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Brief communication

Inhibitory effects of Zinc on hyperglycaemia and metabolic disorders in the liver of alloxan-induced diabetic rats

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Background: Zinc may participate as a component of the antioxidant defense system. Its deficiency induces oxidative damage to cell components and alterations in antioxidants enzymes in both animal and cells models. There are few studies to provide evidence of the action of zinc in diabetic animal models.

Objective: To evaluate the action of oral administration of zinc (Zn) in hyperglycemia and metabolic disorders induced in the liver of alloxan-induced diabetic rats.

Materials and method: Wistar rats (age: two months) were used for this study. Inducing diabetes in rats using alloxan, we obtained the diabetic rats after four weeks. The rats were divided into five groups (each n=8): normal (control) rat, diabetic rats before the beginning of treatment (Diab-ref), diabetic rats at the end of the treatment (Diab-Con), diabetic rats treated with zinc gluconate (Diab+Zn), and diabetic rats treated with insulin (Diab+Ins). Zinc was orally administrated in drinking water at dose 150mg/L, and insulin was administrated at 0.5IU/rat/day. Thiobarbituric acid-reactive substances (TBARS) superoxide dismutase (SOD), glutathione peroxidise (GPX), catalase (CAT), transaminase glutanic pyruvic (TGP), transaminase glutanic oxaloacetic (TGO), total bilirubin, total cholesterol (TCh), triglyeerides (TG), high density lipid-cholesterol (HDL-Ch), plasmatic, and liver glucose were determined in blood and liver samples.

Results: Zn administration significantly decreased glucose level and glycogen content. Activities of SOD, CAT and GPO were significantly increased by Zn-treatment. In addition, the liver toxicity was prevented by significantly lowering in total bilirubin, TARSS, TGP, and TPO.

Conclusion: Zinc supplements may be beneficial for correcting hyperglycemia leading to diabetic complications in the liver.

Keywords: Antioxidant, diabetes, insulin, oxidative stress, lipid profiles, zinc.

Antioxidant micronutrients have been widely studied for their alleged beneficial properties in the prevention of human diseases, including cancer, arthritis, and cardiovascular diseases [1, 2]. Zinc is one of the most important essential metals for human nutrition. It has plays a key role in genetic expression, cell division and growth and is essential for the function of more than 200 enzymes [3]. Zinc may participate as a component of the antioxidant defense system. Zago and Oteriza [4] reported that zinc deficiency induces oxidative damage to cell components and alterations in antioxidants enzymes in both animal and cells models. According to Faurea et al. [5], several complications of diabetes may be related to increased intracellular oxidants and free radicals associated with decreases in intracellular zinc and zinc-dependent antioxidant enzymes. Zinc-deficiency may lower serum insulin content and/or activity [6-9].

Roussel et al. [10] studied antioxidant effects of Zinc supplementation in Tunisian populations with type-2 diabetes mellitus. Plasma thiobarbituric acid-reactive substances (TBARS) decreased by approximately 15% following six months of Zn supplementation, but the decreases were not significant. However, there are few studies to provide evidence of study of the
action of zinc in diabetic animal models. The present work aims to investigate the possible curative action of zinc in insulin dependent diabetes and its complications in liver functions, using alloxon-induced diabetic rats.

**Material and methods**

**Animals and treatments**

Forty Wistar rats aged two months were used for this study. The rats were fed with standard chow and had access to tap water ad libitum during the experimentation.

Diabetes was induced by single injection of alloxan (150mg/kg body weight ip). Four weeks after alloxan injection, the diabetic rats were obtained. Their plasmatic glucose level was greater than 2g/L, measured using commercially available kits from Biomagreb (Tunis, Tunisia). The day of experiments start, eight diabetic rats were scarified before treatment as referent diabetic rats (Diab-Ref). The other twenty diabetic rats were divided into three groups: diabetic control rats Diab-Con, n=8); diabetic rats treated with Zinc gluconate (Dolisasa laboratories, Boulogne, France) in drinking water at dose 150mg/L (Diab-Zn, n=8) [11], and diabetic rats treated with insulin 0.5IU/rat/day (Novo Nordisk AIS, Danmark) (Diab-Ins). Eight normal rats were used as controls.

The Diab+Zn and Diab+Ins rats were sacrificed four weeks after the beginning of administration of Zinc and insulin, respectively. Blood was collected, and the serum was prepared by centrifugation (1500g, 15 minutes) at 4°C. The liver was removed, and stored at -80°C until used.

**Biochemical assays**

The liver and kidney were homogenate in TBS buffer (1g/2 mL) and centrifugation (1500g, 15 minutes) at 4°C. All biochemical parameters were determined in the homogenates.

Lipid peroxidation in the liver was estimated colorimetrically as follows. Thiobarbituric acid-reactive substances (TBARS) (nmoles/mg protein) were measured using the method of Buege and Aust [12]. Superoxide dismutase (SOD) (U/g tissue) was assayed according to the technique of Marklund and Marklund [13]. Activity of glutathione peroxidise (GPX)(μmoles GSH/(min x mg protein),was determined according to the Paglia and Valentine method [14]. Activity of catalase (CAT) (μmoles H₂O₂/(min*g tissues/min)) was determined using the kinetic method by Aebi [15].

Activities of transaminase glutamic pyruvic (TGP) and transaminase pyruvic oxaloacetic (TGO) (μmoles GSH/(min (?).g tissues/min)), and levels of total bilirubin, total cholesterol (TCh), triglyeerides (TG), high density lipid-cholesterol (HDL-Ch), plasmatic, and liver glucose in serum, were determined using commercially available kits from Biomagreb (Biomerieux, Lyon, France).

![Fig. 1](image_url) Levels of blood glucose (g/L) and liver glycogen (g/100 g) in diabetic rats treated with insulin or zinc. Values are given as means±SD for group of eight animals each. *p <0.01 significantly different compared to normal (control). #p <0.01 significantly different compared to Diab-Ref. +p <0.01 significantly different compared to Diab-Con. $p <0.01 significantly different compared to Diab+Ins.
Statistical analysis

Data are presented as means ± SD. The determinations were performed from eight animals per group and the differences were examined by the one-way analysis of variance (ANOVA) followed by the Fisher test (Stat View). The significance was accepted at p < 0.05.

Results

Blood and liver glucose level

Figure 1 shows blood and liver glucose levels measured in diabetic rats. The blood glucose level increased by 167%, compared to the normal (control) level. However, zinc and insulin administration caused a significant decrease in blood glucose by 28 and 44%, respectively. Interestingly, the liver glycogen level was lower in diabetic rats than that in normal (control) rats (p < 0.001). We note that zinc and insulin administration significantly increased glycogen level in the liver by 141 and 250%, respectively, compared to the control (normal) level (p < 0.001).

Lipid peroxidation

Figure 2 shows levels of liver SOD, CAT and GPX activities and TBARs levels after zinc or insulin administration to diabetic rats. We note that activities of SOD, CAT, and GPX in the liver of diabetic rats decreased by 60%, 74%, and 64%, respectively, compared to their normal (control) levels. After administration of Zn, levels of CAT, GSH, and HDL-Ch were lower in plasma and liver of diabetic rats, compared to their normal (control) levels.

![Fig. 2 Activities of liver SOD (U/mg protein), CAT (μmoles H2O2/(min x mg protein)) and GPX (μmoles GSH/(min x mg protein)) and TBARs levels (nmol/g tissues) after administration of zinc or insulin. Values are given as means±SD for group of eight animals each. *p< 0.01 significantly different compared to the normal (control). ^p < 0.01 significantly different compared to Diab-ref. ~p <0.01 significantly different compared to Dian-Con. &p <0.01 significantly different compared to Diab+Ins.]
Liver toxicity and metabolic indices

Table 1 (A, B) shows liver toxicity indices (TGO, TGP and total bilirubin), and metabolic indices (TCh, TG and HDL-Ch) in serum and liver after supplementation of Zn or insulin. Apparently, diabetes increased liver TBARS contents and the indices of liver dysfunction parameters (TGO, TGP activities, and bilirubin content in plasma. Levels of TCh and TG in both plasma and liver significantly increased in diabetic rats. Interestingly, administration of insulin or zinc showed a similar trend in alloxan-induced diabetic rats. This trend was less pronounced in insulin than zinc.

Discussion

In diabetes, chronic elevation of blood glucose will eventually causes a destruction of both non-enzymic and enzymatic antioxidants by several mechanisms, such as glycation of proteins and glucose auto-oxidation, which lead to high production of free radicals, especially reactive oxygen species (ROS) [16, 17]. The increase of the ROS level causes serious diseases such as diabetes and tissue damage found in many organs and systems [11, 17]. Insulin is considered the effective therapy available to cure diabetes, but with some disadvantages such as the increase in stress oxidative [11, 17]. Therefore, it

Table 1. Levels of liver toxicity indices (A) and serum metabolic disorders indices (B) measured after administration of Zn or insulin to diabetic rats.

A) Liver toxicity (TGO, TGP and total bilirubin)

<table>
<thead>
<tr>
<th>Group</th>
<th>Transaminase glutanic oxaloacetic (TPO) (U/L)</th>
<th>Transaminase glutanic pyruvic (TGP) (U/L)</th>
<th>Total Bilirubin (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (Control)</td>
<td>70.25 5.00</td>
<td>56.06 5.58</td>
<td>1.05 0.05</td>
</tr>
<tr>
<td>Diab-Ref</td>
<td>91.06 8.00</td>
<td>76.8 4.4*</td>
<td>16.9 0.12*</td>
</tr>
<tr>
<td>Diab-Con</td>
<td>103.08 6.00*</td>
<td>88.09 8.00***</td>
<td>2.34 0.19**</td>
</tr>
<tr>
<td>Diab+Zn</td>
<td>81.12 2.90**</td>
<td>59.70 7.00**</td>
<td>1.19 0.09**</td>
</tr>
<tr>
<td>Diab+Ins</td>
<td>86.60 5.00*</td>
<td>67.23 4.00**</td>
<td>1.37 0.16**</td>
</tr>
</tbody>
</table>

B) Serum metabolic indices (TCh, TG, and HDL-Ch)

<table>
<thead>
<tr>
<th>Group</th>
<th>Total Cholesterol (TCh) (g/L)</th>
<th>Triglycerides (TG) (g/L)</th>
<th>HDL-cholesterol (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal (Control)</td>
<td>1.66 0.27</td>
<td>0.63 0.07**</td>
<td>0.68 0.11</td>
</tr>
<tr>
<td>Diab-Ref</td>
<td>2.35 0.12*</td>
<td>1.43 0.10</td>
<td>0.46 0.13*</td>
</tr>
<tr>
<td>Diab-Con</td>
<td>3.14 0.22**</td>
<td>1.64 0.15*</td>
<td>0.24 0.02*</td>
</tr>
<tr>
<td>Diab+Zn</td>
<td>2.18 0.10**</td>
<td>0.67 0.09**</td>
<td>0.45 0.14*</td>
</tr>
<tr>
<td>Diab+Ins</td>
<td>2.46 0.21**</td>
<td>0.72 0.02**</td>
<td>0.60 0.05**</td>
</tr>
</tbody>
</table>

Liver (mg/g)

<table>
<thead>
<tr>
<th>Group</th>
<th>Total Cholesterol (TCh) (g/L)</th>
<th>Triglycerides (TG) (g/L)</th>
<th>HDL-cholesterol (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (Control)</td>
<td>0.95 0.15</td>
<td>1.66 0.26</td>
<td>0.60 0.06</td>
</tr>
<tr>
<td>Diab-Ref</td>
<td>2.74 0.17*</td>
<td>2.54 0.43*</td>
<td>0.38 0.04*</td>
</tr>
<tr>
<td>Diab-Con</td>
<td>3.22 0.56**</td>
<td>2.90 0.25*</td>
<td>0.26 0.01*</td>
</tr>
<tr>
<td>Diab+Zn</td>
<td>1.96 0.12**</td>
<td>2.09 0.19**</td>
<td>0.65 0.06**</td>
</tr>
<tr>
<td>Diab+Ins</td>
<td>2.53 0.12**</td>
<td>2.31 0.29**</td>
<td>0.49 0.05**</td>
</tr>
</tbody>
</table>

*p < 0.01 significantly different compared to controls. *p < 0.01 significantly different compared to Diab-Ref. *p < 0.01 significantly different compared to Diab-Con. *p < 0.01 significantly different compared to Diab+Ins.
becomes necessary to discover other anti-diabetics and antioxidants molecules for the treatment of this disease. Many nutritional molecules have been discerned to manage diabetes [18]. In this study, zinc supplementation at dose 150mg/L in drinking water to alloxan-induced diabetic rats caused a significant decrease of the glucose level. This rise by the increased Zn contents might cause: i) protection of pancreatic β-cells from damage and a regeneration of these cells and ii) increases in the SOD activity. The first item may be in agreement with Kenji et al. study [19] indicating an enhancement in the insulin secretion and sensitivity by a Zn increase. The second item is in agreement with a previous study by Faure et al. [5]. In fact, Zinc has numerous effects on antioxidant defense systems, including protection against protein glycation, and induction of metallothioneins.

Administration of hypoglycaemic and antioxidant zinc in diabetic rats improves the protection of cell membranes against free radical damage. The present result showed low level in TBARS, which may increase the stability of biomembranes as hypothesized by Verstraeten et al. [20]. This study also demonstrated deleterious metabolic disorders that might be improved by Zn, leading to an improvement of insulin activity [21]. We showed both the antioxidant and hypoglycaemic actions of Zn. This action may prevent liver toxicity by lowering of lipid peroxidation level and liver dysfunction indices (TGO, TGP, and bilirubin level) [22]. The positive effect of Zn in liver function may prevent such disorders in TG and TCh metabolism so that HDL-Ch anabolism may be near to the normal (control) level.

In conclusion, zinc association showed hypoglycemic action in alloxan-induced diabetic rats. In addition, zinc improved the liver function of diabetic rats by: i) enhancement of the both enzymatic antioxidant capacities and ii) preservation of the liver function by low level in TGO, TGP, and bilirubin. Moreover, zinc administration might protect from metabolic disorders, indicated by low level in TCh, TG, and higher level in HDL-Ch.

The authors have no conflict of interest to declare.

References