ABSTRACT

The present study has been undertaken to investigate the effect of calcium on blood biochemical parameters, tissue zinc levels and antioxidant enzyme activities in alloxan-induced diabetic rats fed on zinc over dose diet. The diabetic rats were divided into three groups (10 rates in each groups); the first group fed on standard diet (control group) while the second group (Zn group) was fed on zinc over dose of Zn (231 µg ZnSO4/g diet) and third group (Zn-Ca group) was fed on zinc-over dose diet supplemented with calcium (35 mg CaCO3 /g diet). Tissue and blood samples were taken from all animals after 21 days of treatment. In the end of the experiment it was reported that the body weight and intake food was not influenced by supplementation of calcium with zinc in diabetes. Blood glucose level of (Zn-Ca group) was higher than the group fed on the zinc only (Zn group). Calcium added significantly decreased serum triglycerides, protein, GSH and zinc level in (Zn) group animals. However, there was no effect of dietary calcium on serum cholesterol concentrations in diabetic animals. A diet supplemented by calcium led to an increase in serum glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, amylase activities and liver glutathione peroxidase and to a decrease serum aldolase and liver glutathione-S-transferase activity. In conclusion, the present study demonstrates that calcium supplementation perturbed carbohydrate metabolism, significantly reduced the severity of zinc over dose zinc in diabetes and demonstrates the role of GSH system in the detoxification of toxic metabolites of the calcium and therefore the protection of the living cell in diabetes.

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1 Introduction

Minerals are important in biochemistry and human physiology. Both deficiencies and excesses availability of these minerals caused health hazardous for human being (Peter, 2009). Zinc is an essential trace element and plays essential roles in metabolisms from human and animals (Mirela et al., 2005). It is an essential component for large numbers of enzymes actively involved in the metabolism of carbohydrates, lipids, proteins and nucleic acids as well as in the metabolism of other micronutrients with this it act as a stabilizing agents for the membranes and cellular components. With this it helps in maintaining the integrity of the cells and organs. (Shankar & Prasad, 1998). Similarly, the involvement of zinc containing enzyme, carbonic anhydrase and lactate dehydrogenase in exercise metabolism and superoxide dismutase in providing protection against free radical damage and oxidative stress was reported by Parvaneh (2009).

The overall utilization of zinc depends on the composition of the diet. Several studies had experimentally proved that soluble organic substances of low relative molecular mass, such as amino and hydroxy acids, facilitate zinc absorption and work as potential promoters or antagonists of zinc absorption Soluble. In addition, competitive interactions between zinc and other ions with similar physicochemical properties can affect the uptake and intestinal absorption of zinc (Sandström & Lönnerdal, 1998). Mirela et al. (2005) reported the various disorders like central nervous system, including alcoholism, Alzheimer-type dementia, amyotrophic lateral sclerosis, Down’s syndrome, epilepsy, Friedreich’s ataxia, Guillaine-Barré syndrome, hepatic encephalopathy, multiple sclerosis, Parkinson’s disease, Pick’s disease, retinitis pigmentosa, retinal dystrophy, schizophrenia, and Werneric-Korsakoff syndrome which is directly associated with the excess of zinc. Overtakes of zinc can translocate other metals and disturbing the minerals’ homeostasy.

Calcium is also an essential element that is only available to the body through dietary sources. Calcium occupying the fifth abundant element in the human body, and it plays a key role in skeletal mineralization. Requirement of calcium is dependent on the state of calcium metabolism, which is regulated by three mechanisms viz intestinal absorption, renal reabsorption, and bone turnover. These in turn are regulated by a set of interacting hormones, including parathyroid hormone (PTH), 1,25-dihydroxyvitamin D [1,25(OH)2D], ionized calcium itself, and their corresponding receptors in the gut, kidney, and bone (Peacock, 2010). Supplementation of calcium helps to overcome the problems of osteoporosis, hypertension, colon cancer, cardiovascular disease, premenstrual syndrome, obesity, stroke and preeclampsia (a complication of pregnancy). There are several forms of calcium salts used as supplements. They vary in their content of elemental calcium, the amount effectively absorbed by the body, and cost. Whatever the specific form, the supplement should be taken with meals to maximize absorption (Ian et al., 2010). Present study has been proposed to find out the effect of calcium supplementation on zinc status, carbohydrate metabolism and the liver activity of detoxifying glutathione enzymatic system in diabetic rats.

2 Materials and Methods

2.1 Animals and diet

Two to three months old Male albino (Wistar) rats with the weight of 220 to 300 g has been used in study. These rats were maintained on control humidity and temperature with a 12-h light/dark cycle. Animals were left to feed and were given water ad libitum. After one week, freshly prepared alloxan monoohydrate solution (Alloxan; Sigma, UK) at a dose of 150 mg/kg of body weight was injected intraperitoneally as described by Awadallah & Dessoukey (1977) to induce diabetes. After seven days of alloxan administration serum glucose concentration was measured and all the alloxan-treated animals those have glucose concentration in the serum lower than 11 mmol/l (1.98 g/l) were excluded from the experiment.

All the rats, those start showing stable diabetes symptoms divided in three groups (10 rates in each groups). The first group received a standard diet (adequate zinc and calcium, group control), the second group fed on the diet containing 231 µg ZnSO4.7H2O/g diet (Dorota et al., 2011) (zinc over-dose, group Zn) while the third group or rats received a diet containing a 231 µg ZnSO4.7H2O /g diet supplemented with calcium (35 mg CaCO3 /g diet) as formulated by Nelson et al., 1987 (group Zn-Ca). The composition of the diet was similar to that described previously by Southon et al (1984). Rats were maintained on the appropriate experimental diet add libitum for 20 days.

Food intake and body weights were recorded regularly. on day 21, animals were fasted overnight and given access to food for two periods of 1 hour between 11.00 - 12.00 hours and 17.00 - 18.00 hours so that time of feeding on day before death was similar for all groups. At the completion of study (22nd Day), rats were killed and the blood was divided in to three parts first part transferred into ice cold centrifuge tubes, second part of the blood used for the blood glucose analysis which was performed immediately after exsanguinations and the third one used for the serum zinc, cholesterol, triglycerides, protein, urea, GOT, GPT, amylase, aldolase assays which is performed by the centrifugation of blood for 10 min at 3000 rpm. .

The liver, kidney, pancreas and testis of experimental animal were removed, washed with ice-cold physiological saline solution, dried and processed for biochemical measurements. Homogenates were prepared on ice in the ratio 4 g tissue for 16 ml of phosphate buffer, pH 7.5, containing 1 mmol/l Na2EDTA. For each sample 10 µl of 500 mmol/l BHT in acetonitrile was added to prevent formation of new peroxides during the assay. The homogenates were centrifuged at 20000 × g for 15 min at 4°C and frozen at –70°C until analysis.
2.2 Analytical methods

Blood glucose level was estimated by the glucose oxidase method using commercial test kit for glucose as described by Ann (1969). Glutamate oxaloacetate, glutamate pyruvate aminotransferases, amylase and aldolase activity were determined by using commercially available test kits as described by Bergmeyer & Walefeld (1978). Similarly, the amylase (Ying et al., 1998) and aldolase (Feissli et al., 1966) were also decided by the available kit. The presence of cholesterol, triglycerides and urea concentrations were also determined using commercial test kits for cholesterol (Trinder, 1969) and triglycerides (Buccoolo, 1973). Serum total protein level was analyzed by the method of Lowry et al (1951).

For the recovery of Zn from the dissected liver, pancreas, testis and kidney, method described by Kechrid & Bouzerna (2001) was used, for this these organs were heated in silica crucibles at 480°C for 48 hours and the ash taken up in hot Ultrex nitric acid (11.7 M) and analyzed by atomic absorption spectrophotometer (SHIMADZU AA-6200) . The accuracy of zinc recovery was checked using standard reference materials; bovine liver and wheat flour. These standards were prepared and analyzed in similar conditions to the test items to assess recovery. The recovery of zinc in the standard reference material exceeded 96%. Zinc in serum was analysed after a twenty-fold dilution of the serum by Flame Atomic Absorption Spectrophotometer (SHIMADZU AA-6200). Zinc standards were prepared from a 1mg/ml zinc nitrate standard solution (BDH) using 5 % glycerol to approximate the viscosity characteristics, and to avoid zinc contamination from exogenous sources. All tubes were soaked in HNO3 (10 % v/v) for 16 h and rinsed with double distilled water. Glutathione (GSH) concentration was measured utilizing the method described by Weckbercker and Cory (1988). GSH-Px (EC 1.11.1.6) activity was measured spectrophotometrically at 340 nm as described by the Pinto and Bartley (1969), as regards the activity glutathione S transferase, it was determined by the Habig et al., (1974). The reported data are the means of measurements and their SEM. values. For statistical evaluation the Student’s t-test and p < 0.05 was considered the limit for the statistical significance.

3 Results and Discussion

The results of present study indicate that the supplementation of calcium with zinc could not affect the body weight and food intake in diabetic zinc over dose rate, (Table 1). The serum, liver, kidney, pancreatic and testis zinc recovery from the group of rats feed on higher zinc dose was significantly (p < 0.05) higher than the control groups. Table 1 have clearly represented the role of calcium supplements on the increasing of zinc concentration in serum, liver, kidney, pancreas and testis in higher dose zinc group. Similarly, higher glucose level, GOT, GPT and amylase activities have been reported in the group fed on the Zn-Ca supplemented as compared to the group of higher zinc fed and control. With this lower triglycerides, protein and aldolase activities have been reported in the animal group fed on the zinc calcium supplements, these activities were maximum in the group which was fed on the higher zinc dose. However, there was no effect of dietary calcium on serum cholesterol concentrations (Table 02).

The liver protein and liver glutathione (GSH) levels in zinc over dose diabetic group (Zn) were higher as compared to the diabetic control group. Supplementation of calcium with zinc showed a significantly reduction in the liver GSH and protein levels. The liver glutathione S transferase (GST) activity in diabetic (Zn) group was increased as compared to the diabetic control group. However, the liver glutathione peroxodase (GPx) activity of (Zn) group animals was lower than those of control group. Addition of calcium significantly reduced liver GST and elevated liver GPx activities (Table 3).

Table 1 Body weight gain, serum zinc and tissues zinc concentrations of diabetic rats fed adequate diet (control), zinc over dose diet (Zn) and zinc over dose supplemented with calcium diet (Zn-Ca) at the end of experimental period.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Experimental groups</th>
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<tr>
<td></td>
<td>Control</td>
<td>Zn</td>
<td>Zn-Ca</td>
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<tr>
<td>Body weight gain (g/day)</td>
<td>3.99±0.51</td>
<td>4.38±0.44</td>
<td>5.03±0.75**</td>
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<td>Food intake (g/day)</td>
<td>17.14±0.04</td>
<td>18.21±0.02</td>
<td>18.62±0.05</td>
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<tr>
<td>Zinc serum (µg/100ml)</td>
<td>90.30 ± 3.40</td>
<td>147.12±9.21</td>
<td>102.31±35.7</td>
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<tr>
<td>liver Zn Concentration (µg/g dry weight)</td>
<td>28.59±1.18</td>
<td>35.48±2.40</td>
<td>30.86±1.02**</td>
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<td>kidney Zn Concentration (µg/g dry weight)</td>
<td>35.19±3.14</td>
<td>45.98±7.72</td>
<td>42.76±5.20</td>
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<tr>
<td>Pancreatic Zn Concentration (µg/g dry weight)</td>
<td>59.82±5.21</td>
<td>76.32±8.52</td>
<td>63.41±7.30</td>
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<tr>
<td>Testis Zn concentration (µg/g dry weight)</td>
<td>81.70±4.05</td>
<td>170.50±7.08</td>
<td>100.18±5.14***</td>
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Data are mean of ten replicates; ± Standard Error of mean; Values followed by the symbol *, **; *** differ significantly according to LSD value P < 0.05; P < 0.01 and P < 0.001 respectively; NS Not Significant.
The results of the study favor the hypothesis that dietary calcium may alter plasma lipid profile favorably and are consistent with previous studies that suggested that dietary calcium reduces serum triglyceride (Malekzadeh et al., 2007). Calcium supplementation suppressed circulating concentrations of parathyroid hormone and 1,25-dihydroxyvitamin D, thereby possibly promoting lipolysis (Shalileh et al., 2009).

The higher levels of transaminase activities in the Zn-Ca group animals, confirm the result of high concentration of their blood glucose. In other words, the gluconeogenic action of GOT and GPT played an important role in provide a new supply of glucose from other sources such as amino acids (Kechnid et al., 2007). It is interesting to note that Grefley and Sandstead (1993) found evidence of decreased oxidation of the carbon chain of alanine when zinc was restricted and led to alanine accumulation in blood. The decrease of serum aldolase activity in rats given over dose zinc diet supplemented with calcium may be attributed to the decrease in serum zinc. Zinc is essential for the activity of aldolase. It serves as structural functions (Matthias & Georg, 1996). In general the present study indicated that some symptoms and signs associated with decrease zinc statute and aldolase activity in diabetic rats may be stimulated by supplementation with calcium. However, the increased activity of amylase in serum is probably a result of increased calcium concentration. Calcium binding is an inhibited effect of calcium on decrease the bioavailability of the zinc. An inhibitory effect of calcium on zinc utilization has been reported by Chao et al. (2006). In this respect, results of present study are in conformity with the finding of Chao et al. (2006) and Mace and Shannon (2000). In the current study, when the time of feeding was strictly controlled and the amount of food eaten by each animal before an overnight fast was known to be similar, the mean fasting blood glucose concentration in rats fed zinc over dose diet supplemented with calcium were found to be higher than that of rats fed zinc over dose diet. This suggests that the calcium are reportedly associated with the development of diabetes, impaired glucose tolerance, and insulin resistance (Elizabeth & Brendan, 1994). The results of the study favor the hypothesis that dietary calcium increases glucose levels.
irreversible process and is required to maintain both the activity and the stability of amylase (Douglas et al., 1989). The increase GSH concentration and GST activity in liver animals fed Zn confirms an efficacious defense of the zinc against oxidative stress under diabetic conditions (Mariani et al., 2008). Zinc is also necessary to mobilize defense against reactive species oxygen and H2O2 that induce apoptosis (Ani et al., 2007).

The data obtained was similar to those presented by Cho & Fong (1990). Supplementation with calcium decrease protein, GSH concentration and increase GPx activity in liver rats. This result confirms the report of Capel et al. (2005) who observed decrease GSH level in liver by calcium in rats.. This Ca2+ induced mitochondrial oxidative stress could lead to a loss of muscle mass through apoptosis, necrosis or stimulation of proteolysis after protein oxidation (Chuan-Chin et al., 2010). Glutathione (GSH) participates in the cellular defense system against oxidative stress by scavengers free radicals and reactive oxygen intermediates (Mosaad & Abd-Allah, 2004). Thus, the decrease in GSH level might reflect a direct reaction between GSH and free radicals generated by calcium supplement. A significant increase in the level of GSH-Px activity during the experiment confirms an efficacious defense of the liver against oxidative stress. The decrease of GST activity of our experiment suggests a zinc deficiency by calcium supplement has been reported to decrease the activity of glutathione transferase in the liver (Cho and Fong, 1990).

Reference


