The protective effects of zinc and vitamin E supplementation against kidney toxicity by lithium in rats

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ABSTRACT

The valuable effects of antioxidants supplementation on lithium-induced nephrotoxicity has not been understood yet. The purpose of this study was to evaluate the renoprotective effect of zinc sulfate (Zn) and/or vitamin E (Vit. E) against lithium chloride (Li)-induced nephrotoxicity in rats. Forty male rats were divided into five groups. The first worked as controls and the other were treated with Li (20 mg/kg daily for 4 weeks). Group I of Li-treated was left without treatment, however, group II, III and IV were treated with Zn (10 mg/kg daily for 4 weeks), Vit. E (10 mg/kg, twice a week for 4 weeks), and the combination of Zn and Vit. E, respectively. Rats were killed for collection of blood and kidneys for biochemical and histological studies. The results showed a significant increase in Li in kidney tissue in all treated groups with Li, however, Zn was only increased in the groups treated with Zn, whereas Cu was similar in all treated and control groups. Plasma levels of creatinine, urea and glucose showed differences among the treated groups. The levels of lipid peroxidation, nitric oxide, glutathione, superoxide dismutase and catalase in renal tissue were significantly increased in Li-treated groups in comparison with the control and ameliorated by treatment with Zn and the combination of Zn and Vit. E. Histological observation showed peri-vascular edema and interstitial lymphocytic cell reaction in kidney of rats treated with Li, however co-treatment with Zn and/or Vit. E resulted in improvement of the histological changes. In conclusion Li-exposure causes a histological and biochemical changes mediated by oxidative stress and Li accumulation and co-treatment with Zn and/or Vit. E may protect against Li toxicity.

Keywords: Lithium toxicity; Kidney; Vitamin E; Zinc; Oxidative stress; Metal accumulation.

1. INTRODUCTION

Toxic heavy metals in water, air and soil are global problems that are a growing threat to humanity. Renal tubular damage has been known to occur by lithium (Li) toxicity in experimental animals [1]. Treatment by Li is the most effective long-term therapy for bipolar disorder and unipolar depression [2, 3]. Moreover, human exposed to Li by dietary grains and vegetables [4] and as an environmental pollutants by production of metal alloys, ceramics, television screens, color films and batteries [5]. Dysfunction of kidney is often irreversible in spite of Li withdrawal, so early detection
is essential to prevent progression to end stage renal disease [6]. Because, intracellular accumulation of Li in the collecting duct inhibits the glycogen synthase kinase type 3β leading to insensitivity of cells to the actions of aldosterone and vasopressin [7].

Renal diseases are associated with oxidative stress and reduction of nitric oxide (NO), however, it is difficult to determine if this relationship contributes to disease or is a consequence of disease [8]. Study on rats found that small doses of Li (15, 30 mg/kg b.w.) caused oxidative stress in erythrocytes, liver and kidney tissues [9]. Accordingly, administration of antioxidants like N-acetyl cysteine and caffeic acid phenethyl ester, a component of honeybee propolis during Li therapy provides significant protective effect in rat model of Li-induced renal failure [10, 11].

Vitamin E (Vit. E) is an important component of human diet and considered the most effective lipid soluble antioxidant which protects the body’s biological systems by preventing lipid peroxidation (LPO) [12]. Zinc (Zn) play important roles in intracellular signaling, cell-mediated immune functions, oxidative stress and inflammation [13]. Because of the health problems induced by many environmental pollutants, much effort has been given in evaluating the relative antioxidant potency of Vit. E and Zn supplementation [13, 14]. Ibrahim et al. [15] found that Zn, Vit. E and their interaction protect brain tissues from Li toxicity with the priority for the combination of Zn and Vit. E.

Regard to the broad way for Li toxicity through medical application and environmental pollutants, the present study was carried out to evaluate the efficacy of Zn and/or Vit. E supplementation on the renal toxicity induced by Li exposure in drinking water.

2. MATERIAL AND METHODS

2.1. Chemicals

Lithium chloride (LiCl), zinc sulphate (ZnSO₄), Vit. E (α-tocopherol), superoxide dismutase, epinephrine, thiobarbituric acid (TBA), naphthyl-ethylene diamine dihydrochloride, and 5,5-dithiobis (2-nitrobenzoic acid (DTNB) were obtained from Sigma Chemical Co. (St. Louis, MO). All other chemicals and reagents were of the highest purity commercially available.

2.2. Animals

Forty adult male albino rats (120-150 grams) were purchased from the Animal House of the Faculty of Medicine, Assiut University, Assiut, Egypt. Rats were housed in cages and kept in a room temperature at 25 ± 3°C with normal 12 h light/12 h dark cycle. They were allowed to acclimatize for one week before the experiments. Rats had free access to water and standard diet.

The experiments were conducted in accordance with ethical guidelines of the Animal Care and Use Committee of Assiut University.

2.3. Animal groups, treatment and collection of samples

Rats were divided into five groups (n = 8). Group I (control) - received normal drinking water, group II - received Li (20 mg/kg b.w.) for 4 weeks in drinking water according to Rana and Parker [16], group III - received Li (20 mg/kg b.w.) and Zn (10 mg/kg b.w.) for 4 weeks in drinking water according to Claverie et al. [17], group IV - received Li (20 mg/kg b.w.) for 4 weeks in drinking water and intraperitoneally injected with Vit. E (100 mg/kg b.w.) twice a week for 4 weeks according to Warren et al. [18], group V - received Li (20 mg/kg b.w.) and Zn (10 mg/kg b.w.) for 4 weeks in drinking water and injected interaperitoneally with Vit. E (100 mg/kg b.w.) twice a week for 4 weeks. After 4 weeks, under anesthesia with diethyl ether, blood was collected in the heparin coated tubes. Plasma specimens were obtained and used for determination of creatinine, urea and glucose. Kidney homogenates were obtained using a tissue homogenizer (IKA Yellow line DI 18 Disperser, Germany). The homogenates (1:10 w/v) were prepared using a 0.1M phosphate buffer (pH 7.4). All homogenates were centrifuged at 6000 rpm for 30 min at 4°C and the supernatants were used for the biochemical assays. Small pieces of kidneys were quickly removed, immersed in 10% formalin, dehydrated and embedded in paraffin, sectioned at 5 μm, stained with hematoxylin and eosin (H&E) and observed by light microscopy according to
Drury and Wallington [19].

2.4. Biochemical measurements

Levels of glucose, uric acid, creatinine in plasma were measured coloimetrically according the manufactured procedures of commercial kits produced by Diamond Diagnostics Egyptian Company for Biotechnology. Total protein content in the supernatant of kidney tissues was measured for calculation the specific enzyme activity by the method of Lowry et al. [20]. Lipid peroxidation products as TBARS were determined according to the method ofOhkawa et al. [21]. Nitric oxide (NO) was measured as nitrite concentration colorimetrically using the method of Ding et al. [22]. Glutathione (GSH) was determined using the method of Beutler et al. [23]. Activity of superoxide dismutase (SOD) was determined according to its ability to inhibit the autoxidation of epinephrine at alkaline medium according to the method of Misra and Fridovich [24]. Activity of catalase (CAT) was determined by the procedure of Gregory and Fridovich [25], basing on its ability to decompose hydrogen peroxide.

Li, Zn and copper (Cu) concentrations in the samples were determined by ICP-MS (Thermo Fisher Scientific (Bremen) GmbH). Standard solutions of multi-elements were prepared from commercial stock standard solutions at concentrations of 100 mg/L deionised water. Working standard solutions were prepared by dilution of stock standard solution with the addition of hydrochloric acid, so that the acid concentration in working standard solutions matched acid concentration in digested solutions.

2.5. Statistical analysis

The statistical analysis was carried out using the Statistical Package for Social Sciences (SPSS) for Windows version 16.0 software. All data were represented as mean ± standard error (SE). Data were subjected to one-way analysis of variance (ANOVA) followed by Student’s t-test. Statistical probability of P < 0.05 was considered to be significant.

3. RESULTS

Plasma levels of creatinine and urea were significantly increased, whereas glucose was significantly decreased in rats exposed to Li compared to control. Co-treatment of rats with Zn and Vit. E resulted in normalization of the plasma levels of creatinine, urea and glucose (Table 1).

The levels of LPO, NO, GSH and the activities of SOD and CAT were significantly increased in kidney tissue of Li treated group in comparison to the control group (Table 2). Co-treatment of rats with Zn or the combination of Zn and Vit. E resulted in normalization of LPO level and the activities of SOD and CAT. However, co-treatment of rats with Vit. E alone failed to normalize LPO, NO, GSH levels as well as SOD activity.

Fig. 1. Photomicrographs of kidney sections from control rat (A), rat exposed to Li showing perivascular edema (arrow) and interstitial lymphocytic cell reaction (star) (B), rat exposed to Li and treated with Zn showing congestion of the glomerular capillaries and edema in the interstitium (arrow) (C), rat exposed to Li and treated with Vit. E showing congestion of the interstitial blood vessels (arrow) (D), and rat exposed to Li and treated with Zn and Vit. E showing slight dilatation of the renal tubules (E) (H&E x 100).
The level of Li, Zn, and Cu in the kidney tissues were significantly increased in Li-treated rats and remained in rats co-treated with Zn and/or Vit. E. However, the level of Zn was increased only in rats co-treated with Zn and the combination of Zn and Vit. E and there is no change in the level of Cu among all groups (Table 3).

Histopathological observation of kidney sections from control rat showed normal histological structure of renal cortex, kidney of rat exposed to Li showed perivascular edema and interstitial lymphocytic cell reaction, kidney of rat exposed to Li and treated with Zn showed congestion of the glomerular capillaries and edema in the interstitium, kidney of rat exposed to Li and treated with Vit. E showed congestion of the interstitial blood vessels, and kidney of rat exposed to Li and treated with Zn and Vit. E showed slight dilatation of the renal tubules (Fig. 1).

### 4. DISCUSSION

It is known that urea and creatinine are renal function markers which increase when the kidney was injured. In the present results elevation of creatinine and urea levels in the plasma of Li treated rats indicates a reduction in glomerular filtration capacity. Moreover, the present reduction in plasma glucose level of rats treated with Li is strongly linked with insufficient ATP generation via glycolysis and TCA cycle [26]. Furthermore, altered level of the glucose and urea in treated rats treated with Li are indicative of hepatic and renal damages [9]. In the present study, treatment with Zn and or Vit. E normalized the levels of glucose, creatinine and urea in plasma. In comparison, treatment with selenium as antioxidant ameliorated the changes in blood parameters induced by exposure of rat to Li [27].

### Table 1. Plasma levels of creatinine, urea and glucose in control and treated groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Control</th>
<th>Li</th>
<th>Li and Zn</th>
<th>Li and Vit. E</th>
<th>Li, Vit. E and Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.10 ± 0.004</td>
<td>0.24 ± 0.03</td>
<td>0.10 ± 0.01</td>
<td>0.10 ± 0.01</td>
<td>0.10 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>32.17 ± 0.78</td>
<td>106.14 ± 1.25</td>
<td>75.34 ± 6.35</td>
<td>33.27 ± 2.25</td>
<td>38.72 ± 2.07</td>
<td></td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>88.10 ± 3.58</td>
<td>57.10 ± 1.79</td>
<td>68.56 ± 3.27</td>
<td>107.77 ± 8.15</td>
<td>81.73 ± 2.83</td>
<td></td>
</tr>
</tbody>
</table>

Letters mean statistically significant difference at p<0.05 between different groups.

### Table 2. Levels of oxidative stress markers in renal tissues of control and treated groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Control</th>
<th>Li</th>
<th>Li and Zn</th>
<th>Li and Vit. E</th>
<th>Li, Vit. E and Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPO (nM of MDA/mg protein)</td>
<td>1.33± 0.26</td>
<td>9.23± 0.96</td>
<td>2.69± 0.40a</td>
<td>4.46± 0.85a</td>
<td>1.16± 0.19a</td>
<td></td>
</tr>
<tr>
<td>NO (nM of sodium nitrite/mg protein)</td>
<td>28.12± 3.02</td>
<td>137.9± 6.76d</td>
<td>55.2± 3.09b</td>
<td>112.4± 4.78b</td>
<td>39.5± 3.49ab</td>
<td></td>
</tr>
<tr>
<td>GSH (µg/mg protein)</td>
<td>3.12± 0.29</td>
<td>5.28± 1.03</td>
<td>3.58± 0.24a</td>
<td>4.73± 1.01b</td>
<td>2.15± 0.29a</td>
<td></td>
</tr>
<tr>
<td>SOD (U/mg protein)</td>
<td>0.45± 0.07</td>
<td>0.92± 0.15</td>
<td>0.23± 0.02a</td>
<td>0.90± 0.12b</td>
<td>0.37± 0.08a</td>
<td></td>
</tr>
<tr>
<td>CAT (U/mg protein)</td>
<td>0.45± 0.08</td>
<td>4.15± 0.60</td>
<td>0.80± 0.13a</td>
<td>0.58± 0.07a</td>
<td>1.02± 0.17a</td>
<td></td>
</tr>
</tbody>
</table>

Letters mean statistically significant difference at p<0.05 between different groups.

### Table 3. Concentration of Li, Zn, and Cu in kidneys of control and treated groups.

<table>
<thead>
<tr>
<th>Element</th>
<th>Control</th>
<th>Li</th>
<th>Li and Zn</th>
<th>Li and Vit. E</th>
<th>Li, Zn and Vit. E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Li (µg/gm tissue)</td>
<td>0.00 ± 0.00</td>
<td>23.30±0.10</td>
<td>18.60±0.90</td>
<td>21.30±0.90d</td>
<td>16.10±0.90b</td>
</tr>
<tr>
<td>Zn (µg/gm tissue)</td>
<td>0.10 ± 0.00</td>
<td>0.10± 0.00</td>
<td>6.40 ± 0.30</td>
<td>0.21±0.01d</td>
<td>7.70± 0.50b</td>
</tr>
<tr>
<td>Cu (µg/gm tissue)</td>
<td>0.04 ± 0.00a</td>
<td>0.03± .001a</td>
<td>0.30± 0.01c</td>
<td>0.20± 0.01b</td>
<td>0.10± 0.01b</td>
</tr>
</tbody>
</table>

Letters mean statistically significant difference at p<0.05 between different groups.
In the present study, exposure of rats to Li caused a significant increase in LPO, NO and GSH levels and the activities of SOD and CAT in renal tissue. These changes in oxidative stress parameters were confirmed by perivascular edema and interstitial lymphocytic cell reaction. Kielczykowska et al. [28] found that Li not caused any change in LPO but caused change in the SOD activity. Increased LPO due to induction of polyunsaturated fatty acids peroxidation may be lead to the degradation of phospholipids and cellular deterioration [29]. Catalase is the most important antioxidant for detoxifying excess H$_2$O$_2$ to prevent hydroxyl production. The imbalance between SOD and CAT activities could lead to an excessive generation of free radicals. In the present study, the increased in SOD activity in kidneys of Li treated rats support the idea of overproduction of superoxide anion by Li. Accordingly, increased CAT activity is probably related to O$_2$ dismutation by SOD and H$_2$O$_2$ accumulation due to decrease in glutathione peroxidase activity. Then, H$_2$O$_2$ react with O$_2$ to generate OH which initiates LPO [30]. The present data showed that Li induced the activities of SOD and CAT in the kidney tissues, however, co-treatment of rats with Zn along Li resulted in decline in the activities of SOD and CAT in comparison with Li treated alone. This is because zinc as structural element of non-mitochondrial form of SOD has antioxidant properties [31-33]. Moreover, Zn is involved in destruction of free radicals through Zn-metallothioneins which may serve as an efficient antagonist in inhibiting LPO to stabilize biomembranes [34, 35] and inhibit NADPH oxidase [13]. Cabre et al. [36] found that Zn supplementation for 12 weeks caused a decrease in lipid peroxidation, together with an increase in metallothionein concentration in alcoholic rats. Also, Zn brings down the elevated levels of SOD, CAT and GPx to within normal limits in rats given ethanol [37]. Antioxidant Vit. E breaks the chain of free radical reactions by allows free radicals to abstract a hydrogen atom from the antioxidant molecule rather than from polyunsaturated fatty acids [38]. Therefore, Vit. E supplementation protects the kidney and testicular tissue against peroxidative damage due to free radical damage in time consuming process [39, 40] and Zn was effective in reversing the oxidative stress induced by Li in the rat brain [15].

In the current study, the level of NO in kidneys of rats treated with Zn and or Vit. E showed a significant decrease with improvement in the histological structure as compared with Li treated rats. Siles et al. [41] and Bashandy [42] found a significant increase in the expression of inducible nitric oxide synthase in the brain of rats treated with Li. The antioxidant effect of Zn and Vit. E may be due to its role in reducing NO because it is mostly involved in regulation of redox status under physiological conditions [43].

The levels of elements in tissues reflect dietary concentrations of these elements [44]. In the present study, Li concentration was increased in kidney tissue of all treated groups, but, Zn was increased in the rats co-treated with Zn or the combination of Zn and Vit. E. Compartmental analysis by Jaeger et al. [46] found that Li was rapidly distributed and accumulated in kidney and liver following ingestion. Moreover, Zn supplementation increased serum Zn levels and decreased oxidative stress [47]. Pharmacologic doses of Li increased Li content in the liver and Cu content of the brain, and showed no effect in liver and kidney. Moreover, it increased Zn content in liver and kidney but did not affect Zn in the brain. Accordingly, Li therapy may induce major changes in the storage of Cu in the brain and Zn in liver and kidney, respectively [48]. The morphological examination of kidney revealed that cellular architecture was disturbed following Li treatment. Similar to our results, several histopathological examination revealed congestive or degenerative lesions, large areas of focal fibrotic lesions and interstitial accumulations of leukocytes, glomerulosclerosis, peritubular infiltration with lymphocytes and mononuclear cells in the kidney of Li treated rats and co-treatment with antioxidant improved those changes [15,49-51].

In conclusion, renal tissues of kidney exposed to Li in drinking water for 4 weeks showed alterations in oxidative stress markers and the concentration of Li with histological changes. Co-treatment of rats with Zn and/or Vit. E ameliorated the oxidative stress and improved the pathological changes in the kidney with the priority for the combination.
AUTHORS’ CONTRIBUTIONS

All authors contributed equally to this work. The final manuscript has been read and approved by both authors.

TRANSPARENCY DECLARATION

The authors declare that they have no competing interests.

REFERENCES


