Beneficial Effect of Zinc on diabetes induced kidney damage and liver stress oxidative in rats

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ABSTRACT
The present study was designed to investigate the effects of Zn supplementation on kidney function and liver oxidative stress in Alloxan diabetic rats. Male albino Wistar rats were randomly divided into 4 groups (n=10): Control, Non Diabetic + Zinc, Diabetic and Diabetic + Zinc. Diabetes was induced by alloxan (150 mg/kg). Zinc (231 mg/kg feed) as ZnSO4 7H2O was added to the feed of the animals in the zinc groups for 21 days. Alloxan Diabetes caused a significant decrease in body weight gain, serum and tissue zinc, serum protein concentrations, liver GSH, GPx and GST activities. In contrast, it led to an augmentation in urea, creatinine, uric acid and MDA in rats. Zinc supplementation in the diet for diabetic rats ensured a partial correction of the previous parameters. In conclusion, this study indicated that zinc act as powerful antioxidants which may exercise adverse effect against severity and complication of diabetes.

Keywords
Diabetic rats, zinc, liver, kidney, stress oxidant

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) [4]. The liver plays a central and crucial role in the regulation of carbohydrate metabolism. Its normal functioning is essential for the maintenance of blood glucose levels and of a continued supply to organs that require a glucose energy source. This central role for the liver in glucose homeostasis offers a clue to the pathogenesis of glucose intolerance in liver diseases but little insight into the mechanisms of liver disease in diabetes mellitus [5]. Diabetic nephropathy (DN) is the leading cause of chronic kidney disease (CKD) in the UK, and the second most common cause of end-stage renal disease (ESRD) requiring renal replacement therapy (RRT) or kidney transplantation. This patient group are at significant cardiovascular risk and usually succumb to this, rather than progress to ESRD, especially patients with type 2 diabetes (T2DM). [6]. Zinc is an essential trace element that exists in all cells and is required by thousands of proteins for catalytic, structural, or transcriptional functions. Since the 1930s when zinc was first demonstrated to be an integral element of the insulin crystalline structure [7], Zinc is important in insulin action and carbohydrate metabolism. Oxidative stress plays an important role in the pathogenesis of diabetes and its complications. Zinc is a structural part of key anti-oxidant enzymes such as superoxide dismutase, and Zinc deficiency impairs their synthesis, leading to increased oxidative stress [8]. Present study has been proposed to find out the effect of zinc supplementation on some biochemical marker of kidney function and liver oxidative stress in Alloxan diabetic rats.

MATERIALS AND METHODS
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/12h 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.

Induction of diabetes
Diabetes was induced with fresh alloxan monohydrate solution using a previously described method [9]. Alloxan was administered intraperitoneally (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 established when the glucose concentration exceeded 2.5g/l, confirmed by a glucose-meter (Accu-Check, Roche Diagnostics, Paris, France).
Blood collection and preparation of tissue sample

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 glucose, urea, creatinine uric acid and total proteins assays. Liver was rapidly excised, weighed and stored at -20°C for oxidative stress parameters analysis.

Measurement of biochemical parameters

The level of serum glucose, urea, creatinine and uric acid were determined with commercial kits from Spinreact, Spain (refs: glucose-41011, urea-1001331, creatinine-1001113, uric acid-1001013). The total proteins concentrations were also measured using commercial kits (Spinreact, refs: total proteins-1001291).

Serum, liver and kidney zinc analyses

Dried liver or kidney was heated in silica crucibles at 480°C for 48 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 [10].

Antioxidants measurement.

Preparation of homogenates

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

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) [11]. Thiobarbituric acid 0.67% (w/v) was added to a liquots 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.

Determination of reduced glutathione (GSH) level

GSH concentration was performed with the method described by Ellman [12], based on the development of a yellow color when DTNB is added to compounds containing sulfhydryl groups. In brief, 0.8 mL of tissue homogenate was added to 0.2 mL of 0.25% sulphasalic 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.

Determination Glutathione-S-transferase (GST) activity

Glutathione-S-transferase (GST) activity of tissues was measured spectrophotometrically by the method of Habig et al. [13], 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 CDNBGSH conjugate formed/min/mg protein.

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 [14]. 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 250°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.
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.

RESULTS

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) increase both body weight gain and food intake of diabetic rats compared to the control and diabetic rats respectively (Table 1).

Table 1. Initial body weight, body weight gain, food intake in control and experimental groups

<table>
<thead>
<tr>
<th>parameters</th>
<th>Control (n=10)</th>
<th>Non diabetic +Zn (n=10)</th>
<th>Diabetic (n=10)</th>
<th>Diabetic + Zn (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight (g)</td>
<td>273.4 ±3.8</td>
<td>275.2 ±1.5</td>
<td>286± 7.4</td>
<td>279.6± 1.6</td>
</tr>
<tr>
<td>Body weight gain (g/day)</td>
<td>5.03±0.09</td>
<td>5.42±0.12</td>
<td>3.99±0.04</td>
<td>4.38±0.10</td>
</tr>
<tr>
<td>Food intake (g/day)</td>
<td>12.4± 0.18</td>
<td>13.4±0.46</td>
<td>17.1±0.17</td>
<td>18.22±0.46</td>
</tr>
</tbody>
</table>

* p<0.05, ***p<0.001: significantly different from control group.
a p<0.05: significantly different from Diabetic group.
Values are mean ± SEM, n=number of observations.

Zinc concentrations

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

Table 2. Zinc concentration in serum, liver and kidney of control and experimental rats.

<table>
<thead>
<tr>
<th>parameters</th>
<th>Control (n=10)</th>
<th>Non diabetic +Zn (n=10)</th>
<th>Diabetic (n=10)</th>
<th>Diabetic + Zn (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum zinc (µg/100ml)</td>
<td>153,8±3,10</td>
<td>172.4±4.23</td>
<td>90.3±1,43</td>
<td>147,12±4,31</td>
</tr>
<tr>
<td>Liver zinc (µg/g)</td>
<td>35,19±1,75</td>
<td>56.36±1.29</td>
<td>28,59±1,18</td>
<td>35,48±1,97</td>
</tr>
<tr>
<td>Kidney zinc (µg/g)</td>
<td>39,70±1,31</td>
<td>54.67±1.50</td>
<td>35,19±0,82</td>
<td>45,98±1,06</td>
</tr>
</tbody>
</table>

* p<0.05, ** p<0.01, ***p<0.001: significantly different from control group.
a p<0.05, c p<0.001: significantly different from Diabetic group.
Values are mean ± SEM, n=number of observations.

Blood biochemical parameters

As shown in Table 3, diabetes caused in a significant increase (p < 0.001) of serum glucose, urea, creatinine and uric acid concentrations. Also diabetes resulted in a significant decrease (p < 0.001) of serum total protein concentration when compared with control animal. Meanwhile, zinc supplementation made a recovery in the above mentioned blood glucose and Biochemical Markers of Renal Function either in normal or diabetic rats.
Table 3. Mean blood glucose levels and Biochemical Markers of Renal Function value in control and experimental groups

<table>
<thead>
<tr>
<th>parameters</th>
<th>Control (n=10)</th>
<th>Non diabetic +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>1.089±0.027</td>
<td>0.91±0.045</td>
<td>2.08±0.028</td>
<td>1.61 ± 0.040</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>41±1</td>
<td>32.4±2.86</td>
<td>60±2.8</td>
<td>46±2.9</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.75±0.01</td>
<td>0.69±0.027</td>
<td>0.87±0.017</td>
<td>0.70 ±0.025</td>
</tr>
<tr>
<td>uric acid (mg/dl)</td>
<td>7.19±0.21</td>
<td>7.019±0.188</td>
<td>8.99±0.19</td>
<td>8.44±0.189</td>
</tr>
<tr>
<td>total Protein (g/dl)</td>
<td>8.45±0.04</td>
<td>8.97±0.16</td>
<td>6.95±0.11</td>
<td>8.01±0.16</td>
</tr>
</tbody>
</table>

* p<0.05, ** p<0.01, ***p<0.001: significantly different from control group.

b p<0.01, c p<0.001: significantly different from Diabetic group.

Values are mean ± SEM, n=number of observations.

Liver antioxidants statue

Seen from Table 3, diabetes induced a significant increase (p < 0.001) in liver MDA concentration and a significant decrease (p < 0.001) in liver GSH level, GST and GPx activities compared to the corresponding control values. Treatment with Zinc partially restored the levels of MDA, GSH, GPx and GST activities in non-diabetic and diabetic rats.

Table 4. malondialdehyde (MDA), Glutathione (GSH), Glutathione S transferase (GST) and Peroxydase Glutathione (GPx) activities in liver 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>Liver MDA (nmol/mg prot)</td>
<td>0.185±0.0085</td>
<td>0.18±0.0088</td>
<td>0.30±0.0051</td>
<td>0.16±0.01</td>
</tr>
<tr>
<td>Liver GSH (nmol/mg prot)</td>
<td>39.43±1.04</td>
<td>49.07±2.94</td>
<td>30.05±1.30</td>
<td>45.92±2.58</td>
</tr>
<tr>
<td>Liver GST (nM/min/mg prot)</td>
<td>0.42±0.07</td>
<td>0.43±0.02</td>
<td>0.38±0.03</td>
<td>0.44±0.08</td>
</tr>
<tr>
<td>Liver GPx (µM GSH/mg prot)</td>
<td>39.25±3.5</td>
<td>0.41±0.07</td>
<td>31.4±3.6</td>
<td>38.18±2.93</td>
</tr>
</tbody>
</table>

* p<0.05, **p<0.01, ***p<0.001: significantly different from control group.

b p<0.01, c p<0.001: significantly different from Diabetic group.

Values are mean ± SEM, n=number of observations.

DISCUSSION

In this experiment diabetic rats weighed less than control rats, this is consistent with some previously published reports [15, 16]. This raises the possibility of the metabolic state disturbance of animal, suggesting that the diabetic condition had exacerbated 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 [17]. 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 [18]. 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 [19]. 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 Zn supplementation have a beneficial effect in an elderly population with low Zn levels by improving Zn status [20]. The mean fasting blood glucose, concentration in diabetic were found to be higher than that of normal rats. Meanwhile, the current research showed that glucose concentration was ameliorated after zinc supplementation in both normal and diabetic animals. Several molecular mechanisms are believed to be involved in the regulation of blood glucose levels following zinc supplementation [21]. 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 [22]. In the present study, the findings indicated the raise of urea and uric acid level and the diminution of total serum protein in rats with diabetes compared to those without diabetes. This might be due to mesangial cells are exquisitely sensitive to the toxic effects of...
hyperglycaemia [23] and due to microproteinuria, which is an important clinical marker of diabetic nephropathy [24]. Zinc supplementation corrects the diabetes-induced decreased proteins and increase urea and uric acid in rats, indicating the importance of zinc treatment for protection against diabetic nephropathy. In our experimental model of DM, it was observed that alloxan administration produced a significant increase in liver MDA level and a significant decrease in GSH content and GPx and GST activities of liver tissue. Several studies showed that alloxan produces a decrease in the activity of the antioxidant enzymes during the development of alloxan-induced type 1 DM in liver [25, 26]. There is a complex interaction between antioxidants and oxidants such as reactive oxygen species, which modulates the generation of oxidative stress [27]. The diminished of GSH may due to the higher levels of superoxides and free radicals GSH converts more to oxidized glutathione (GSSG) or, is a high 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 [28]. In contrast, zinc supplementation decrease MDA and raised liver 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 [29]. Zinc is also necessary to stimulate defense against reactive species oxygen and H2O2 that induce apoptosis and superoxide dismutase (SOD) activity [30].

CONCLUSION

In conclusion, the study clearly demonstrated the supplementation of zinc to diabetic rats change glucose and biochemical marker of kidney function, which play an important role in oxidative stress, reducing the renal and hepatic complication of diabetes.

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REFERENCES


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