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ORIGINAL ARTICLE

Antioxidant effect of zinc, selenium and their combination on the liver and kidney of alloxan-induced diabetes in rats

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Abstract Zinc and selenium have been shown to singly act in normalising glycaemia and are also postulated to possess insulin-like functions. Supplementation with their combination in the diets of rats with alloxan-induced diabetes was investigated with the aim of investigating their effects on glucose homeostasis and their antioxidant properties on the liver and kidney of alloxan-induced diabetic rats. Thirty-five rats were randomly assigned to five groups and four groups were made diabetic by the administration of 150 mg/kg body weight of alloxan monohydrate, after which three diabetic groups were fed with diets supplemented with zinc, selenium and a combination of the two. Zinc, selenium and the combination significantly reduced blood glucose concentration, restored hepatic functions, increased the antioxidant status of the diabetic rats and reduced lipid peroxidation in both the hepatic and renal tissues. It was concluded that supplementation with zinc, selenium and the combination facilitated glucose uptake, prevented oxidative stress, reduced lipid peroxidation and preserved hepatic function in diabetes.

Keywords Zinc · Selenium · Diabetes · Free radicals · Catalase · Glutathione

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Introduction

A number of studies have reported an association between diabetes mellitus (DM) and alterations in the metabolism of several trace minerals (e.g., chromium, magnesium, selenium, vanadium and zinc) [1]. Some of these minerals (e.g., zinc, chromium, magnesium) are excreted at higher than normal rates in patients with DM, often leading to excessive urinary mineral wasting [2]. The general characteristic polyuria in patients with DM that results from the glucose-mediated hyperosmotic glomerular filtration may be largely responsible for the urinary mineral losses. A lowered availability of minerals may affect optimal insulin secretion and/or action. Increased dietary intake of these minerals or utilising supplemental sources of the minerals may replenish the losses.

In patients with DM, hypozincaemia and hyperzincuria are usually observed, most likely because of an increased filtered load of zinc and possibly a decreased absorption of the mineral [3]. Patients with diabetes are more likely to have suboptimal zinc status and a negative correlation has been observed between zinc intake and prevalence of DM. It has been reported that zinc in very high doses can mimic some of the actions of insulin [4].

Control of free radical production by selenium has been postulated to be of benefit in preventing glucose intolerance and the complications of DM. The insulin-like properties of selenium (translocation of glucose transporters to the plasma membrane) have been demonstrated in *in vitro* studies using adipocytes. Selenium also improves polydipsia and polyuria to controllable levels. [5] Moreover, the normally observed cardiac function decrements seen in such animals were prevented by selenium.

Zinc and selenium have been shown to singly act in normalising glycaemia and are also postulated to have

insulin-like functions. A restored Zn and Se status in people with DM may counteract the deleterious effects of oxidative stress and help to prevent complications associated with diabetes. Therefore, this study was aimed at investigating the effects of single and combined Zn and Se supplementations on variables associated with glucose metabolism and their antioxidative properties on the liver and kidney of alloxan-induced diabetic rats.

Material and methods

Animals

Thirty-five albino rats (75–95 g) of the Wistar strain were obtained from the disease-free stock of the Animal Unit of the Biological Sciences Department, University of Agriculture, Abeokuta, Ogun State, Nigeria. The rats were housed in metabolic cages. They were weighed and acclimatised to animal house condition for seven days. They were fed semi-purified standard diet for normal rats (Table 1) and had free access to water.

After acclimatisation, the rats were reweighed and fasted overnight (deprived of food for 12–14 h but had free access to water). Fasting blood glucose level was determined with the aid of a glucometer (Accu-chek Active, Roche Diagnostics, Indianapolis). Four groups of rats were made diabetic (diabetic groups) by the intraperitoneal administration of alloxan monohydrate (Sigma, St Louis, MO, USA) (dissolved in physiological saline) at a concentration of 150 mg/kg body weight while the fifth group (non-diabetic control (NDC)) were injected with an equivalent amount of saline. The feed intake was recorded daily and the body weight taken weekly. The cages were cleaned daily to maintain a proper hygienic conditions for the rats.

Table 1 Composition of diet in g/100 g of diet

Constituents	Normal diet	Zinc diet	Selenium diet	Zinc+ selenium diet
Corn starch	50.00	49.90	49.9992	49.8992
Sucrose	15.00	15.00	15.00	15.00
Soy protein	20.00	20.00	20.00	20.00
Groundnut oil	10.00	10.00	10.00	10.00
Mineral mix[a]	3.70	3.70	3.70	3.70
Vitamin mix[b]	1.30	1.30	1.30	1.30
Zinc	–0.10	–	0.10	
Selenium	–	–	0.0008	0.0008

[a]Mineral mix contained the following in g/100 g: calcium phosphate (49.50), sodium powder (11.80), potassium sulphate (5.20), sodium chloride (7.40) magnesium oxide (2.40), potassium citrate (22.40), ferric citrate (0.60), manganous carbonate (0.35), cupric carbonate (0.03), zinc carbonate (0.16), chromium potassium sulfate (0.055), potassium iodate, (0.001), sodium selenate (0.001), choline chloride (0.50)

[b]Vitamin mix contained the following in g/100 g: thiamine HCl (0.06), riboflavin (0.06), niacin (0.30), calcium pantothenate (0.16), biotin (0.010), vitamin B_{12} (0.10), vitamin D_3 (0.025), vitamin E acetate (1.00), pyridoxine (0.07), folic acid (0.02), vitamin A acetate (0.08)

After one week of alloxan administration, the preprandial blood glucose concentration was again determined. The rats with blood glucose concentration 3–5 times the initial blood glucose concentration of the rats prior to the administration of alloxan were considered diabetic. The four groups of diabetic rats were regrouped based on equalised mean blood glucose concentration. The 5 groups of rats were designated as follows:

1. Group 1. Non-diabetic control (NDC): Rats fed normal diet.
2. Group 2. Diabetic control (DC) group: Diabetic rats fed normal diet as the NDC.
3. Group 3. Zinc (Zn) group: Diabetic rats fed normal diet supplemented with 1000 mg/kg of diet of zinc as zinc sulphate.
4. Group 4. Selenium (Se) group: Diabetic rats fed normal diet supplemented with 8 mg/kg of diet of selenium as sodium selenate.
5. Group 5. Zinc+selenium (ZnSe) group: Diabetic rats fed normal diet supplemented with a combination of zinc (1000 mg/kg diet) and sodium selenate (8 mg/kg diet).

The rats were fed for four weeks on a semi-purified diet for experimental rats (Table 1). In the experimental diets, zinc and selenium were included and the percentage of carbohydrate was modified according to the composition of zinc and selenium to establish the same total percentage. Fasting blood glucose concentration was determined at the end of the experiment. The rats were killed after anaesthesia with ether and the livers and kidneys were removed; weights were recorded and any morphological changes were noted. The hepatic and renal tissues were homogenised in normal saline (1 g tissue in 4 ml of saline) and kept at 4°C before use.

Biochemical analysis

Fasting blood glucose concentration was determined with a One Touch Glucometer (Lifescan, Mulpital, CA) after blood was drawn from the tail. Tissue protein concentration was determined by the method of Lowry et al. [6]. Catalase activity was determined according to the method described by Tukahara et al. [7] where the rate of decomposition of hydrogen peroxide (H_2O_2) was measured at 570 nm. Reduced glutathione (GSH) was measured by the method of Sedlak and Lindsay [8] where protein-bound sulphydryl group in tissues was estimated

with the Ellman reagent (5,5[I]-dithionitrobenzoic acid). Lipid peroxidation in tissue homogenate was determined using the procedure of Okhawa et al. [9]. Lipid peroxidation was assessed as the amount of thiobarbituric acid reactive substances (TBARS) produced. Alkaline phosphatase activities, alanine aminotransferase activities and aspartate aminotransferase activities were determined using commercially available kits prepared by Cromatest Diagnostics, Joaquim Costal (Montgat, Barcelona, Spain).

Statistical analysis

All the results were expressed as mean ± SEM for seven animals in each group. All the grouped data were statistically evaluated with SPSS 10.0 software. Hypothesis testing methods included one-way analysis of variance (ANOVA) and subsequent comparisons between groups were made using Duncan's Multiple Range Test (DMRT). Statistical significance was set at $p \leq 0.05$.

Results

Figure 1 shows the body weight of control and experimental rats. A significant decrease in body weight was observed in untreated alloxan-induced diabetic rats when compared with the control group. However, rats fed on zinc (Zn), selenium (Se) and zinc plus selenium (ZnSe) supplemented diets showed progressive significant increases in body weight compared with the diabetic controls (DC).

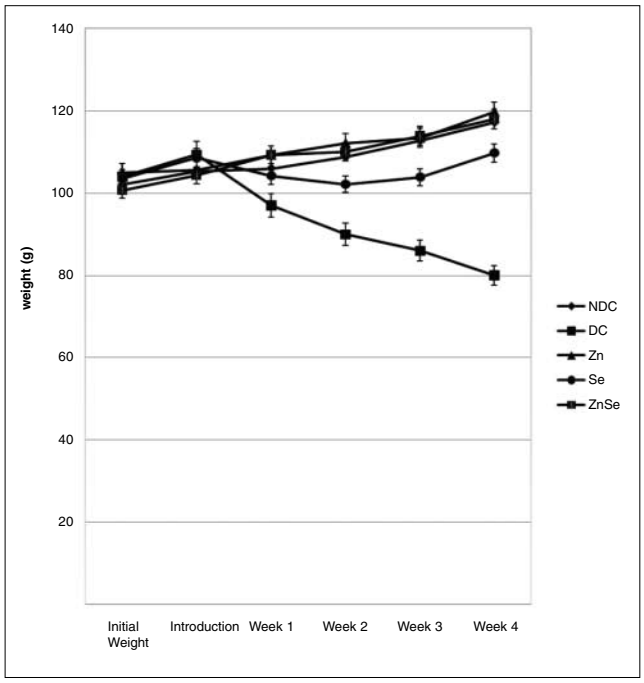

Fig. 1 Body weight changes of experimental and control rats

Figure 2 shows the blood glucose levels in control and experimental rats. There was no significant difference ($p > 0.05$) in the blood glucose levels at the beginning of the experiment (before induction). There was, however, a significant increase in the blood glucose of the diabetic groups of rats when compared with the NDC ($p < 0.05$). Supplementation of the diets with zinc, selenium and the metal combination significantly reduced the blood glucose levels when compared with the untreated diabetic rats, although the reduction was not as great as in the NDC ($p < 0.05$).

Table 2 shows the activity of catalase and the concentrations of TBARS and GSH in the liver and kidney of the experimental rats. There was a significant reduction in the hepatic and renal catalase activity of diabetic rats when compared with the NDC. The supplementations led to significant increases in the hepatic and renal catalase activity when compared with the DC ($p < 0.05$). However, the supplementation with selenium did not lead to any significant difference with the DC in the kidney ($p > 0.05$). The concentrations of TBARS in the liver and kidney of diabetic rats were significantly higher than that of the NDC rats ($p < 0.05$). The supplementation with zinc, selenium and the combination led to a significant reduction in the hepatic and renal TBARS levels when compared with the DC rats. A significant decrease was

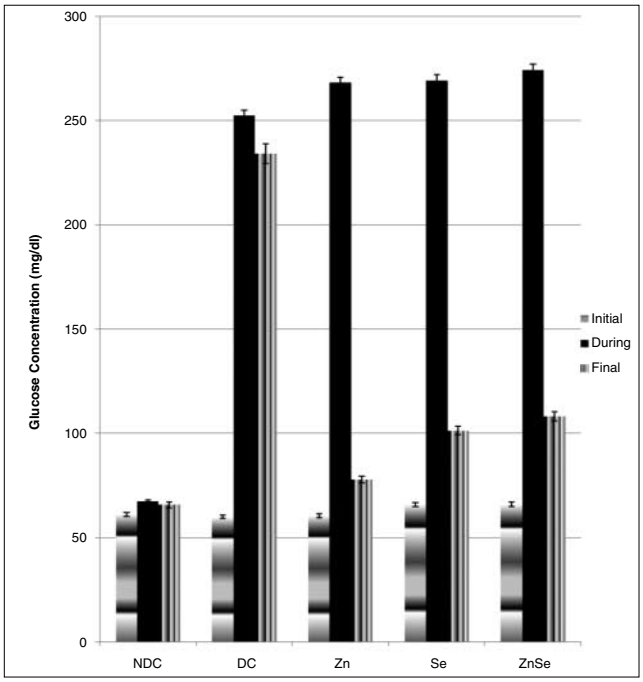

Fig. 2 Blood glucose concentrations of experimental and control rats. Values are expressed as mean ± SEM. Mean values are compared using one-way ANOVA. Level of significance was evaluated using Duncan's Multiple Range Test (DMRT) at $p \leq 0.05$. Bars of the same colour with different letters are significantly different ($p < 0.05$)

Table 2 Catalase activity and glutathione and TBARS concentrations in organs of experimental rats

	Catalase activity* (U/100 g)		Glutathione conc. (mg/100 g)		TBARS conc. (μM/100 g)	
	Liver	Kidney	Liver	Kidney	Liver	Kidney
NDC	3.66 ± 0.080^{d}	3.88 ± 0.080^{d}	45.66 ± 7.84^{b}	40.50 ± 3.02^{b}	51.50 ± 0.48^{a}	43.79 ± 0.40^{a}
DC	2.01 ± 0.041^{a}	1.98 ± 0.042^{a}	16.03 ± 3.25^{a}	21.37 ± 2.53^{a}	104.11 ± 0.75^{e}	116.99 ± 0.48^{e}
Zn	3.13 ± 0.097^{c}	3.18 ± 0.073^{c}	40.47 ± 3.41^{b}	41.19 ± 3.80^{b}	59.57 ± 0.66^{c}	76.25 ± 0.65^{c}
Se	2.05± 0.164^{a}	3.08± 0.087^{b}	42.93 ± 6.60^{b}	41.07 ± 5.77^{b}	65.39 ± 0.40^{d}	81.49 ± 0.44^{d}
ZnSe	3.06 ± 0.049^{b}	3.10 ± 0.036^{c}	42.70 ± 5.03^{b}	43.33 ± 1.19^{bc}	57.18 ± 0.50^{b}	69.41 ± 0.69^{b}

Values are expressed as mean ± SEM. Mean values are compared using one-way ANOVA. Level of significance was evaluated using Duncan's Multiple Range Test (DMRT) at $p \leq 0.05$. Values in the same column with the same superscript are not significantly different ($p > 0.05$)

*Catalase activity is expressed as micromoles of hydrogen peroxide decomposed per minute per milligram of protein

observed in the GSH level of the diabetic rats when compared with the NDC. However, the supplementations were able to restore the GSH concentration in the liver and kidney of treated groups.

Table 3 summarises the activities of key hepatic enzymes of experimental and control rats. The results show that there was a significant decrease in the alkaline phosphatase activities in the diabetic rats compared with the NDC group ($p < 0.05$). However, the treated diabetic groups showed significant increases in the alkaline phosphatase activities ($p < 0.05$) when compared with the DC. However, the activities were not fully restored to the normal level.

There were also significant decreases in the aspartate aminotransferase and alanine activities in the diabetic rats compared with the NDC group ($p < 0.05$). The treated diabetic rats all showed significant increases in the aspartate and alanine aminotransferase activities toward the value found in the NDC ($p < 0.05$).

Table 3 Activities of key hepatic enzymes in livers of experimental rats

	Alkaline phosphatase (U/l)	Aspartate aminotransferase (U/l)	Alanine aminotransferase (U/l)
NDC	155.64 ± 1.74^{d}	133.70 ± 0.52^{c}	134.92 ± 0.85^{d}
DC	92.60 ± 1.66^{a}	105.43 ± 0.06^{a}	106.99 ± 0.09^{a}
Zn	146.59 ± 0.70^{c}	124.03 ± 0.09^{b}	118.88 ± 1.37^{c}
Se	142.58 ± 0.17^{bc}	124.07 ± 0.10^{b}	112.23 ± 0.06^{b}
Zn/Se	142.10 ± 0.35^{b}	123.14 ± 0.13^{b}	111.96 ± 0.06^{b}

Values are expressed as mean ± SEM. Mean values are compared using one-way ANOVA. Level of significance was evaluated using Duncan's Multiple Range Test (DMRT) at $p \leq 0.05$. Values in the same column with the same superscript are not significantly different ($p > 0.05$)

Discussion

Alloxan-induced experimental diabetes is a valuable model for type I diabetes. It has been stated that alloxan-induced diabetic animals may exhibit most of the diabetic complications mediated through oxidative stress [10]. Studies also suggest those free radicals are involved in pancreatic cell destruction [11]. Alloxan-induced diabetes has been reported to be characterised by severe loss in body weight and this was also seen in the present study [12]. Zn, Se and ZnSe supplementations controlled this loss in body weight; however, it remained less than in normal control rats. The decrease in body weight observed in diabetic rats might be the result of protein wasting due to unavailability of carbohydrate for utilisation as an energy source [12]. In the treated groups, glucose utilisation was enhanced, hence the rate of weight loss in alloxan-induced diabetic rats was halted.

The mechanism by which Zn, Se and ZnSe caused the decrease in blood glucose sugar may be the potentiation of the insulin effect in increasing either the pancreatic secretion of insulin from β cells of islets of Langerhans or its responsiveness [11]. The increase in free radical-mediated toxicity is well documented in clinical diabetes [13] and alloxan-induced diabetes [14]. The elevated levels of toxic oxidants in diabetes are due to processes such as glucose oxidation and lipid peroxidation [15]. The reduction of oxidative stress as assessed by the catalase activities and glutathione and TBARS concentration in this study by the trace elements showed their antioxidant potentials. Also hyperglycaemia results in free radical formation through various biochemical reactions. Free radicals may be formed via the auto-oxidation of unsaturated lipids in plasma and membrane lipids.

Free radicals produced react with polyunsaturated fatty acids in cell membranes leading to lipid peroxidation. Lipid peroxidation will in turn result in elevated production of free radicals [16]. Lipid peroxide-mediated tissue damage has been observed in the development of both type I and type II diabetes. It has been observed that insulin secretion is closely associated with lipoxygenase-derived peroxides [17]. The increased lipid peroxidation in diabetic animals may be due to the observed remarkable increase in the concentration of TBARS (lipid peroxidative markers) in the liver and kidney of diabetic rats [18]. Nakakimura and Mizuno [19] also reported that the concentration of lipid peroxides increases in the kidney of diabetic rats. In the present study, TBARS levels in liver and kidney were significantly lower in the mineral-

treated group compared to the diabetic control group. The above result suggests that Zn, Se and ZnSe may exert antioxidant activities and protect the tissues from lipid peroxidation.

In the present study, the elevation of GSH levels was observed in liver and kidney of diabetic rats given diets supplemented with Zn, Se and ZnSe. This indicates that Zn, Se and ZnSe can either increase the biosynthesis of GSH or reduce the oxidative stress leading to less degradation of GSH, or have both effects. GSH has a multifunctional role in antioxidant defence. It is the most important biomolecule against chemically induced toxicity and can participate in the elimination of reactive intermediates by reducing hydroperoxides in the presence of glutathione peroxidase [20]. GSH also function as free radical scavengers and in the repair of radical-induced biological damage [21]. Loven et al. [22] suggested that the decrease in tissue GSH could be the result of decreased synthesis or increased degradation of GSH by oxidative stress in diabetes. Increased oxidative stress, resulting from significant increase in aldehydic products of lipid peroxidation, has probably decreased hepatic GSH content.

Catalase is a hemeprotein which catalyses the reduction of hydrogen peroxides and protects tissues from highly reactive hydroxyl radicals [23]. This decrease in catalase activity could result from inactivation of the enzyme by glycation [24]. Reduced activities of catalase in the liver and kidney have been observed during diabetes and this may result in a number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxides [25]. Reductions of hepatic catalase activities in alloxan-induced diabetic rats when compared with normal rats have been reported [26], whereas mineral-treated groups showed a significant increase in the hepatic and renal catalase activities of the diabetic rats. This means that Zn, Se and ZnSe can possibly reduce the glycation of enzymes or they may reduce reactive oxygen free radicals and improve the activities of antioxidant enzymes.

Tissue alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase are usually used in the diagnosis of liver necrosis and damage. Increased levels of these markers in the serum is usually indicative of a disease state. However, increased levels of these biomarkers could mean a decreased level in the liver. The decreased levels of these enzymes in the diabetic rats could be a result of loss of hepatic integrity due to the effect of excess free radicals and lipid peroxidation. Zinc and selenium may have prevented the increase in free radicals and ensured antioxidative protection of the liver, which, in turn, prevented lipid peroxidation of the organs.

Conflict of interest The authors declare that they have no conflict of interest related to the publication of this manuscript.

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