Original Article
Changes in metabolism of Zinc and carbohydrate and testis oxidative stress of diabetic rats fed zinc-over dose diet.

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ABSTRACT

The present study was designed to evaluate the effect of dietary zinc on blood biochemical parameters, zinc levels and testis antioxidant status in Alloxan-induced diabetic rats. The diabetic rats were divided into four groups (10 rats in each group): the first group normal rats fed adequate diet (control group), the second group normal rats fed adequate diet supplemented with zinc (231 mg ZnCO₄ 7H₂O /kg diet) (normal +Zn group), the third group diabetic rats fed adequate diet (diabetic group) and the fourth group diabetic rats fed adequate diet supplemented with zinc (231 mg ZnCO₄ 7H₂O /kg diet) (diabetic +Zn group). Diabetes was induced by Alloxan (150 mg/kg) and zinc was added to the diet of the animals for 21 days. Results showed that Diabetes caused a significant decrease in body weight gain, serum and testis zinc, serum amylase, alkaline phosphatase, aldolase and LDH activities, testis GSH, GPx and GST activities. In contrast, it led to an augmentation in blood glucose, triglycerides, cholesterol and testis MDA in rats. Zinc treatment in the diet for diabetic rats ensured a partial correction of the previous parameters. In conclusion, this study indicated that zinc act as powerful protective and antioxidants which may exercise adverse effect against metabolic disorder and testis complication of diabetes.

Introduction

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia and insufficiency of secretion or action of endogenous insulin [1]. Diabetes is associated with an extensive list of late complications involving nearly every tissue [2]. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels [3]. Oxidative damage due to free radicals is associated with vascular disease in people with type 1 and those with type 2 diabetes mellitus (DM). There are several potential sources of increased free radical production in diabetes including auto-oxidation of plasma glucose, Intercellular activation of sorbitol pathway and non-enzymatic protein glycation[4]. Increased oxidative stress is a widely accepted participant in the development and progression of diabetic tissue damage and induced changes in the activities of antioxidant enzymes in various tissues [5]. The interrelationship between diabetes and various minerals is characterized by a high degree of reciprocity. Chronic uncontrolled hyperglycemia can cause significant alterations in the status of these nutrients, and conversely, some of these substances, especially those that have been characterized as micronutrients, can directly modulate glucose homeostasis [6]. Zinc is the second most abundant trace element in the body [7]. It is one of the important essential trace metals that are required for many cell vivan as cofactor of numerous enzymes which are involved with the metabolism of protein, carbohydrate and lipids [8]. Zinc is involved in a myriad of biological processes that include catalysis, stabilisation of cell membranes and regulation of gene expression [9]. Zinc homeostasis always is related to certain disorders such as metabolic syndrome, diabetes and diabetic complications [10]. Zinc also interacts with important hormones involved in testis and bone growth such as somatomedin-c, osteocalcin, testosterone, thyroid hormones, and insulin [11]. Correction of zinc deficiency in patients with type 1 DM by zinc over dose also leads to decreased lipid peroxidation and improvements in glucose homeostasis. Therefore, Zinc act in normalizing glycemia and is postulated to function as antioxidants, a restored zinc status in people with type 2 DM may counteract the deleterious effects of oxidative stress and help to prevent complications associated with diabetes [12]. Present study has been proposed to find out the effect of zinc supplementation on some biochemical parameters and oxidative stress in alloxan diabetic rats.

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2. Materials and Methods

2.1. Animals and Handling.

Forty adult male albino rats, weighing 269–294 g, were taken from the animal house of Pasteur institute, Algeria. They were placed in four groups of ten rats in each and kept in animal’s house of Molecular and cellular biology Department, University of El Oued, Algeria. Standard rat food and tap water were available ad libitum for the duration of the experiments. Animals were adapted for two weeks under the same laboratory conditions of photoperiod (12h light/12 h dark) with a relative humidity 62% and room temperature of 24 ± 1°C. The experimental procedures were carried out according to the National Institute of Health Guide-lines for Animal Care and approved by the Ethics Committee of our Institution.

2.2. Induction of diabetes

Diabetes was induced with fresh alloxan monohydrate solution using a previously described method [13]. Alloxan was administered intraperitonially (i.p.) at a dose of 150 mg/kg body weight dissolved in citrate buffer (0.01 M, pH 4.5). Blood glucose was measured 7 days after induction of diabetes on samples taken from tail vein. The diabetic state was confirmed by a glucose-meter for glucose concentration exceeded 2.5g/l, when glucose was measured 7 days after induction of diabetes on samples taken from tail vein. The diabetic state was established when the glucose concentration exceeded 2.5g/l, confirmed by a glucose-meter [13].

2.3. Blood collection and preparation of tissue samples

At the end of 3 weeks of zinc treatment, rats were fasted for 16 hrs, anaesthetized with chloroform by inhalation, rats were decapitated and blood samples were transferred into ice cold centrifuge tubes. The serum was prepared by centrifugation, for 10 min at 3000 revolutions/min and utilized for triglyceride and total cholesterol concentration and GOT, GPT, amylase, ALP, LDH and aldolase assays. The blood glucose was measured by glucometer. Testis was rapidly excised, weighed and stored at -20°C for oxidative stress parameters analysis.

2.4. Measurement of biochemical parameters

The activities of GOT, GPT, amylase, alkaline phosphatase, lactic dehydrogenase and aldolase were determined with commercial kits from Spinreact, Spain (refs: GOT-1001161, GPT-1001171, LDH-1001260, amylase-41201). ALP-1001131 and aldolase-1001075). Total cholesterol and triglyceride concentrations were also measured using commercial kits (Spinreact, refs: triglyceride-100131 and cholesterol-1001093).

2.5. Serum and testis zinc analyses

Dried testis was heated in silica crucibles at 480°C for 4 h and the ash was dissolved in hot 12 M hydrochloric acid for zinc using a flame atomic absorption spectrophotometer (SHIMADZU AA-6200). In the serum samples zinc was determined after 20-fold dilution. In this case the zinc standards were prepared from a 1mg/ml zinc nitrate standard solution, using 5 % glycerol to approximate the viscosity characteristics and to avoid zinc contamination from exogenous sources. All tubes were soaked in HCl (10% v/v) for 16 h and rinsed with doubly distilled water [14].

2.6. Antioxidants measurement

2.6.1. Preparation of homogenates

About 1g of testis was homogenized in 9 ml of buffer solution of Tris buffer saline (TBS, pH=7.4). Homogenates were centrifuged at 10000g for 15 min at 4°C, and the obtained supernatant was used for the determination of antioxidant activity.

2.6.2. Determination of malondialdehyde (MDA) level

Tissue homogenates were prepared at 10% (w/v) in 0.1 mol/L Tris-NaCl buffer, pH 7.4, and MDA steady-state level was determined. MDA was measured according to the method described by Sastre et al. (2000) [15]. Thiobarbituric acid 0.67% (w/v) was added to a liquor of the homogenate previously precipitated with 10% trichloroacetic acid (w/v). Then the mixture was centrifuged, and the supernatant was heated (100°C) for 15 min in a boiling water bath. After cooling, n-butanol was added to neutralize the mixture, and the absorbance was measured at 532 nm. The results were expressed as nmol of MDA/g tissue.

2.6.3. Determination of reduced glutathione (GSH) level

GSH concentration was performed with the method described by Ellman [16], based on the development of a yellow color when DTNB is added to compounds containing sulphydryl groups. In brief, 0.8 mL of tissue homogenate was added to 0.2 mL of 0.25% sulphyosalicylic acid and tubes were centrifuged at 2500 g for 15 min. Supernatant (0.5 mL) was mixed with 0.025 mL of 0.01 M DTNB and 1 mL TBS (pH 7.4). Finally, absorbance at 412 nm was recorded. Total GSH content was expressed as nmol GSH/mg prot.

2.6.4. Determination of Glutathione-S-transferase (GST) activity

Glutathione-S-transferase (GST) activity of tissues was measured spectrophotometrically by the method of Habig et al. [17], using CDNB as electrophilic substrate that binds to GSH with the participation of the enzyme and forms a colored GSH-substrate complex, detected at 340 nm. The activity of GST was expressed in terms of μmol CDNB-GSH conjugate formed/min/mg protein.

2.6.5. Assay of Glutathione peroxidase (GSH-Px) activity

Glutathione peroxidase (GSH-Px) catalyzes the reduction of hydroperoxides using GSH as a reductant. Determination of tissue GSH-Px activity was carried out according to the method of Flohe and Gunzler [18]. The reaction mixture contained 0.2 mL of TBS (Tris 50 mM, NaCl 150 mM, pH 7.4); 0.4 mL of GSH (0.1 mM), 0.2 mL of homogenate was added and allowed to equilibrate for 5 min at 25°C. The reaction was initiated by adding 0.2 mL of H2O2 (1.3 mM); reaction was terminated by addition of 1 mL of 1% Trichloroacetic acid (TCA). Tubes were centrifuged at 1500 g for 5 min and the supernatant was collected. To 0.48 mL of resultant supernatant, 2.2 mL of TBS (pH 7.4) and 0.32 mL of DTNB (1.0 mM) were added. After mixing, absorbance was recorded at 412 nm and the specific activity of this enzyme is expressed as μmol GSH/mg protein.
2.7. Statistical Analysis

carried out by using 1-way analysis of variance followed by the Student t test to compare means among the groups. Differences were considered statistically significant at p<0.05.

3. Results

3.1. Body weight and food intake

Induction of experimental diabetic state caused a decrease in body weight gain (P<0.001) and increase in food intake (P<0.001) compared to the control rats. Zinc was also significantly (P<0.05) increased both body weight gain and food intake of diabetic rats compared to the control and diabetic rats respectively (Table 1).

3.2. Serum and testis zinc concentrations

Figure 1 and 2 showed that serum and testis zinc contents were significantly lower (p < 0.001, p < 0.01) in the diabetic group than the control. Zinc treatment in both diabetic and non-diabetic rats was resulted in an increase (p < 0.001) of serum and testis zinc concentrations when compared with control and diabetic rats.

Fig. 1. Zinc concentration in serum of experimental rats.*p<0.05, ***p<0.001: significantly different from control group; c p<0.001: significantly different from Diabetic group.

Fig. 2. Zinc concentration in testis tissue of experimental rats.***p<0.001: significantly different from control group; c p<0.001: significantly different from Diabetic group.

3.3. Blood biochemical values

As shown in Table 2, diabetes caused a significant increase (p < 0.001) of serum glucose, triglyceride and cholesterol concentrations. Also diabetes resulted in a significant increase (p < 0.001) of serum GOT and GPT activities and a decrease (p < 0.001) of serum amylase, alkaline phosphatase, aldolase and LDH activities. Meanwhile, high zinc feed made a recovery in the above mentioned biochemical parameters either in normal or diabetic rats.

Table 2. Mean blood glucose levels and blood biochemical value in control and experimental groups

<table>
<thead>
<tr>
<th>parameters</th>
<th>Control (n=10)</th>
<th>Nomal + Zn (n=10)</th>
<th>Diabetic (n=10)</th>
<th>Diabetic + Zn (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood glucose (g/l)</td>
<td>1.09±0.027</td>
<td>0.91±0.045</td>
<td>2.08±0.028</td>
<td>1.61±0.040**</td>
</tr>
<tr>
<td>Serum triglycerides</td>
<td>0.87±0.037</td>
<td>0.59±0.017</td>
<td>1.14±0.032</td>
<td>0.61±0.013**</td>
</tr>
<tr>
<td>Serum cholesterol (g/l)</td>
<td>1.58±0.52</td>
<td>1.94±0.46</td>
<td>2.31±0.25</td>
<td>2.05±0.25**</td>
</tr>
<tr>
<td>Serum GOT (U/l)</td>
<td>81.4±2.22</td>
<td>72.1±3.18</td>
<td>109.3±3.18</td>
<td>91.3±2.34**</td>
</tr>
<tr>
<td>Serum GPT (U/l)</td>
<td>42.1±3.35</td>
<td>40.5±1.38</td>
<td>55.0±1.15</td>
<td>49.9±1.12</td>
</tr>
<tr>
<td>Serum Amylase (U/l)</td>
<td>73.7±13.3</td>
<td>83.1±3.17</td>
<td>93.7±13.3</td>
<td>93.7±13.3</td>
</tr>
<tr>
<td>Serum ALP (U/l)</td>
<td>350.7±1.47</td>
<td>518±1.27</td>
<td>212±1.46</td>
<td>259±1.27**</td>
</tr>
<tr>
<td>Serum LDH (U/l)</td>
<td>143±1.39</td>
<td>147.9±1.66</td>
<td>132±1.06</td>
<td>128±1.06</td>
</tr>
<tr>
<td>Serum Aldolase (U/l)</td>
<td>7.4±0.15</td>
<td>8.7±0.16</td>
<td>6.0±0.12</td>
<td>7.0±0.12</td>
</tr>
</tbody>
</table>

3.4. Oxidative stress parameters

Seen from Table 3, diabetes induced a significant increase (p < 0.001) in MDA concentration and a significant decrease in GSH level (p < 0.001), GST (p < 0.05) and GPx (p < 0.001) activities compared to the corresponding control values. Treatment with Zinc partially restored the levels of MDA, GSH, GPx and GST activities. In fact, MDA concentration in the diabetic + Zn groups was significantly higher than in the control group (p<0.05).

Table 3. Malondialdehyde (MDA), Glutathione (GSH) level, Glutathione S transferase(GST) and Peroxydase Glutathione (GPx) activities in testis of Control and experimental groups.

<table>
<thead>
<tr>
<th>parameters</th>
<th>Control (n=10)</th>
<th>Normal + Zn (n=10)</th>
<th>Diabetic (n=10)</th>
<th>Diabetic + Zn (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tests MDA (g/mg prot)</td>
<td>0.12±0.08</td>
<td>0.11±0.089</td>
<td>0.20±0.057</td>
<td>0.14±0.019</td>
</tr>
<tr>
<td>Tests GSH (g/mg prot)</td>
<td>32.9±0.8</td>
<td>39.9±2.94</td>
<td>24.0±1.30</td>
<td>34.0±1.09</td>
</tr>
<tr>
<td>Tests GST (g/mg prot)</td>
<td>0.43±0.07</td>
<td>0.43±0.07</td>
<td>0.41±0.09</td>
<td>0.45±0.03</td>
</tr>
<tr>
<td>Tests GPx (g/mg prot)</td>
<td>0.25±0.06</td>
<td>0.31±0.02</td>
<td>0.20±0.04</td>
<td>0.24±0.01</td>
</tr>
</tbody>
</table>

4. Discussion

In this experiment diabetic rats weighed less than control rats, this is consistent with some previously published reports [19, 20]. This raises the possibility of the metabolic state disturbance of animal, suggesting that the diabetic condition had exacerbated reduced the ability of the diabetic rats to utilize food intake as normal subjects. However, the effective mechanisms of zinc supplementation on weight can be due to the role of zinc in appetite regulation through changes in hypothalamic neurotransmitter metabolism of leptin system and its receptors, in other words zinc can induce synthesis of leptin[21]. In addition,
zinc may enhance levels of growth factor-I (IGF-I), particularly it may contribute to elevating serum testosterone. Both IGF-I and testosterone are anabolic factors that may be enhancing body weight gain [22]. Serum and testis zinc concentrations in diabetic rats were lower than that of non-diabetic rats. These findings, indicating the effect of diabetes on body zinc status. It has been postulated that low level of zinc in diabetic patients may be due to excessive urinary output and gastrointestinal malabsorption [23]. Similarly with zinc supplementation, a total correction of zinc status in diabetic rats was noticed. These results are in line with study of Sharif et al, who suggested that zinc supplementation have a beneficial effect in an elderly population with low Zn levels by improving Zn status [24], the mean fasting blood glucose, cholesterol and triglyceride concentration in diabetic were found to be higher than that of normal rats. Meanwhile, the current research showed that glucose, cholesterol and triglyceride concentration were ameliorated after zinc supplementation in both normal and diabetic animals. These observations indicate that carbohydrate metabolism of these animals is sensitive to zinc variation Several molecular mechanisms are believed to be involved in the regulation of blood glucose levels following zinc supplementation [25]. The protein tyrosine phosphatase 1B (PTP 1B), a key regulator of the phosphorylation state of insulin receptor is known to be a target of zinc ions activation. Studies have shown that zinc may play a role in improving peripheral insulin sensitivity, as it can potentiate insulin which stimulated glucose transport [26]. In other words, it has been stated that zinc can play an important role on enzymes involved in lipids metabolism [27]. In this experiment it was also found a significant rise in serum GOT and GPT activities in diabetic rats, which could relate to excessive accumulation of amino acids (glutamic and alanine) in serum of diabetic animals as a result of amino acids mobilization from protein stores [28]. These excessive amino acids are then converted to ketonic bodies (a keto-glutaric and pyruvate) for which the enzyme GOT and GPT are needed, leading to increased enzyme activity, all these changes could be prevented with zinc treatment, which was associated with the up regulation of hepatic metallothionein expression [29]. The decrease in serum amylase, alkaline phosphatase, aldolase and LDH in diabetic rats may be attributed to the decrease in serum zinc associated with diabetes. Zinc is essential for the activity of these enzymes. It serves as one or more structural, regulatory or catalytic functions. In general, the present study indicated that some symptoms and signs associated with zinc deficiency and decrease metallo-enzyme in diabetic rats can be ameliorated by supplementation with zinc. In our experimental model of DM, it was observed that alloxan administration produced subsequent increase in testis MDA level and a significant decrease in GSH content and GPx and GST activities of testis tissue. Several studies showed that alloxan produces a decrease in the activity of the antioxidant enzymes during the development of alloxan-induced type I DM in testis [30, 31]. There is a complex interaction between antioxidants and oxidants such as reactive oxygen species, which modulates the generation of oxidative stress [32]. The diminished of GSH may due to the higher levels of superoxides and free radicals GSH converts more to oxidized glutathione (GSSG) or; is alight rate of conversion of GSH to hydrogen-sulfide (H2S) during the diabetes process as GSH converts to H2S in the liver and is ultimately excreted out [33]. In contrast, zinc supplementation decrease MDA and raised testis GSH level, GPx and GST activities as compared to diabetic group. these changes confirms an efficacious defense of the zinc against oxidative stress under diabetic conditions [34]. Zinc is also necessary to stimulate defense against reactive species oxygen and H2O2 that induce apoptosis and superoxide dismutase (SOD) activity [35].

Conclusion
In conclusion, the study clearly demonstrated the administration of zinc sulfate to diabetic rats change glucose and biochemical parameters, which play an important role in oxidative stress, reducing the severity and complication of diabetes.

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REFERENCES


