Zinc, insulin and diabetes. J. Am Coll Nutr 17(2): 109-115.

Cunningham, J., Fu, A., Mearkle, P. (1994): Hyperzincuria in individuals with insulin-dependent diabetes mellitus: concurrent zinc status and the effect of high-dose zinc supple- mentation. Metabolism; 43(12):1558-1562.

Derouiche, S., Kawther, A., Manel, D. (2013): The effects of copper supplement on zinc status, enzymes of zinc activities and antioxidant status in alloxan -induced diabetic rats fed on zinc over dose diet. Int. J. Nutr. Metab. .5(5), 82-87.

Davis, C., Klevay, L., Milne, D. (1998): Extracellular superoxide dismutase activity: a promising indicator of zinc status in humans. FASEB J. 12: A346.

Dawud, F., Eze, E., Ardja, A. (2012): Ameliorative Effects of Vitamin C and Zinc in Alloxan-induced Diabetes and Oxidative Stress in Wistar Rats Current Research J. Biol Sci 4(2): 123-129.

El-Missiry, M ., El Gindy, A. (2000): Amelioration of alloxan-induced diabetes mellitus and oxidative stress in rats by oil of Eruca sativa seeds. Ann Nutr Metab. ; 44(3):97-100.

Faure, P., Benhamou, P., Perard, A. (1995): Lipid peroxidation in insulin-dependent diabetic patients with early retina degenerative lesions: Effects of an oral zinc supplementation.Eur J Clin Nutr, 49:282-288.

Gwarzo, M., Ahmadu, J., Ahmad M. (2014): Serum Glucose and Malondialdehyde Levels in Alloxan-Induced Diabetic Rats Supplemented with Methanolic Extract of Tacazzea Apiculata. Int. J. Biomed Sci., 10(4): 236.

Haase, H., Maret, W. (2005): Protein tyrosine phosphatases as targets of the combined insulinomimetic effects of zinc and oxidants. Biometals. 18:333-338.

Jarald, E., Sddaheswari, C., Dharam, S. (2008): Diabetes and herbal medicines. Iranian J. Pharmacol. Therap., (7): 97-106.

***Jayawardena, R., Ranasinghe, P., Galappatthy, P. (2012)*:** Effects of zinc supplementation on diabetes mellitus: a systematic review and meta-analysis. Diabetol. Metab. Syndr., 4(1): 13-14.

Jelodar, G., Mohsen, S. Shahram, A. (2003): Effect of Walnut leaf, Coriander and Pomegranate on blood glucose and hispathology of pancreas of Alloxan-induced diabetic rats. Afr J Tradit Complement Altern Med., 3: 299-305.

Jurowski, K., Szewczyk, B., Nowak, G. (2014): Biological consequences of zinc deficiency in the patho- mechanisms of selected diseases. J. Biol Inorg. Chem., 19 (7): 1069-1079.

Kakkar, R., Mantha, S., Radhi, J. (1998): Increased oxidative stress in rat liver and pancreas during progression of streptozotocin-induced diabetes. Clin Sci., 94(6): 623-632.

Kanthlal, S., Kumar, B., Joseph, J. (2014): Amelioration of oxidative stress by Tabernamontana divaricata on alloxan-induced diabetic rats. Anc. Sci. life, 33(4): 222-223.

Kechrid, Z., Bouzerna, N., Zio, M. (2001): Effect of low zinc diet on (65) Zn turnover in non-insulin dependent diabetic mice. Diabetes. Metab., 27(5): 580-583.

***Kechrid, Z., El-Hadjla, D., Layachi, N. (2007)*:** The beneficial effect of vitamin E supplementation on zinc status, carbohydrate metabolism, transaminases and alkaline phosphatase activities in alloxan-diabetic rats fed on zinc deficiency diet. Int. J. Diab. Metab., 15(2): 46-49.

Kelleher, S., McCormick, N.,Velasquez, V. (2011) : Zinc in specialized secretory tissues: Roles in the pancreas, prostate, and mammary gland. Adv Nutr. 2: 101-111.

***Kim, E., Noh, S., Koo, S. (1998)*:** Feeding of a marginally low level of dietary zinc lowers the concentration of alpha -tocopherol in selected organs. J. Nutr., 12: 217-220.

Kumar, P., Clark, M. (2002) :Textbook of Clinical Medicine. Pub: Saunders (London), p 1099-1121.

***Mahomed, K., Bhutta, Z., Middleton, P. (2007)*:** Zinc supplementation for improving pregnancy and infant outcome. Cochrane Database Syst. Rev., 2:CD000230.

Maritim, A., Sanders, R., Watkins, J. (2003) : Diabetes, oxidative stress, and antioxidants: a review. J. Biochem Mol Toxicol., 17(1): 24-38.

***Mishima, T., Kuroki, T., Tajima, Y. (2014)*:** Dietary zinc supplementation to the donor improves insulin secretion after islet transplantation in chemically induced diabetic rats. Pancreas. Mar; 43(2):236-239.

***Nouf M., Hala, A., Raeesa, A. (2013)*:** Preventive Effects of Selenium Yeast, Chromium Picolinate, Zinc Sulfate and their Combination on Oxidative Stress, Inflammation, Impaired Angiogenesis and Atherogenesis in Myocardial Infarction in Rats. J Pharm Pharm Sci., 16(5): 848 – 867.

***Nishikimi, M., Roa, A., Yagi, K. (1972)*:** The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem Biophys. Res. Commun., 64 (2): 849-854.

***Ohkawa, K., Ohishi, W., Yagi, K. (1979)*:** Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 95(2):351-358.

Ojezele, M., Abatan, O. (2011): Hypoglycemic and coronary risk index lowering effects of Bauhinia thoningii in alloxan-induced diabetic rats. Afr Health Sci 11 (1):85-89.

Preciado-Puga, M., Malacara, J., Fajardo-Araujo, M. (2014): Markers of the Progression of Complications in Patients with Type 2 Diabetes: A One-year Longitudinal Study. Exp. Clin. Endocrinol Diabetes 122(8): 484-490.

Prince, P., Menon, V. (2000): Hypoglycemic and other related action of Tinospora codifolia roots in alloxan rats. J. Ethnopharmacol 70: 19-25.

Ranasinghe, P., Jayawardena, R., Pigera, A. (2013): Zinc supplementation in pre-diabetes: study protocol for a randomized controlled trial. Trials 19 (1): 14-52.

Ravikumar, R., Krishnamoorthy, P., Kalidoss, A. (2010): Antidiabetic and Antioxidantefficacy of Andrographis paniculata in Alloxanized Albino rats. Int. J. Pharm. Technol., 2(4): 1016-1027.

Roussel, A., Kerkeni, A., Zouari N. (2003): Antioxidant effects of zinc supplementation in Tunisians with type 2 diabetes mellitus. J. Am. Coll. Nutr., 22:316-321.

Rutter, G. (2010): Think zinc: New roles for zinc in the control of insulin secretion. Islets, 2:49-50.

Sakanashi, T., Keen, C., Hong, K. (1993): Zinc deficiency associated alterations in the chemotactic and respi-ratory burst responses of rat peripheral blood neutrophils. FASEB J. 7: A723.

Seet, R., Lee, C., Lim, E. (2011): Oral zinc supplementation does not improve oxidative stress or vascular function in patients with type 2 diabetes with normal zinc levels. Atherosclerosis; 219(1):231-239.

Shaw, J., Sicree, R., Zimmet, P. (2010): Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res Clin Pract., 87(1), 4-14.

Stanley, P., Mainzen, P., Venugopal, M. (2001): Anti-oxidant action of Tinospora cordifolia root extract in alloxan diabetic rats. Phytother. Res, 15, 213-218.

Tang, X., Shay, N. (2001): Zinc has an insulin-like effect on glucose transport mediated by phospho-inositol-3-kinase and Akt in 3T3-L1 fibroblasts and adipocytes. J Nutr, 131:141-1420.

Tripathi, U., Chandra, D. (2010): Anti-hyperglycemic and anti-oxidative effect of aqueous extract of Momordica charantia pulp and Trigonella foenum graecum seed in alloxan-induced diabetic rats. Indian. J. Biochem. Biophys., Aug; 47(4):227-233.

Zalewski, P., Millard, S., Forbes, I. (1994): Video image analysis of labile zinc in viable pancreatic islet cells using a specific fluorescent probe for zinc. J. Histochem. Cytochem 42(7): 877-884.

Zargar, A., Bashir, M., Masoodi, S. (2002): Copper, zinc and magnesium levels in type 1 diabetes mellitus. Saudi. Med. J.; 23:539–542**.**

Zheng, Y., Li, X., Wang, Y. (2008): The Role of Zinc, Copper and Iron in the Pathogenesis of Diabetes and Diabetic Complications: Therapeutic Effects by Chelators. Hemoglobin 32(1-2): 135-145.