Lithium Induced Toxicity in Rats: A Hematological, Biochemical and Histopathological Study

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Lithium (Li⁺) salts are commonly used in treating bipolar diseases. As physicians frequently keep the patients on long-term lithium therapy, awareness of the numerous side effects and pathogenesis of this lightest alkali metal is needed for such treatments. The current study was designed to evaluate the toxic effect of small doses of lithium nitrate in rats. In the present study we showed that the oral gavage feeding of lithium nitrate (20 mg Li/kg body wt) for 7 weeks on every alternate day to male albino wistar rats elicited a significant alterations in gross hematological values owing to hypochromic anemia and leucocytosis. Erythrocyte sedimentation rate (ESR) and clotting time depicted higher values and animals exhibited icteric condition. Serum levels of hemoctine, cholesterol and blood urea elevated; however, proteins depleted markedly. A significant increase in serum calcium and phosphorous has also been registered in lithium salt treated animals. The enzyme activities of alkaline phosphatase (Alpase) and acid phosphatase (AcPase) diminished depicting the disturbed general physiological status while there was a marked rise in the activities of transaminases (GOT and GPT) reflecting a stimulating transamination reaction in hepatic and renal tissues. The histopathological picture of the kidney tissues revealed many deformative alterations. Necrosis, binucleated cells and Kuffer’s cells are visible in renal tissue. The epithelium lining of renal tissue was damaged and there were also some marked changes in glomerular region apart from intracellular alterations in corticomedulary region. The results of present study suggest that small doses of lithium induce toxicity in rats.

Key words  lithium salt; hypochromic anemia; leucocytosis; blood urea nitrogen; phosphatase; transaminase

The effect of long-term lithium treatment on renal function has been a matter of concern for about a quarter of century and remains controversial. For the last few decades researchers have been paying much attention to the lithium salts due to their therapeutic uses in controlling a variety of neurotic and psychosomatic manic depressions, neurotoxicity, affecting muricidal behavior and lithium sickness.1—4) Extensive usage of lithium and its compounds in pharmaceuticals, dehumidifying and air conditioning units, ceramics and metallurgical processes, lubricants and in a number of chemical and biological laboratories brings this lightest alkali metal in close contact of human.

Toxicity of lithium may be caused while taken as medicine.5,6) Lithium salts cause ocular side effects,7) polyuria, polydipsia, loss of body mass8) and impaired renal concentration capacity after water deprivation.9) Lithium salts used as medicine can readily cross the placental barriers and produce teratogenic effects and toxicity.10) In early pregnancy, lithium salt therapy elicited to be associated with a several fold increase in the incidence of cardiovascular anomalies in newborn, including tricuspid valve abnormalities.11) Cerebellar atrophic changes related to lithium therapy12) and peri-follicular inflammation and follicular plugging13) even with the therapeutic limit of lithium level of blood have been observed. The occurrences of choreoathetosis14) and abnormalities in lung and respiratory tract have also been reported by intake of lithium.15) Hypochromic microcytic anemia, hypercholesterolaemia, hyperglycemia, glycogenolysis, disturbance in TCA cycle and stimulation in transamination reaction in liver and kidney following repeated intramuscular injections of lithium nitrate in laboratory animals have been reported previously.16,17) Renal insufficiency in long term lithium treatment has also been reported recently.18) This is an endeavor to study the histopathological and biochemical impacts of intake of small doses of lithium nitrate on renal tissue along with some blood parameters in male albino rats. Our results suggest that small doses of lithium induces toxicity in rats and therefore, studies evaluating its long-term tolerability are important.

MATERIALS AND METHODS

Chemicals  Lithium nitrate, sodium citrate, diethylbarbiturate, 3,5-dinitrosaliicilate, ammonium molybdate, citric acid, diacetylmonoxime, β sodium glycerophosphate, α ketoglutarate, α l-alanine, l-aspartate, sodium hypochlorite, trichloroacetic acid, and dinitrophenyl hydrazine were purchased from Sigma Chemical Company (St Louis, MO, U.S.A.). All other chemicals and reagent used were either of analytical grade or of highest purity, commercially available.

Animals and Treatments  Animals experiments were approved by animal care committee of Jamia Hamdard, care and handling of animals were in accordance with Indian Council of Medical Research guidelines. Male albino wistar rats (4—6 weeks) weighing 125—150 g, from Jamia Hamdard Central Animal House Colony were used throughout this study. The rats were housed in polypropylene cages in a group of twelve rats per cage and kept in a room maintained at 22±2 °C with a 12-h light/dark cycle. They were given standard laboratory feed (Hindustan Lever Ltd, Bombay, India) and tap water ad libitum. The rats were used after one week of acclimatization.

For studying the effect of lithium nitrate on hematological, biochemical and histopathological changes of renal tissues, 24 male albino wistar rats were taken and divided into two groups of 12 animals in each. Animals of group I received saline and served as control. Animals of group II received oral gavage feeding of lithium nitrate (20 mg Li/kg body wt)

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for 7 weeks on every alternate day. The dose and time of lithium nitrate administration was selected based on previously published data where significant changes were observed.16,17 All of these animals were killed 3 h after the last dose of lithium nitrate or saline by cervical dislocation.

For studying the dose dependent effect of lithium nitrate on changes in serum blood urea nitrogen and transaminases, 36 animals were taken and divided into three groups of 12 animals in each. Animals of group I received saline and served as control. Animals of group II and III received oral gavage feeding of lithium nitrate, 12 and 24 mg Li/kg body wt, respectively for 7 weeks on every alternate day. All of these animals were killed 3 h after the last dose of lithium nitrate or saline by cervical dislocation.

For studying the time dependent effect of lithium nitrate on changes in serum blood urea nitrogen and transaminases, 36 animals were also taken and divided into three groups of 12 animals in each. Animals of group I received saline and served as control. Animals of group II and III received oral gavage feeding of lithium nitrate, 24 mg Li/kg body wt, for 4 weeks and 8 weeks respectively on every alternate day. All of these animals were also killed 3 h after the last dose of lithium nitrate or saline by cervical dislocation.

**Hematological and Biochemical Estimations** Just before killing, blood samples from control and lithium nitrate treated animals were taken from retro-orbital sinus puncture and was used for the estimation of total erythrocyte count (TEC), white blood corporules (WBCs) count, hemoglobin content, packed cell volume (PCV) and calculations were done for their absolute values viz. mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC).19 Erythrocyte sedimentation rate (ESR) and clotting time (CT)20 were also determined in the fresh blood of control and lithium nitrate treated animals. Serum was used for the blood urea nitrogen21 and enzymological estimations.22

**Enzymatic Activities** The activities of phosphatases (AlPase and AcPase) in terms of inorganic phosphate liberated per mg of protein,23 transaminases24,25 and urease26 have been determined in the liver, kidney and blood of control and lithium nitrate treated animals. Protein content in all samples was estimated by the method of Lowry et al.27 using bovine serum albumin as standard.

**Histopathological Evaluation** From the sacrificed animals, kidney tissues were taken and fixed in 10% neutral buffered formalin for 24 h and then embedded in paraffin, and cut into 4-μm sections for histopathological evaluations. The slides were dewaxed and stained with haematoxylin and eosin using conventional methods for light microscopic examination.

**Statistical Analysis** The statistical significance of the difference between control and lithium nitrate treated groups was evaluated by analysis of variance (ANOVA), followed by Dennett’s t-test. The differences in mean value were considered as statistically significant at \( p<0.05, 0.01 \) and \( p<0.001 \). Data are presented as mean±S.E. of twelve animals.

**RESULTS**

Experimental data in comparison to controls are presented in Table 1. Oral feeding of lithium nitrate induced significant decrease in TEC value (29.4%), Hb content and PCV along with marked increase in white blood corporules (WBC) (40.7%) in the test animals. ESR and CT increased significantly (\( p<0.001 \)) as did the icteric index. Serum levels of cholesterol, hexose, blood urea nitrogen, phosphorus and calcium content elevated significantly. The serum proteins, however, depicted a marked decline (\( p<0.01 \)).

Dose dependent effect of lithium nitrate on changes in serum blood urea nitrogen and transaminases in male albino rats is shown in Table 2. Serum levels of blood urea nitrogen and transaminases elevated dose dependently (\( p<0.05 \)). Time dependent effect of lithium nitrate on changes in serum blood urea nitrogen and transaminases is shown in Table 3. Serum levels of blood urea nitrogen and transaminases also elevated time dependently (\( p<0.05 \)) as shown in Table 3.

Table 1. Hematological and Sero-chemical Alterations in Male Wistar Albino Rats Following Lithium Nitrate Administration

<table>
<thead>
<tr>
<th>Blood values</th>
<th>Control</th>
<th>Lithium nitrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (%)</td>
<td>14.33±0.18</td>
<td>12.04±0.21</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>42.67±0.14</td>
<td>36.83±0.83</td>
</tr>
<tr>
<td>RBC (10^6/mm^3)</td>
<td>7.18±0.10</td>
<td>5.07±0.15</td>
</tr>
<tr>
<td>WBC (10^5/mm^3)</td>
<td>11.80±0.08</td>
<td>16.60±0.14</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>19.56±0.08</td>
<td>23.45±0.29</td>
</tr>
<tr>
<td>MCV (μm^3)</td>
<td>59.63±0.90</td>
<td>72.57±1.43</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>33.72±0.24</td>
<td>31.91±0.68</td>
</tr>
<tr>
<td>LI</td>
<td>3.64±0.24</td>
<td>4.82±0.17</td>
</tr>
<tr>
<td>CT (s)</td>
<td>206.46±2.10</td>
<td>238.60±2.48</td>
</tr>
<tr>
<td>ESR (mm/h)</td>
<td>2.46±0.07</td>
<td>2.77±0.06</td>
</tr>
<tr>
<td>Glucose (mg/ml serum)</td>
<td>8.67±0.04</td>
<td>10.04±0.09</td>
</tr>
<tr>
<td>Protein (mg/ml)</td>
<td>12.11±0.09</td>
<td>10.38±0.21</td>
</tr>
<tr>
<td>Cholesterol (mg/ml)</td>
<td>6.33±0.05</td>
<td>7.67±0.08</td>
</tr>
<tr>
<td>BUN (mg N/ml)</td>
<td>4.38±0.14</td>
<td>6.23±0.10</td>
</tr>
<tr>
<td>Calcium (mg/ml)</td>
<td>2.44±0.08</td>
<td>3.08±0.07</td>
</tr>
<tr>
<td>Phosphorus (mg/ml)</td>
<td>0.30±0.004</td>
<td>0.352±0.002</td>
</tr>
</tbody>
</table>

a) \( p<0.05 \), b) \( p<0.01 \), c) \( p<0.001 \). Hb, hemoglobin; PCV, packed cell volume; RBC, red blood corpuscle; WBC, white blood corpuscle; MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume; MCHC, mean corpuscular hemoglobin concentration; I.I. icteric index; CT, clotting time; ESR, erythrocyte sedimentation rate; BUN, blood urea nitrogen. Each value represents mean±S.E. of twelve animals. Dose regimen and treatment protocols are described in text.

Table 2. Dose Dependent Effect of Lithium Nitrate Administration on Changes in Serum Blood Urea Nitrogen and Transaminases in Male Wistar Albino Rats

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Blood urea nitrogen (N/ml)</th>
<th>SGOT (IU/ml)</th>
<th>SGPT (IU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (saline)</td>
<td>3.2±0.10</td>
<td>1.7±0.10</td>
<td>1.5±0.12</td>
</tr>
<tr>
<td>Lithium nitrate (12 mg Li/kg body weight)</td>
<td>5.9±0.12*</td>
<td>2.7±0.12*</td>
<td>3.0±0.17*</td>
</tr>
<tr>
<td>Lithium nitrate (24 mg Li/kg body weight)</td>
<td>8.2±0.14*</td>
<td>3.2±0.17*</td>
<td>3.9±0.19*</td>
</tr>
</tbody>
</table>

\( * p<0.05 \) vs. saline treatment control group. Each value represents mean±S.E. of twelve animals. Dose regimen and treatment protocols are described in text.
elevated in liver and kidney, significantly and showed more than two folds GOT in blood.

Effect of lithium nitrate on renal histopathological changes in male albino wistar rats is shown in Fig. 2. The epithelial lining of both, proximal and distal tubules in medullary region were found ruptured. Renal tubular necrosis was observed at many sites (Fig. 2b). Further, the capsular wall of glomerulus was observed thickened accompanied by the shrinkage in glomerular capillary network, resulting in widened the space between the wall and the network. Only a few alterations were observed in cortical region. Interstitial haemopoietic tissue was severely damaged. Cytoplasmic vacuolization and extrinsic position of nuclei were specially noted in corticomedullary region. However, no significant nuclear changes of shape and size were there. Peripheral part was almost unaffected.

**DISCUSSION**

Reduction in TEC is considered due to the direct injurious action of the toxin on the animals. According to earlier reports, lithium can cross the erythrocyte membrane by several routes and causes ill effects therein.\(^2\)\(^8\)\(^\) Erythropenia along with decreased hemoglobin content is an indication of decrease in oxygen carrying capacity in the animals, resulting in insufficient supply of oxygen to the tissues causing adverse effects on animal health. Here the gross hematological picture reveals the occurrence of hypochromic macrocytic anemia. Further induction of leucocytosis is considered of immunological significance to meet the adverse situation developed by the introduction of foreign bodies (Li\(^+\)/H\(_2\)\(O\)\(_2\)) in the blood. It may also be for the removal of the debris of tissue damaged by the toxin. Increase in ESR and hypercoagulability are correlated to the alterations of plasma proteins and coagulating factor(s), respectively.\(^2\)\(^9\),\(^3\)\(^0\) The icteric condition of the animals is attributed to the hepatic dysfunctioning where more bile pigments may be produced.\(^3\)\(^1\)

Significant elevation in blood urea nitrogen and decrease in serum proteins seems related to the observed kidney damage. An increase up to 30 to 60% urinary excretion of proteins associated with decreased serum proteins has also been reported in mammals intoxicated with mercury salt.\(^3\)\(^2\) Hypercholesterolaemia in animals reflects the disturbed carbohydrate metabolism. Rise in serum hexose level may be due to the pancreatic manifestations (insulin insufficiency). Earlier observations made with laboratory animals following lithium nitrate intoxication, the glycogen content in the liver and kidney was found decreased\(^3\)\(^6\) along with a marked increase in blood glucose.\(^3\)\(^1\) One possible reason for this decrease may be that the toxin interferes the carbohydrate metabolism re-

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<th>SGOT (IU/ml)</th>
<th>SGPT (IU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (saline)</td>
<td>4.1±0.13</td>
<td>2.2±0.21</td>
<td>1.7±0.12</td>
</tr>
<tr>
<td>Lithium nitrate (24 mg Li/kg body weight for 4 weeks)</td>
<td>6.5±0.21*</td>
<td>4.0±0.19*</td>
<td>2.9±0.17*</td>
</tr>
<tr>
<td>Lithium nitrate (24 mg Li/kg body weight for 8 weeks)</td>
<td>8.9±0.17*</td>
<td>5.6±0.17*</td>
<td>5.4±0.13*</td>
</tr>
</tbody>
</table>

\(^\ast\)\(p<0.05\) vs saline treatment control group. Each value represents mean±S.E. of twelve animals. Dose regimen and treatment protocols are described in text.
sulting in a significant fall in blood glucose utilization and gluconeogenesis and accelerating glycogenolysis, due to which the serum level of sugar increases. A significant increase in calcium and phosphorus is considered as a result of demineralization of skeletal bones. Also due to kidney disorders in intoxicated state, filtration may also alter for these constituents. As blood is the overall reflector of body health, the altered level of the hexose, urea and cholesterol in treated rats are suggestive of some degree of hepatic and renal damage. Furthermore, the macrocytic and leucocytic changes in animals may be considered as an adaptive response to such tissue damages.

Alkaline phosphatase is involved in transphosphorhylation reaction and acid phosphatase has functional importance in cell autolysis. So, a loss in phosphatases activities reflected the cytotoxic effects of the toxin. It has also been reported that the enzymes may also come out of the inflamed tissue to the cytotoxic effects of the toxin. It has also been reported that the enzymes may also come out of the inflamed tissue to

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