Effect of Mercuric Chloride on Biochemical and Hematological Parameters in Male Albino Mice

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Abstract: This study was designed to investigate the effect of inorganic mercury (mercuric chloride) on biochemical, hematological parameters, and examining the histological changes in liver, and kidney tissues in male albino mice as a laboratory animal model. The animals used for the sub-chronic study for mercuric chloride received 1/100th, 1/50th, 1/20th and 1/10th of the calculated LD50 which were equivalent to 0.25, 0.5, 1.25, and 2.5 mg/kg respectively. At four concentrations, levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), urea and creatinine were high significantly increase in mice treated with HgCl2 compared with control group. Whereas, the parameters cholesterol, glutathione (GSH), total protein, hemoglobin (Hb), red blood cells (RBC), and white blood cells (WBC) were significantly decrease in examined samples for animals treated with HgCl2 as compared with control group. It can be concluded that the altered biochemical and hematological parameters can be used as efficient bioindicators on the toxicity of mercury. Also, Mercuric chloride induced various histological alterations in liver, and kidney of mice.

Keywords: mercuric chloride, blood parameters, Glutathione

1. Introduction

Heavy metals are hazardous substances that cause serious health risk to ecosystems and organisms due to their high toxicity conferred by nature of their environmental persistence [1]. Mercury is a well-known toxic heavy metal to animals as well as humans [2,3]. Mercury occurs naturally in the environment in different chemical forms. Elemental mercury is the form used in dental amalgams. Forms more commonly found in nature are inorganic mercury and organic mercury [4]. All mercury forms are considered toxic [5]. It is being widely used in the industrial, medical, agriculture and other fields [6]. Mercury toxicity is associated to its high affinity for sulphydryl groups (–SH), forming stable complexes and causing several alterations, such as structural changes of sulphydryl enzymes and the inactivation of their active sites [7]. Mercury treatment enhanced lipid peroxidation in all tissues [8]. These lipid peroxides and hydroxyl radical may cause the cell membrane damage and thus destroy the cell [9]. Once absorbed, mercury distributes widely to all tissues. The principal target organs of the inorganic mercury are kidney and liver [10]. The accumulated mercury could produce certain hematological and biochemical changes in blood [11]. Biochemical parameters are still more indicative of early physiological changes following sub-chronic and chronic Hg exposure [12].

2. Methodology

2.1 Chemical

Mercury in the form of inorganic mercury (mercuric chloride) was used for the present study. It was dissolved in distilled water to prepare the concentrations that used in the experiment.

2.2 Animals

Healthy adult male albino mice at the age of 8-10 weeks and average weight 25±5 g were obtained from Al-Razi center in Baghdad City, Iraq. The mice were housed in polypropylene cages under controlled conditions of temperature (25±5°C), and normal photoperiod (12 to 12 h light-dark cycles). Diet and drinking water were given ad libitum.

2.3 Determination of the median lethal dose (LD50)

Dixon’s up and down method was used for estimation of median lethal dose (LD50) [13]. In Dixon’s method, the LD50 determination begins with a dose of the test chemical. The survival or death of the animal at this concentration decides for the next dose either to be increased or decreased accordingly. In this method survival is represented as “O” and death as “X”, and the score is made accordingly. In the present study, male mice weighing 25±5 g were injected with increasing doses of mercuric chloride to get the result (the loss or survival of mouse) during 24 h of injection. If the animal survives, the dose for the next animal is increased. If it dies, the dose for the next animal is decreased. The results are evaluated as follows:-

\[ \text{LD}_{50} = X_f + Kd \]

Where \( X_f \) is the final dose administered, \( K \) is the tabular value, \( d \) the difference between two doses. The LD50 for mercuric chloride was calculated to be 25 mg/kg.

2.4 Treatment

The mice were divided into five experimental groups; each consists of ten mice. The first group was served as the control. The other animal groups used for the sub-chronic study for mercuric chloride injected intraperitoneally 1/100th, 1/50th, 1/20th and 1/10th of the calculated LD50 which were equivalent to 0.25, 0.5, 1.25, and 2.5 mg /kg body weight, respectively for 30 days. The study was conducted following approval from the animal ethics committee of department of biology, University of Baghdad, Baghdad, Iraq. The animals
were injected for three days in the week for month, always at the same time, from 9:00 to 11:00 o’clock. Blood samples were collected from animals by heart puncture and placed into EDTA containing tubes, to determine the following: RBC count, WBC count, and Hb concentration. The other amount of blood was centrifuged at 3000 rpm for 10 minutes. Serum was separated for assessment of various biochemical parameters like cholesterol, urea, creatinine, AST, ALT, ALP, total protein, and GSH. Subsequently, mice were sacrificed, liver, and kidney were quickly removed for the histopathological examinations.

2.5 Histopathological assessment

Animals were dissected after the end of the exposure period. Liver, and kidney were quickly removed and kept in formalin solution 10% for histological study. The tissue sections were prepared according to the method described by [14]. After the specimens were dehydrated in serial ethanol 60, 70, 80, 90, and 100% and xylene, they were embedded in paraffin wax. Paraffin blocks of the tissues were sectioned at 5μm thickness by using a rotary microtome. Tissue sections were stained with hematoxyline and eosin, drops of material canada balsam was placed on the slides to install a cover slide. For histological alterations these slides were observed under light microscope.

2.6 Statistical Analysis

The Statistical Analysis System- SAS program [15] was used to determine the effect of difference factors in study parameters. Least significant difference-LSD test or T-Test was used to significant compare between means in this study.

3. Results and Discussion

3.1 Biochemical parameters

Table (1) shows the effect of different concentrations from mercuric chloride (HgCl2) on biochemical parameters in mice. The HgCl2-treated mice showed a high significant increase (p<0.01) in serum AST, ALT, ALP enzyme activities, urea and creatinine serum levels compared to the control. While, the present investigation demonstrates that HgCl2 induced high significant decreasing (P<0.01) in cholesterol, and total protein level. There was a significant reduction in GSH level in the HgCl2-treated mice.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Concentration of HgCl2 (mg/kg)</th>
<th>T-Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control groups</td>
<td>0.25</td>
</tr>
<tr>
<td>S. Cholesterol (mg/dl)</td>
<td>128.67 ±11.21</td>
<td>109.00 ± 1.00</td>
</tr>
<tr>
<td>S. AST (U/L)</td>
<td>135.33 ± 1.76</td>
<td>149.67 ± 1.76</td>
</tr>
<tr>
<td>S. ALT (U/L)</td>
<td>27.00 ± 1.52</td>
<td>38.67 ± 1.76</td>
</tr>
<tr>
<td>S. ALP (U/L)</td>
<td>42.33 ± 5.23</td>
<td>58.33 ± 3.48</td>
</tr>
<tr>
<td>B. Urea (mg/dl)</td>
<td>50.67 ± 0.88</td>
<td>56.67 ± 1.45</td>
</tr>
<tr>
<td>S. Creatinine (mg/dl)</td>
<td>0.613 ± 0.04</td>
<td>0.743 ± 0.02</td>
</tr>
<tr>
<td>Total protein (g/dl)</td>
<td>8.73 ± 0.12</td>
<td>7.60 ± 0.01</td>
</tr>
<tr>
<td>GSH (µmol/L)</td>
<td>4.87 ± 0.20</td>
<td>3.67 ± 0.13</td>
</tr>
</tbody>
</table>

(*) refer to significant differences between means at (P < 0.05), (**) refer to high significant differences between means at (P < 0.01).

3.2 Hematological parameters

The results of the Hematological parameters showed a high significant decrease (P<0.01) in RBC, Also a significant decrease (P<0.05) in Hb concentration, and WBC count compared to non-treated mice (Table 2).

Table 1: Mean value ± standard error (SE) of biochemical parameters in mice exposed to different subchronic doses of mercuric chloride and control group after 30 days

Decrease in cholesterol level can also interfere with its function in building blocks for steroids and vitamin D [16]. Our data agree with that reported by [12] who referred that plasma cholesterol levels were significantly reduced following eight weeks of HgCl2 exposure in neonatal rats. The increase in AST and ALT in serum may be due to hepatocellular necrosis, which causes increase in the permeability of the cell membrane resulting in the release of transaminase in the blood stream [17]. The Increased serum ALP has been explained by pathological processes such as liver impairment [18, 19]. These results are in agreement with [20] who mentioned that mercury intoxication produced significant hepatic damage as evidenced by increase in the leakage of AST, and ALT. Urea augmentation in this study could also come from protein catabolism acceleration because of oxidative stress provoked by mercury [21]. Our study showed an increase in urea and creatinine levels in serum of mice after mercuric chloride treatment, reinforcing inorganic mercury as a nephrotoxic agent [22]. The depletion of protein content may be due to degradation and the possible utilization of the degraded products for metabolic purposes [23]. These results are in agreement with [24] who mentioned that mercury intoxication produced significant decrease in total protein of rat Albino Wistar. Mercury has high affinities for glutathione (GSH), which is the primary intracellular antioxidant and conjugating agent [25]. The metal-GSH conjugation process is desirable in that it results in the excretion of the toxic metal into the bile. However, it can deplete the cell of GSH and thus decrease antioxidant capacity [26]. [27] reported that mice exposed to HgCl2 showed significant changes at the levels of glutathione.

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Table 2: Mean value ± standard error (SE) of hematological parameters in mice exposed to different sub-chronic doses of mercuric chloride and control group after 30 days.

<table>
<thead>
<tr>
<th>Concentrations of mercuric chloride (mg/kg)</th>
<th>Mean ± SE</th>
<th>RBC (x10^6/µL)</th>
<th>Hb (g/dl)</th>
<th>WBC (x10^3/µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8.50 ± 0.42</td>
<td>12.2 ± 0.75</td>
<td>9.0 ± 0.34</td>
<td></td>
</tr>
<tr>
<td>0.25</td>
<td>8.17 ± 0.37</td>
<td>12.6 ± 0.61</td>
<td>8.2 ± 0.41</td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>8.15 ± 0.29</td>
<td>11.7 ± 0.67</td>
<td>3.2 ± 0.09</td>
<td></td>
</tr>
<tr>
<td>1.25</td>
<td>8.15 ± 0.29</td>
<td>13.1 ± 0.70</td>
<td>3.4 ± 0.12</td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td>7.75 ± 0.30</td>
<td>11.7 ± 0.56</td>
<td>2.1 ± 0.06</td>
<td></td>
</tr>
<tr>
<td>LSD value</td>
<td>0.663 **</td>
<td>1.772 *</td>
<td>2.884 *</td>
<td></td>
</tr>
</tbody>
</table>

(*) refer to significant differences between means at (P < 0.05), (**) refer to high significant differences between means at (P < 0.01).

[28] demonstrated that the decrease in red blood cell can attributed to the decrease iron within erythrocytes or its content of hemoglobin and this causes decrease carrying capacity of oxygen by blood. According to [29], mercury exposure caused a reduction in the erythrocyte count. The reduction in Hb can be probably due to the production of reactive oxygen species (ROS) under the influence of mercuric chloride [30]. [31] observed decrease Hb concentration could be due to either an increase in the rate at which Hb is destroyed or a decrease in the rate of Hb synthesis. Mercury exposure caused a reduction in leukocyte count in mice [29, 32]. The decreased number of white blood cells (leucopenia) may be the result of bioconcentration of the tested metal in the kidney and liver [28].

3.3 Histological Study

The histological examination of the liver section in the control untreated mice group showed a normal histological structure, which contains several hepatic lobules. The central vein lies at the center of the lobule surrounded by the hepatic cells (hepatocytes) which arranged as cords radially and these cords separated from each other by vascular canals (sinusoids). The sinusoids house an important part of livers defense system and populated by numerous type of fixed macrophage (kupffer cells) are exhibited as shown in (Figure 1). Mercuric chloride induced various pathological alterations in liver of mice. Figure (2) represents the histological changes in liver obtained from mice exposed for 0.25 mg / Kg of mercuric chloride, these alterations were characterized by inflammatory with necrosis of hepatocytes, congestion in central vein, and slight dilation in sinusoids. Also, Figure (3) shows abundant inflammatory cells, necrosis of hepatocytes, increase of Kupffer cells, and dilation in sinusoids in mice treated with 0.5 mg /Kg of mercuric chloride. Figure (4) shows the hepatic tissue from mice treated with 1.25 mg /Kg of mercuric chloride, necrosis of hepatocytes with inflammatory cells infiltration, dilation in sinusoids, and increase of kupffer cells were seen. In a group of mice which were treated with 2.5 mg /Kg from mercuric chloride, the section of liver showed chronic inflammatory cells infiltration, necrosis of hepatocytes, and severe congestion in central vein (Figure 5). Under the conditions of liver toxicity, some cells become infiltrated close to the damaged hepatocytes and play a major role in the development of fibrosis, including the fibrosis secondary to alcoholic liver disease [33, 34]. Our results are agreement with [35] who found that a 30-days exposure of male mice to mercuric chloride at doses of 0.16, 0.32, and 0.48 mg/kg body weight caused degenerative hepatocytes, inflammatory cells, and extensive necrosis of cell in the liver.
The kidney of the control mice showed the normal histological structure of the renal corpuscles and renal tubules. The renal corpuscle consisted of tuft of blood capillaries surrounded by the Bowmann’s capsule. The renal tubules included proximal convoluted tubules lined by large pyramidal cells with a brush border of microvilli and appears with small lumen, and distal convoluted tubules lined by cuboidal cells without brush border so its appears with large and clear lumen, Figure (6). Light microscope evaluation of kidney in mice treated with dosing 0.25 mg/kg from mercuric chloride showed inflammatory cells, degeneration and necrosis in epithelial cells (Figure 7). The section of kidney in mercuric chloride-treated mice with 0.5 mg/kg showed inflammatory cells, necrosis of cells, shrinking of glomerular, and degeneration in proximal convoluted tubules (Figure 8). While, in mercuric chloride treated-mice at concentration 1.25 mg/kg, kidney showed inflammatory cells, necrosis in epithelial cells, degeneration in proximal convoluted tubules, and degeneration in distal convoluted tubules (Figure 9). On the other hand, the group of mice which were treated with mercuric chloride 2.5 mg/kg, the renal tissue showed necrosis in epithelial cells, degeneration in proximal convoluted tubules, and apoptosis in distal convoluted tubules (Figure 10). All forms of mercury have toxic effects in a number of organs, especially in the kidneys. The interaction of mercury with protein sulfhydryl groups is thought to play an important role in nephrotoxicity induced by mercury at cellular level [36]. Changes in mitochondrial morphology and function are very early event which follow mercuric chloride administration, which suggests that mitochondrial dysfunction and oxidative stress have an important role in mercury induced renal toxicity [37].

4. Conclusions

The present data showed that the exposure of mice to mercuric chloride is capable of inducing alterations in some enzymatic activities, liver functions, renal functions and some biochemical parameters. Whereas, results of the differences in urea and creatinine concentrations in blood
between the exposed mice and unexposed group suggest that exposure to mercury could cause renal dysfunction. Also, increase in serum AST, ALT and ALP can be used as potential enzyme biomarkers for mercury-induced hepatotoxicosis which ultimately affects the general health by altering the functional and structural integrity of liver. Reduction in glutathione levels (GSH) for examined samples in comparison to control sample gave a sign that mice may suffer from oxidative stress as a result of exposure to mercury. Also, mercuric chloride induced hematological disturbances in mice, where anemia associated with leucopenia were recorded in different concentration of mercuric chloride. The present findings clearly demonstrate that mercuric chloride is capable of inducing dose dependent histopathological changes in the liver, and kidney of the exposed mice.

5. Conflicts of interest

The authors declare that there are no conflicts of interest.

6. Acknowledgments

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References


