MODULATORY ACTIVITY OF ZINC AND/OR CHROMIUM ON GLYCEMIC CONTROL, ANTIOXIDANT ENZYMES AND MITOGENESIS OF LYMPHOCYTES OF STREPTOZOCIN-INDUCED DIABETIC RATS

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ABSTRACT
Zinc (Zn) and chromium (Cr) appeared to be the most promising elements in the therapeutics of diabetes mellitus (DM). The objective of the study was to identify the modulating activities of Zn and/or Cr in vivo and in vitro mitogenic of lipopolysaccharide (LPS) and phytohaemagglutinin (PHA) on peripheral lymphocyte and glycemic control and some anti oxidant enzymes in streptozocin-induced diabetic rats. Sixty rats were equally divided into five equal groups. The first group was considered as non-diabetic control. The second group was considered as DM control. Third and fourth groups were administered with 80 µg/Kg/day Cr and 30 mg/kg/day Zn by oral intubations respectively. The last group was supplemented with both Cr and Zn. Rats’ weights and glucose levels were determined at 2 and 8 weeks. On day 60, livers were removed for weighting and blood was used for biochemical Analysis, anti oxidant activities and Lymphocyte transformation assay. The results revealed that Zn and/or Cr supplementation were significantly altering plasma glucose level, body weight gains and glycogen concentration of DM rats. TBA-RS level was significantly increased in DM and Cr treated groups. Anti-oxidant enzymes activity is significantly lower in DM while it is significantly ameliorated with Cr and/or Zn supplementation. Glucose consumption of LPS and PHA stimulated lymphocytes in DM was significantly decreased invivo and invitro while it was significantly increased after 8 weeks. The above results indicated important implications for the development of therapeutic strategies aimed at manipulating Zn and Cr in protection from different oxidative stress and immune events during diabetes.

Keywords: Diabetes, Zinc, Chromium, Oxidative Stress, Lymphocyte Transformation Assay

INTRODUCTION
Diabetes mellitus (DM) is considered to be one of the most common chronic diseases worldwide, and recognized as one of the leading causes of morbidity and mortality (American Diabetes Association, 2004). DM in domestic animals has been most commonly reported in dogs (Kimmel et al., 2000) and cats (Nelson et al., 2000), but has also been reported rarely in other species (AL Haj et al., 2006; Philip et al., 2012). The main factors in the pathophysiology of this disease are the development of insulin resistance, dysfunction and / or loss of β cells, deficient secretion of insulin and islet amyloidosis (Nicole et al., 2010).

As is characteristic of diabetic condition, hyperglycemia was associated with an increase in oxidative stress in liver in terms of increased lipid peroxidation and decreased glutathione levels (Turk, 2010). Studies advancing the role of oxidative stress in vascular endothelial cells proposed that oxidative stress mediated the diversion of glycolytic intermediates into pathological pathways (El Faramawy and Rizk, 2011; Samanthi et al., 2011). Oxidative stress is increased in DM owing to an increase in the production of oxygen free radicals and a deficiency in antioxidant defense mechanisms (Rodifio-Janeiro et al., 2010). This altered parameters could lead to damage of cellular organelles and enzymes, increased lipid peroxidation and development of insulin resistance (Ceriello, 2006). Also, it leads to structural damages of liver, kidney and pancreas (Özkaya et al., 2011). Oxidative stress induced by high glucose concentration plays a pivotal role in complications of diabetes, though it is not clear yet whether increased...
oxidative stress has a primary role in the pathogenesis of diabetic complications, or it is simply a consequence of them (Niedowicz and Daleke, 2005; Miranda et al., 2007).

Impaired lymphocyte functions and enhanced infections were a common feature of diabetes and stress. Through hyperglycemia, a high rate of apoptosis was observed in lymphocytes isolated from alloxan-induced diabetic rats (Otton et al., 2004). Some of the lymphocytes might result from elevated glucose concentrations, and normal proliferation of lymphocytes had been restored following insulin administration (Rubinstein et al., 2008). A non hormonal manipulation of DM especially trace elements was discussed by several investigators. Out of all the trace elements studied, zinc (Zn) and chromium (Cr) appeared to be the most promising elements in the therapeutics of DM. Cytoplasmic Zn concentrations were influenced by cell activation and by oxidative stress (Maret, 2006). Undulations in free Zn ions were likely to influence signalling pathways, including the protein tyrosine phosphatases (Ho et al., 2008). Several complications of diabetes might be related to decreases in intracellular Zn and Zn-dependent antioxidant enzymes (Powell, 2000; Brandao-Neto et al., 2003). Zn supplementation resulted in amelioration of glycemic control of diabetic patients and animals indicating insulin-like effects (Özcelik et al., 2012). Zn supplementation reduces the lipid peroxidation levels in the kidney of diabetic rats by virtue of their inherent antioxidant properties, suggesting that Zn plays an essential biochemical function that retards the oxidative processes and it serves as a potential antioxidant (Özkaya et al., 2011). The body needs Cr for normal health and growth and to enhance the action of insulin (NRC, 2001). Feeding the organic form of Cr had an impact on the level of blood glucose and on the activity of insulin (Horky et al., 2012) as it was considered a cofactor for insulin action (Patal et al., 2010; Sundaram et al., 2012a; b) and increased number of insulin receptors (Patal et al., 2010). Several complications of diabetes may be related to increased intracellular oxidants and free radicals associated to decreases in intracellular Zn and Zn-dependent antioxidant enzymes (Brandao-Neto et al., 2003).

Zn and Cr are essential trace elements important for the efficiency of the immune system in reason of its widespread role in the activity of enzymes, transcription factors and cytokines. It is of utmost importance to develop novel therapeutic strategies for diabetic patients that efficiently prevent diabetic complications (Tang et al., 2010). In addition to an antioxidant role, Zn may affect immunity via its important role in cell replication and proliferation (Weiss and Spears, 2006). Zn deficiency is, in part, responsible for the compromised immune function in Third World countries, leading to increased morbidity and mortality from infections (Fischer and Black, 2004). Zn affects multiple aspects of the immune system (Prasad, 2009). Phagocytosis, intracellular killing and cytokines production are all affected by Zn deficiency. The growth and function of T and B cells are also affected adversely due to Zn deficiency (Prasad 2009).

Dietary Cr had received attention as a potential nutrient involved in immune competence (Arthington, 2006). Terpilowska and Siwicki (2010) observed the immune protective role of Cr. They showed the increase of IL-1α concentration and decrease of IL-6 concentration after incubation of mouse fibroblasts with Cr chloride. Indeed, Cr had important structural roles that were directly relevant for T cells (Kim et al., 2003).

Numerous data indicated that Zn and/or Cr ameliorate diabetes without clarifying the mechanism. Therefore the aim of the present study is to identify the modulating activities of Zn and/or Cr on glycemic control, some antioxidant enzymes, in vivo and in vitro mitogenesis of LPS and PHA on peripheral lymphocytes in STZ-induced diabetic rats.

**MATERIALS AND METHODS**

**Animals and Experimental Protocol:** Sixty male albino rats were used and kept at 22°C and at a 12 h light/dark cycle. The experimental protocol met the care and use of laboratory animals throughout the experimental duration (Guide for the Care and Use of Laboratory Animals, 1996). The rats were equally divided into five groups (n=12), as follow: Control group (CO): non-diabetic rats. Diabetic group (DM): diabetes was induced in the rats by a single-dose (70 mg/kg) IP injection of streptozotocin (STZ) (Sigma, St. Louis, MO, USA) (Peschke et al., 2000). Blood samples were collected for glucose determination and animals with blood glucose level more than 190 mg/dl were considered diabetic. Diabetic group treated
with Cr (DM+Cr): diabetic rats were administered 80 µg/Kg/day Cr (Sigma, St. Louis, MO, USA) dissolved in distilled water by oral intubations (Soheir, 2013). Diabetic group treated with Zn (DM+Zn): diabetic rats were administered 30 mg/kg/day Zn (ZnSO₄) (Sigma, St. Louis, MO, USA) dissolved in distilled water by oral intubations (Özelik et al., 2012). Diabetic group treated with Cr and Zn (DM+Cr+Zn): diabetic rats were supplemented with both Cr (same as Cr group) and Zn (same as Zn group).

**Biochemical Analysis:** Rats’ weights were recorded before the first injection and after 2 and 8 weeks. Blood samples were taken at 2 and 8 weeks after manipulation for glucose determination. Mean plasma glucose for each rat was calculated from blood samples and immediately analyzed with a glucometer (GlucoSurePlus; Haemedic AB, Munka Ljungby, Sweden) at 2 and 8 weeks after the initial injection. On day 60, the animals were sacrificed and their livers were removed. The livers were weighted and homogenized with 9ml of phosphate buffer (KCL 140mM, phosphate 20mM pH 7.4) per tissue gram. The determination of total liver glycogen was performed as that of glucose following an enzymatic hydrolysis with amyloglucosidase (Asperiguillus niger; Sigma) (Bergmeyer, 1963). Thiobarbituric acid reactive substance (TBA-RS) was determined colorimetrically by the method of (Buege and Aust, 1978).

**Determination of Zn and Cr:** A whole blood Zn and Cr was analyzed using the 4500 ICP-MS –Oes-Varian. 710. Full quantitative analysis was performed for elements following simple dilution of the whole blood. Appropriate matrices will be selected for each element.

**Determination of Anti Oxidant Enzymes:** Catalase (CAT) (Bergmayer et al., 1983), glutathione peroxidase (GPx) (Pagila and Valentine, 1967) and superoxide dismutase (SOD) (Madesh and Balasubramanian, 1998) was analyzed in the normal, diabetic induced and treated rats using biodiagnostic kits.

**Lymphocyte Transformation Assay:** It was done using glucose consumption test as previously described by Walters et al., (2003). Briefly, citrated blood was diluted 1:1 and layered over Ficoll histopaque solution (Scharlau Chemie S.A). The blood was centrifuged and the mononuclear cell layer was aspirated, washed and resuspended in medium RPMI-1640 (Sigma Aldrich, St. Louis, MO, USA). The cells were examined for lymphocyte purity and viability using trypan blue dye exclusion method. Phytohaemagglutinin-P (PHA) was used for T cell mitogen and lipopolysaccharide (LPS) was used for B cell mitogen. Lymphocytes were cultured in triplicate in the presence of either 5 µg/ml PHA or 10 µg/100ul LPS in 24-well plates. Each well contained 200µl of culture suspension containing 2 × 10⁶ cells. For the mitogenic Zn and Cr effect assay, treatments in vitro were made with Zn (15µM) as zinc sulfate (ZnSO₄, Sigma, St. Louis, MO, USA) and with Cr as chromium chloride (CrCl₂. 6H₂O, Merck) (Stella et al., 1982) to test cells. The cell cultures were incubated for 72 hours at 37 °C in a humidified atmosphere with 5% CO₂. Glucose was estimated in the terminal medium using glucometer. The lymphocyte stimulation was estimated as the quantity of glucose (mg/dl) consumed minus the concentration of stimulated cell culture of control samples.

**Statistical Analysis:** Data were presented as mean ± standard error (SE) and analyzed with the Statistical Package for the Social Sciences version 15.0 (SPSS-15.0).

**RESULTS AND DISCUSSION**

**Results**

Induction of a type I diabetic state caused a decrease in body weight gain in the diabetic rats at 8 weeks compared to the non-diabetic rats (P<0.05). Cr supplementation significantly affected body weight gains of diabetic rats only after 8 weeks, while Zn supplementation alone or in combination with Cr were significantly altering body weight gains of diabetic rats after both 2 and 8 weeks treatment. hyperglycemia was induced after 2 and 8 weeks in diabetic group while combination of Zn and Cr supplementation decreased plasma glucose level after 8 weeks. No significant change in liver weights was observed in control and diabetic rats. Glycogen concentration was significantly increased in diabetic than control rats while i
groups treated with Cr and those treated with Cr in combination with Zn. TBA-RS level was significantly increased in DM than control group and also increased in group treated with Cr than DM group (Table 1). In table (2) there is a significant decrease in serum Zn and Cr in diabetic group. Supplementation of Zn or Cr alone for diabetic rats increased Zn and Cr levels respectively, while their combination increased Cr level. CAT level was significantly lower in DM group than control while there was significant increase in DM+Zn and DM+Cr+Zn groups than DM group. GPx level was significantly lower in DM group than control while there was significant increase in DM+Cr+Zn groups than DM group.

In table (3) glucose consumption of LPS and PHA stimulated lymphocytes in DM group was significantly decreased than control. Glucose consumption of LPS and PHA stimulated lymphocytes of the different treated groups of rats is significantly increased after than DM group.

In table (4) invivro glucose consumption of LPS stimulated lymphocytes in DM group was significantly decreased than control. Invivro glucose consumption of LPS stimulated lymphocytes was significantly increased in all treated groups of rats while PHA stimulated lymphocytes was significantly increased in Zn treated group than DM group.

Table 1: Effect of Zn and/or Cr on body weight, body weight gain, glucose, liver wet weight, liver glycogen concentration, and TBA-RS of diabetic rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CO</th>
<th>DM</th>
<th>DM+Cr</th>
<th>DM+Zn</th>
<th>DM+ Cr+Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Weight (g)</td>
<td>126.8</td>
<td>131.18</td>
<td>129.22</td>
<td>130.41</td>
<td>130.34</td>
</tr>
<tr>
<td>± 5.2</td>
<td>±4.91</td>
<td>±3.63</td>
<td>±5.61</td>
<td>±4.31</td>
<td></td>
</tr>
<tr>
<td>Body weight gain at 2 weeks (g/week)</td>
<td>6.42</td>
<td>4.32</td>
<td>10.53</td>
<td>13.19 b</td>
<td>13.18 a</td>
</tr>
<tr>
<td>±1.70</td>
<td>±0.54</td>
<td>±2.54</td>
<td>±2.00</td>
<td>±1.54</td>
<td></td>
</tr>
<tr>
<td>Body weight gain at 8 weeks (g/week)</td>
<td>24.34</td>
<td>12.00 a</td>
<td>28.43 b</td>
<td>30.12 b</td>
<td>30.65 b</td>
</tr>
<tr>
<td>±4.23</td>
<td>±2.63</td>
<td>±4.22</td>
<td>±2.43</td>
<td>±3.45</td>
<td></td>
</tr>
<tr>
<td>Glucose at 2 weeks (mmol/L)</td>
<td>7.42</td>
<td>13.52 a</td>
<td>11.02</td>
<td>10.22</td>
<td>10.42</td>
</tr>
<tr>
<td>±1.62</td>
<td>±1.01</td>
<td>±2.31</td>
<td>±2.43</td>
<td>±1.55</td>
<td></td>
</tr>
<tr>
<td>Glucose at 8 Weeks (mmol/L)</td>
<td>8.43</td>
<td>22.12 a</td>
<td>20.53</td>
<td>19.17</td>
<td>9.40 b</td>
</tr>
<tr>
<td>±1.22</td>
<td>±2.87</td>
<td>±2.23</td>
<td>±2.80</td>
<td>±2.90</td>
<td></td>
</tr>
<tr>
<td>Liver wet weight (g)</td>
<td>10.43</td>
<td>9.36</td>
<td>11.28</td>
<td>10.90</td>
<td>10.24</td>
</tr>
<tr>
<td>±2.14</td>
<td>±2.34</td>
<td>±3.31</td>
<td>±2.25</td>
<td>±2.65</td>
<td></td>
</tr>
<tr>
<td>Liver glycogen concentration (mg/g fresh wt)</td>
<td>11.23</td>
<td>5.32 a</td>
<td>11.22 a</td>
<td>8.27</td>
<td>11.03 a</td>
</tr>
<tr>
<td>±1.34</td>
<td>±1.64</td>
<td>±1.53</td>
<td>±1.03</td>
<td>±1.34</td>
<td></td>
</tr>
<tr>
<td>TBA-RS (nmols/mg prot.)</td>
<td>0.22</td>
<td>0.33 b</td>
<td>0.40 b</td>
<td>0.20</td>
<td>0.31</td>
</tr>
<tr>
<td>±0.03</td>
<td>±0.01</td>
<td>±0.01</td>
<td>±0.09</td>
<td>±0.01</td>
<td></td>
</tr>
</tbody>
</table>

* Values of the diabetic group were different significantly from the values of control group in the same row at P < 0.05
a,b Values of the treated groups were different significantly from the values of diabetic group in the same row at P<0.05 and P<0.01 respectively.
Research Article

Table 2: Effect of Zn and/or Cr on Zn, Cr, CAT, GPx and SOD of diabetic rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>CO</th>
<th>DM</th>
<th>DM+Cr</th>
<th>DM+Zn</th>
<th>DM+Cr+ZN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn (PPm)</td>
<td></td>
<td>0.194±0.005</td>
<td>0.162*±0.013</td>
<td>0.176±0.026</td>
<td>0.295c±0.019</td>
<td>0.192±0.050</td>
</tr>
<tr>
<td>Cr (PPm)</td>
<td></td>
<td>0.099±0.008</td>
<td>0.073*±0.007</td>
<td>0.103 c±0.016</td>
<td>0.072±0.003</td>
<td>0.195c±0.018</td>
</tr>
<tr>
<td>CAT (MU/L)</td>
<td></td>
<td>152.20±5.80</td>
<td>116.43**±2.45</td>
<td>119.03±4.06</td>
<td>134.54b±4.11</td>
<td>139.80 c±2.57</td>
</tr>
<tr>
<td>GPx (U/mL)</td>
<td></td>
<td>8.31±1.21</td>
<td>4.53*±0.08</td>
<td>4.26±0.21</td>
<td>5.76±2.07</td>
<td>7.12 a±1.04</td>
</tr>
<tr>
<td>SOD (U/mL)</td>
<td></td>
<td>105.14±2.65</td>
<td>87.73**±2.07</td>
<td>84.58±1.89</td>
<td>98.18 b±1.09</td>
<td>104.70 a±2.86</td>
</tr>
</tbody>
</table>

Table 3: Effect of Zn and/or Cr on glucose consumption (mg/dl) of LPS - and - PHA stimulated lymphocytes of diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>CO</th>
<th>DM</th>
<th>DM+Cr</th>
<th>DM+Zn</th>
<th>DM+ZN+Cr</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPS</td>
<td>Without LPS</td>
<td>31.48±4.02</td>
<td>27.22±3.65</td>
<td>32.34±2.44</td>
<td>35.21±4.11</td>
</tr>
<tr>
<td></td>
<td>With LPS</td>
<td>14.15±3.57</td>
<td>20.13±4.11</td>
<td>12.53±2.73</td>
<td>12.44±2.73</td>
</tr>
<tr>
<td>Glucose consumption</td>
<td>17.52±2.20</td>
<td>7.76*±2.52</td>
<td>15.77 a±2.67</td>
<td>22.16 b±3.10</td>
<td>25.43 c±3.19</td>
</tr>
<tr>
<td>PHA</td>
<td>Without PHA</td>
<td>33.44±2.48</td>
<td>28.43±3.77</td>
<td>30.63±3.76</td>
<td>33.18±5.22</td>
</tr>
<tr>
<td></td>
<td>With PHA</td>
<td>18.31±2.60</td>
<td>19.38±2.09</td>
<td>13.33±1.45</td>
<td>14.09±2.04</td>
</tr>
<tr>
<td>Glucose consumption</td>
<td>16.25±2.25</td>
<td>8.63 *±2.06</td>
<td>17.47 a±2.12</td>
<td>23.48 b±3.80</td>
<td>25.53 b±2.75</td>
</tr>
</tbody>
</table>

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Previously, a significant decrease in body weight gains and increased concentration of glycerogen content of diabetic rats was also associated with liver dysfunction. As reported by Kechrid et al., 2007, the fasting liver glycogen content of diabetic rats was lower compared to that of control group, although liver weight was similar in different groups as previously mentioned by Kechrid et al., 2007. This might be related to liver dysfunction (Vallee et al., 1959). Zn and Cr supplementation alone or with combination were significantly altering body weight gains and increased concentration of glycogen of diabetic rats after both 2 and 8 weeks treatment. This was in a good agreement and similar with some previously published reports of Kechrid et al., 2007. TBA-RS level was significantly increased in DM than control group and also increased in group treated with Cr than DM group. The formation of TBA-RS in plasma and organs of adults (liver, kidney and brain) and of fetuses (placenta and liver) was monitored as an index of lipid peroxidation according to a previously described colorimetric method (Zama et al., 2007).

Anti oxidant enzymes activity was significantly lowered in DM group than respective control that confirmed previous studies of Likidililid et al., 2007 who reported alteration in the activity of antioxidant enzymes was also associated with liver oxidative injury. As reported by Aly et al., 2010, hyperglycemia-evoked oxidative stress played a crucial role in the development of diabetic complications.

**DISCUSSION AND CONCLUSION**

Trials for diabetes control showed that, even with intensive insulin treatment, a substantial proportion of patients still develop complications. These clinical observations suggested that factors other than hyperglycemia might contribute to the development of complications in diabetes (Sjöquist et al., 1998). The aim of the present study was to identify the modulating activities of Zn and/or Cr in vivo and in vitro mitogenesis of LPS and PHA on peripheral lymphocyte and some anti oxidant enzymes in STZ-induced diabetic rats.

In this study, a significant increase of blood glucose was observed at 8 weeks in STZ-treated rats as previously reported by (Dervis et al., 2012). Also, these rats showed a significant decrease in body weight relative to untreated controls, a result previously confirmed by study of Olofsson et al., 2009. The diabetic rats given had a lower feed efficiency than the non diabetic rats although the food intake of these animals was higher than that of the non-diabetic rats (Kechrid et al., 2007). The daily mean of consumed diet by rat was 14 g (Goss et al., 1970). This raised the possibility of the metabolic state disturbance of animals, suggesting that the diabetic condition had exacerbated reduced the ability of the diabetic rats to utilize food intake as normal subjects (Kechrid et al., 2007).

The fasting liver glycogen content of diabetic rats was lower compared to that of control group, although liver weight was similar in different groups as previously mentioned by Kechrid et al., 2007. This might be related to liver dysfunction (Vallee et al., 1959). Zn and Cr supplementation alone or with combination were significantly altering body weight gains and increased concentration of glycogen of diabetic rats after both 2 and 8 weeks treatment. This was in a good agreement and similar with some previously published reports of Kechrid et al., 2007. TBA-RS level was significantly increased in DM than control group and also increased in group treated with Cr than DM group. The formation of TBA-RS in plasma and organs of adults (liver, kidney and brain) and of fetuses (placenta and liver) was monitored as an index of lipid peroxidation according to a previously described colorimetric method (Zama et al., 2007).

Anti oxidant enzymes activity was significantly lowered in DM group than respective control that confirmed previous studies of Likidililid et al., 2007 who reported alteration in the activity of antioxidant enzymes was also associated with liver oxidative injury. As reported by Aly et al., 2010, hyperglycemia-evoked oxidative stress played a crucial role in the development of diabetic complications.

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which resulted from augmented reactive oxygen species generation, which probably resulted from decreased antioxidant defenses. The activities of antioxidant enzymes like SOD, CAT and glutathione reductase were significantly reduced in liver of diabetic animals (Davi et al., 2005; Lai, 2008; Sundaram et al., 2013). These might suggest that the ability to scavenge or inactivate free radicals was weakened in the diabetic animals (Sundaram et al., 2013). Moreover oxygen radicals also interacted with the lipid bilayer and produced lipid peroxides particularly in cell membranes (Miranda et al., 2007).

The present study showed that CAT activity was a significantly increased in DM+Zn and DM+Cr+Zn groups than DM group. GPx activity was significantly increased in DM+Cr+Zn groups than DM group. SOD activity was significantly increased in DM+Zn and DM+Cr+Zn groups than DM group. These results indicated that Zn and/or Cr supplementation may be resulted in amelioration of glycemic control of diabetic patients and animals indicating insulin-like effects (Aguilar et al., 2007; Jansen et al., 2009; Özcelik et al., 2012). Also, it had been proposed that Cr supplementation increased a Cr-containing oligopeptide present in insulin-sensitive cells that increased insulin binding to cells due to increased number of insulin receptors and enhances insulin receptor phosphorylation (Pattar et al., 2006; Patal et al., 2010). Jain et al., (2007) reported that Cr supplementation was resulted in a significant inhibition of oxidative stress and pro-inflammatory cytokines in diabetic rats. Cr picolinate administration was found to have beneficial effect in normalizing glucose levels; lipid peroxidation and antioxidant status by restoring antioxidant profile, without any hepatotoxicity, as well as it improved the impaired glucose tolerance and prevents hyperglycemia-induced oxidative damage (Refaie et al., 2009; Sundaram et al., 2013).

Induction of a type I diabetic state caused a decrease in serum Zn and Cr. Supplementation of Cr alone for diabetic rats increased Cr level while its combination with Zn increased Zn level. The present results suggested that the Zn and Cr alteration were involved in STZ-induced mice diabetes. The reduced serum Zn in diabetic rats was probably due to the degranulation, cytolysis and to other pathological changes, associated with progression of the condition (Coleman and Hummel, 1967), or to the high excretion of Zn in the urine (Kechrid et al., 2007). The concept of Zn being an ionic signalling molecule had increased attention to the bio available Zn that was not tightly bound to proteins and was exchangeable within individual cells (Rink and Haase, 2007). Cytoplasmic Zn concentrations were influenced by cell activation and by oxidative stress (Maret, 2006). Undulations in free Zn ions were likely to influence signalling pathways, including the protein tyrosine phosphatases (Ho et al., 2008). Several complications of diabetes might be related to increased intracellular oxidants and free radicals associated to decreases in intracellular Zn and Zn-dependent antioxidant enzymes (Brandao-Neto et al., 2003).

Results of immune status of diabetic rats in this study indicated that glucose consumption of stimulated lymphocytes was significantly decreased that indicated impairment of both cellular and humoral immune response. Glucose consumption of LPS and PHA stimulated lymphocytes both in vivo and in vitro were significantly increased than DM group. These results were in agreement with Arthington (2006) and Terpilowska and Siwicki (2010) who observed the immune protective role of both Zn and Cr respectively. Indeed, Cr had important structural roles that were directly relevant for T cells (Kim et al., 2003). Zn affects multiple aspects of the immune system (Overbeck et al., 2008; Prasad, 2009). Zn is essential for normal development and function of cell-mediating innate immunity, neutrophils, and natural killer cells. Macrophages were also affected by Zn deficiency. Phagocytosis, intracellular killing and cytokines production were all affected by Zn deficiency.

**Conclusion**

In conclusion, these results indicated that action of Zn and Cr on lymphocyte function in diabetes and stress may be important target for modulation of immune responses and understanding of mechanisms leading to several pathologies of immune cells observed in stress and STZ-Induced rat diabetes. Finally, It is suggested that the humoral and cell mediated immune responses are impaired by diabetes and could be significantly reversed by zinc and/ chromium. The above results indicated important implications for the development of therapeutic strategies aimed at manipulating zinc and chromium in protection from different oxidative stress and immune events during diabetes.
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