Original Article

Mercury Intoxication in Rats: Iron and Vitamin B₆ as A Potential Therapy

Mohammad Taimur Islam¹, Anup Kumar Talukder², Milton Talukder³, Mohammad Rohul Amin³, Khondoker Jahengir Alam⁴, Mohammad Golam Haider¹

ABSTRACT

Background: Mercury in any form is poisonous and mercury toxicity most commonly affects the nervous, gastrointestinal (GI) and urinary systems. The aim of this study was to investigate the specific effect associated with mercury toxicity and to evaluate the effectiveness of iron and vitamin B₆ supplement on mercury-induced toxicities in rats.

Methods: This experiment was performed on 25 rats. All rats were randomly divided into five equal groups (5×5). Toxic signs and body weight change, hematological parameters like total erythrocyte count (TEC), total leukocyte count (TLC), hemoglobin content (Hb%) and packed cell volume (PCV) and postmortem changes in rats were investigated.

Results: Rats treated with mercury intoxication showed severe toxic signs and significantly (P<0.01) reduced TEC, TLC, Hb content and PCV. However, rats treated with mercury intoxication in combination with iron and vitamin B₆ showed physiological levels of hematological parameters. Mercury intoxication induced the congestion and necrosis in lung, liver, heart and kidney, whereas combined use of mercury intoxication, iron and vitamin B₆ recovered the condition.

Conclusion: Combined use of iron and vitamin B₆ is highly protective against mercury toxicity.

Keywords: Hematological Parameter, Iron, Mercury Intoxication, Vitamin B₆.

INTRODUCTION

The heavy metals such as mercury, lead, cadmium etc. are considered as silent deadly killers which along with radiation (atmospheric fallout, industrial waste, medical and dental procedures), exert a cumulative toxic effect upon living organisms. Public health concern due to mercury exposure, caused by ingestion of fish contaminated with methylmercury and the elemental mercury content of dental amalgams [1–3], has long been a topic of debate. Dispute of more complexity of mercury toxicology, antioxidant protection in the prevention of neurological and renal damage caused by mercury toxicity is demonstrated [4].

The barometers, batteries, bronzing, calibration instruments, dental amalgams, electroplating, fingerprinting products, fluorescent and mercury lamps, infrared detectors, the jewelry industry, manometers, neon lamps, paints, paper pulp production, photography, silver and gold production, semiconductor cells, and thermometers are responsible for elementary mercury toxicity [5]. The acute ingestion of inorganic mercury salts can cause gastrointestinal disorders such as abdominal pain, vomiting, diarrhea, and hemorrhage. Chronic exposure may cause severe disturbances in the central nervous system, gastrointestinal tract, kidneys, and liver [6–10]. Ingestion of an inorganic mercury-containing laxative may lead to dementia, colitis, and renal failure in chronic poisoned condition [11]. Inorganic mercury toxicity via inhalation may cause a large scale of pathological conditions such as corrosive bronchitis, interstitial pneumonitis, renal disorders, fatigue, insomnia, loss of memory, excitation, chest pains, impairment of pulmonary function and gingivitis [12]. Repeated and prolonged inhalation of inorganic mercury compounds may result in a reduction of sensory and motor nerve function,

1. Department of Pathobiology, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh.
2. Department of Gynecology, Obstetrics and Reproductive Health, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh.
3. Department of Pathobiology, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh.
4. Department of Pathology and Pharmacology, Patuakhali Science and Technology University, Babugonj, Barisal, Bangladesh.

*Corresponding Author: E-mail: shiplu_bau@yahoo.com
depression, visual and auditory hallucinations, muscular tremors, sleep disorders, alterations in autonomic function (heart rate, blood pressure, reflexes), impaired vasomotor coordination, speech disorders, dementia, coma and death [13-18]. “Hg toxicity is now considered as a new independent cardiovascular risk factor” [19].

Foods such as fish, milk, meat, and wheat bran; minerals such as Se, zinc (Zn), copper (Cu), and magnesium (Mg) and Vitamin B₆ have been implicated in the alteration of Hg metabolism. However, evidence for protective or antagonistic effects is often complex and highly dependent on metabolic conditions.

Anti-oxidant therapy is important to reduce oxidative stress and to raise glutathione levels. Lead, vitamin B₆ and iron are especially needed, in addition to a broad-spectrum vitamin. By considering the above facts this study was undertaken to observe the toxic symptoms and to determine the hematological parameter i.e. total erythrocyte count (TEC), hemoglobin (Hb) content, packed cell volume (PCV) and total leukocyte count (TLC) of mercury intoxicated rat. Furthermore, postmortem changes in different organs of the body i.e. liver, heart, lung, kidney, spleen, testes of rats after feeding mercuric chloride alone and in combination with iron and vitamin B₆ were examined.

MATERIALS AND METHODS

Experimental Designs

A total number of 25, forty days-old pathogen free Wister male rats were used in this experiment. The rats were purchased from Livestock Research Institute (L.R.I), Dhaka, Bangladesh.

Animal experiments described in this article were conducted in accordance with the guiding principles for the care and use of research animals promulgated by Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh.

All rats were kept under close observation in order to acclimatize to the new environment for a period of one week. After acclimatization, they were randomly divided into 5 equal groups (5×5) each comprising of five rats and were marked as group A, B, C, D and E. All group of rat were kept separately in a compartmented rectangular metallic cage wrapped with wire mesh. The rat cages were kept in well-ventilated room at 25 °C and the relative humidity of 70-80%. Room lighting consisted of alternate 12 h light and dark periods. One group of rat (group A) was kept as control. They were supplied normal feed and water. Rats of group B were supplied only mercuric chloride (HgCl₂) @75mg/lit.d.w. Rats of group C were administered with mercuric chloride @75 mg/lit.d.w plus ferrous sulphate (FeSO₄) @100 mg/lit.d.w. Rats of group D were administered with mercuric chloride @ 75 mg/lit.d.w plus vitamin B₆ @ 30mg/lit.d.w. Rats of group E were administered with mercuric chloride @ 75 mg/lit.d.w and ferrous sulphate @100 mg/lit.d.w plus vitamin B₆ @ 30 mg/lit.d.w. All the chemicals i.e. Mercuric chloride, ferrous sulphate and vitamin B₆ were fed to the different groups of rat with drinking water daily for 42 days and all the rat were kept under close observation.

Toxic Signs and Body Weight

After feeding mercuric chloride alone and in combination with iron and vitamin B₆ to 5 groups of rat for 42 consecutive days, all the control and treated rats were observed carefully for appearance of any toxic signs and mortality if any. Body weight of the control and treated rats were measured before starting of feeding and at 14 days interval during the entire period of experiment.

Procedure of Blood Collection for Measuring Hematological Parameters

For studying hematological parameters, blood was collected directly from the heart of the control and treated rats at 0 day and 42 days of feeding. The blood was collected with sterile syringe and needle from chloroform-anaesthetized rat. Immediately after collection, blood was transferred to sterile tube containing anticoagulant (4% sodium citrate solution) at a ratio of 1:10.the collected blood was used for different hematological parameters within two hours after collection.

Hematological Parameters

Blood was collected at day 0 and 42. TEC, Hb concentration, PCV and TLC were determined by calculating mean value ± SD [20].

Gross Pathological Changes

At the end of the experiment, all rats were euthanized by using anesthetic chloroform. The carcasses were examined systematically. Gross pathological changes and extent of damage in individual organs were noted. The main organs were liver, heart, kidney, spleen, lung, testes and they were collected and the gross pathological lesions were observed [21].

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**Statistical Analysis**

Data obtained from the experiment on hematological parameters such as TEC, TLC, PCV and Hb content was analyzed statistically by one-way analysis of variance (ANOVA) test using SPSS version 11.5 (Chicago, IL, USA).

**RESULTS**

The experiment was conducted on 5 groups (each group consisting of 5 rats) of 40 days-old rats to study the effect of administration of mercuric chloride alone and in combination with iron and vitamin B6 by investigation of clinical, hematological parameter and pathomorphology of postmortem changes in rats.

**Toxic Signs**

In group A (control group), all rats looked healthy without any toxic signs during the whole experimental period. Rats belonged to group B (mercuric chloride alone) were apparently normal up to 15th day feeding but from 16th day of mercuric chloride administration, rats showed very severe toxic signs i.e. irritability, excitability, restlessness, salivation, incoordination, muscle tremor, ataxia, ruffled coat and anuria.

Rats of group C (HgCl2+FeSO4) were apparently normal without any visible toxic signs up to 14 days of HgCl2+FeSO4 feeding. From 21st day onward of HgCl2+FeSO4 administration, all rats showed very mild toxic signs i.e., excitement, restlessness, anorexia, anuria, diarrhea and salivation.

Rats of group D (HgCl2 + Vitamin B6) showed very mild toxic signs i.e. excitement, restlessness, anorexia, anuria, and salivation.

Rats of group E (HgCl2 + FeSO4 + Vitamin B6) were apparently normal which showed only excitement, restlessness and mild anorexia from 22nd day to 28th day of administration of mercuric chloride, iron and vitamin B6. After that, rats were quite normal without showing any visible toxic sign.

**Effect on Body Weight**

The effect of mercuric chloride on body weight of five different experimental groups of rat is presented in Table 1. The body weight of rats of each group was measured just before giving treatment and body weight gain or loss was recorded every 14 days interval. The body weight of rats in control group A was found to increase significantly but in treated group the body weight was found to decrease significantly. In group B (only HgCl2), the body weight was reduced significantly to the extent of 17.06% and 26.63% at 28th and 42 days of treatment, respectively (Table 1).

In group C (HgCl2+FeSO4) and group D (HgCl2+Vitamin B6), the significant \(P<0.05\) body weight reduction (13.47% and 7.477%, respectively) was recorded at 28 days of treatment. In group C and D, the significant \(P<0.05\) body weight reduction (20.18% and 12.83%, respectively) was recorded at 42 days of treatment (Table 1).

In group E (HgCl2+FeSO4 + Vitamin B6), there was no significant effect on body weight. The body weight of rats of this group was found to increase gradually (Table 1).

**Total Erythrocyte Count (TEC)**

In group B (only HgCl2), highly significant decrease (24.36%) of TEC was observed at 42 days of only mercuric chloride administration (Table 2).

In group C (HgCl2 + FeSO4) and D (HgCl2+ Vitamin B6), TEC was reduced significantly to the extent of 19.50% and 7.00%, respectively at 42 days of treatment (Table 2). On the other hand, there was no significant reduction of TEC was observed in group E (HgCl2 + FeSO4+ Vitamin B6) (Table 2).

**Hemoglobin (Hb) Content**

In group B (only HgCl2), C (HgCl2 + FeSO4) and D (HgCl2+ Vitamin B6), Hb content was reduced significantly to the extent of 27.36%, 16.66% and 9.68% respectively at 42 days of treatment. However, there was no significant decrease of Hb content was observed in group E (Table 3).

**Total Leukocyte Count (TLC)**

In group B (only HgCl2), C (HgCl2 + FeSO4) and D (HgCl2+ Vitamin B6), TLC was reduced significantly to the extent of 22.98%, 17.04% and 8.93%, respectively at 42 days of treatment. No significant effect was found on TLC in group E (Table 4).

**Packed Cell Volume (PCV)**

In group B (only HgCl2), C (HgCl2 + FeSO4) and D (HgCl2+ Vitamin B6), PCV was reduced significantly \(P<0.05\) at 42 days of treatment to the extent of 12.58%, 7.65% and 4.10%, respectively. However, no significant reduction of PCV was observed in group E (Table 5).
Table 1. Effect of feeding mercuric chloride alone and in combination with iron and Vitamin B<sub>6</sub> supplementation on mean body weight in rats (gm/rat).

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<tr>
<th>Groups</th>
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<td>0</td>
<td>14</td>
<td>28</td>
<td>42</td>
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<tr>
<td>Group A (Untreated/ Control)</td>
<td></td>
<td>25.18±0.73</td>
<td>29.80±0.74</td>
<td>33.98±0.64</td>
<td>39.27±0.43</td>
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<tr>
<td>Group B (Only HgCl&lt;sub&gt;2&lt;/sub&gt; @ 75 mg/lit.d.w)</td>
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<td>25.08±0.61</td>
<td>25.07±0.64</td>
<td>20.80±0.54*</td>
<td>18.4±0.36**</td>
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<td>Group C (HgCl&lt;sub&gt;2&lt;/sub&gt; @ 75 mg/lit.d.w+FeSO&lt;sub&gt;4&lt;/sub&gt; @100mg/lit.d.w)</td>
<td></td>
<td>26.56±0.24</td>
<td>25.26±0.25</td>
<td>22.98±0.67</td>
<td>21.20±0.71*</td>
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<td>Group D (HgCl&lt;sub&gt;2&lt;/sub&gt; @ 75 mg/lit.d.w + Vitamin B6 @ 30 mg/lit.d.w)</td>
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<td>24.87±0.456</td>
<td>25.02±0.23</td>
<td>23.51±0.69</td>
<td>21.20±0.71*</td>
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<tr>
<td>Group E (HgCl&lt;sub&gt;2&lt;/sub&gt; @ 75 mg/lit.d.w + FeSO&lt;sub&gt;4&lt;/sub&gt; @ 100 mg/lit.d.w + Vitamin B6 @ 30 mg/lit.d.w)</td>
<td></td>
<td>26.56±0.24</td>
<td>28.20±0.35</td>
<td>32.90±0.65</td>
<td>38.30±0.66</td>
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Values above represent the mean ±SEM of 5 rats
* Significant at P<0.05 ** Significant at P<0.01
(%) percent of increase (% b) percent of decrease

Table 2. Effect of feeding mercuric chloride alone and in combination with iron and Vitamin B<sub>6</sub> supplementation on TEC (million/cu.mm) in rats.

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<th>Groups</th>
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<tr>
<td>Group A (Untreated/ Control)</td>
<td></td>
<td>8.48±0.23</td>
<td>8.91±0.11</td>
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<td>Group B (Only HgCl&lt;sub&gt;2&lt;/sub&gt; @ 75 mg/lit.d.w)</td>
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<td>8.62±0.22</td>
<td>6.52±0.23**</td>
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<tr>
<td>Group C (HgCl&lt;sub&gt;2&lt;/sub&gt; @ 75 mg/lit.d.w+FeSO&lt;sub&gt;4&lt;/sub&gt; @100mg/lit.d.w)</td>
<td></td>
<td>8.82±0.09</td>
<td>7.10±0.05*</td>
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<tr>
<td>Group D (HgCl&lt;sub&gt;2&lt;/sub&gt; @ 75 mg/lit.d.w + Vitamin B6 @ 30 mg/lit.d.w)</td>
<td></td>
<td>8.43±0.20</td>
<td>7.84±0.24*</td>
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<tr>
<td>Group E (HgCl&lt;sub&gt;2&lt;/sub&gt; @ 75 mg/lit.d.w + FeSO&lt;sub&gt;4&lt;/sub&gt; @ 100 mg/lit.d.w + Vitamin B6 @ 30 mg/lit.d.w)</td>
<td></td>
<td>8.52±0.20</td>
<td>8.80±0.18</td>
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Values above represent the mean ±SEM of 5 rats
* Significant at P<0.05 ** Significant at P<0.01
(%) percent of increase (% b) percent of decrease

Table 3. Effects of feeding mercuric chloride alone and in combination with iron and Vitamin B<sub>6</sub> supplementation on hemoglobin (gm%) content in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Days</th>
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<td>0</td>
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<tr>
<td>Group A (Untreated/ Control)</td>
<td></td>
<td>9.27±0.13</td>
<td>10.40±0.36</td>
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<tr>
<td>Group B (Only HgCl&lt;sub&gt;2&lt;/sub&gt; @ 75 mg/lit.d.w)</td>
<td></td>
<td>8.55±0.58</td>
<td>6.21±0.11**</td>
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<tr>
<td>Group C (HgCl&lt;sub&gt;2&lt;/sub&gt; @ 75 mg/lit.d.w+FeSO&lt;sub&gt;4&lt;/sub&gt; @100mg/lit.d.w)</td>
<td></td>
<td>9.54±0.21</td>
<td>7.95±0.48*</td>
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<tr>
<td>Group D (HgCl&lt;sub&gt;2&lt;/sub&gt; @ 75 mg/lit.d.w + Vitamin B6 @ 30 mg/lit.d.w)</td>
<td></td>
<td>9.30±0.07</td>
<td>8.40±0.14*</td>
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<tr>
<td>Group E (HgCl&lt;sub&gt;2&lt;/sub&gt; @ 75 mg/lit.d.w + FeSO&lt;sub&gt;4&lt;/sub&gt; @ 100 mg/lit.d.w + Vitamin B6 @ 30 mg/lit.d.w)</td>
<td></td>
<td>9.32±0.20</td>
<td>10.30±0.15</td>
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Values above represent the mean ±SEM of 5 rats
* Significant at P<0.05 ** Significant at P<0.01
(%) percent of increase (% b) percent of decrease
Table 4. Effect of feeding mercuric chloride alone and in combination with iron and Vitamin B6 supplementation on TLC count (thousand /cu.mm) in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Days</th>
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<tr>
<td>Group A (Untreated/ Control)</td>
<td>9.36±0.16</td>
</tr>
<tr>
<td>Group B (Only HgCl2 @ 75 mg/lit.d.w)</td>
<td>9.31±0.18</td>
</tr>
<tr>
<td>Group C (HgCl2 @ 75 mg/lit.d.w + FeSO4 @ 100 mg/lit.d.w)</td>
<td>9.31±0.13</td>
</tr>
<tr>
<td>Group D (HgCl2 @ 75 mg/lit.d.w + Vitamin B6 @ 30 mg/lit.d.w)</td>
<td>9.48±0.05</td>
</tr>
<tr>
<td>Group E (HgCl2 @ 75 mg/lit.d.w + FeSO4 @ 100 mg/lit.d.w + Vitamin B6 @ 30 mg/lit.d.w)</td>
<td>9.32±0.10</td>
</tr>
</tbody>
</table>

Values above represent the mean ±SEM of 5 rats

* Significant at P<0.05
** Significant at P<0.01
(%a) percent of increase
(%b) percent of decrease

Table 5. Effect of feeding mercuric chloride alone and in combination with iron and Vitamin B6 supplementation on packed cell volume (%) in rats.

<table>
<thead>
<tr>
<th>Groups</th>
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<tr>
<td>Group A (Untreated/ Control)</td>
<td>39.79±0.35</td>
</tr>
<tr>
<td>Group B (Only HgCl2 @ 75 mg/lit.d.w)</td>
<td>40.38±0.32</td>
</tr>
<tr>
<td>Group C (HgCl2 @ 75 mg/lit.d.w + FeSO4 @ 100 mg/lit.d.w)</td>
<td>40.36±0.35</td>
</tr>
<tr>
<td>Group D (HgCl2 @ 75 mg/lit.d.w + Vitamin B6 @ 30 mg/lit.d.w)</td>
<td>41.00±0.01</td>
</tr>
<tr>
<td>Group E (HgCl2 @ 75 mg/lit.d.w + FeSO4 @ 100 mg/lit.d.w + Vitamin B6 @ 30 mg/lit.d.w)</td>
<td>40.20±0.35</td>
</tr>
</tbody>
</table>

Values above represent the mean ±SE of 5 rats

* Significant at P<0.05
** Significant at P<0.01
(%a) percent of increase
(%b) percent of decrease

Gross Pathological Changes

After 42 days of feeding, all control and treated rats were sacrificed and were subjected to post-mortem examination with a view to study the gross pathological changes in some vital organs such as kidney, liver, heart, stomach, spleen and lung. In group A, all vital organs were apparently normal. In group B, pin point hemorrhages were found in liver. Severe congestion was found in kidney. Slight hemorrhages were found in stomach, heart and lung. In group C, pin point hemorrhages were found throughout the liver and kidney with severe congestion. Hemorrhages and congestion were found in stomach, heart and lung. In group D, slight hemorrhage was found in lung, liver, kidney, heart and testes. On the other hand, in group E, all vital organs were apparently normal except few hemorrhagic points noticed in liver.

DISCUSSION

The present study investigated the specific effect associated with mercury toxicity and efficacy of iron and vitamin B6 as a therapy against mercury intoxication using an experimental model in rats. Rats belonged to group A (control group) were normal during the whole experimental period. Rats of group B (only mercuric chloride) showed various toxic signs like excitement, muscle tremor, ataxia, restlessness,
salivation incoordination, ruffled hair coat, stomatitis and polyuria. Rats of group C (HgCl\textsubscript{2}+FeSO\textsubscript{4}) and group D (HgCl\textsubscript{2}+Vitamin B\textsubscript{6}) showed very mild toxin sign like excitement, anorexia, restlessness depression and salivation. Among the two treated group (C and D), in group D (HgCl\textsubscript{2}+Vitamin B\textsubscript{6}) the toxic sign was very mild in nature. On the other hand, rats of group E showed only excitement, restlessness and mild anorexia in the middle of experiment just for seven days. In accordance to the present findings, several other researchers [22-24] observed the similar toxic sign i.e. gastro-intestinal pain, vomiting, salivation, diarrhea, paresisemia, excitement, muscle tremor, ataxia, restlessness, anorexia and incoordination. Mercury toxicity occurs due to severe irritation to gastrointestinal tract and inhibition of –SH groups in different tissues of the body [1, 25]. However, in chronic poisoning, the irritation is not severe; So, GIT symptoms were not prominent in the present findings.

Oral administration of mercuric chloride alone and in combination with iron and vitamin B\textsubscript{6} separately significantly reduced the body weight of adult rats in group B, C and D. Among the two treated group (C and D), in group D (HgCl\textsubscript{2}+Vitamin B\textsubscript{6}) the reduction of body weight was less than other treated groups. However, no effect was observed on the body weight after oral administration of mercuric chloride in combination with iron plus vitamin B\textsubscript{6} in group E. In this group, body weight of rats was increased accordingly. Similar to the present study, several other researchers [23, 24] also observed the reduction of body weight. On the other hand, the body weight of rat did not increase when given mercuric chloride and selenium alone [26]; however, when given combination of mercuric chloride and selenium the body weight of rat was increased.

TEC, Hb level, TLC and PCV of rats in group B, C and D were decreased significantly, following administration of mercuric chloride alone and in combination with Iron and Vitamin B\textsubscript{6} separately. Among the two treated group (C and D), in group D (HgCl\textsubscript{2}+Vitamin B\textsubscript{6}) TEC, Hb content, TLC and PCV was reduced less than other treated groups. The decrease value of Hb content may be due to decrease in the value of TEC. The possible cause of this result might be due to adverse effect of mercuric chloride on hematopoietic system and on the absorption of essential vitamins and minerals from the gut and the destruction of the RBC. On the other hand, no significant difference was observed regarding hematological parameter in the rats of group E, following administration of mercuric chloride in combination with Iron plus Vitamin B\textsubscript{6}. There was not so much difference on hematological parameter between group A (control group) and group E. Collectively, these data indicated that iron plus vitamin B\textsubscript{6} have performed synergistically against mercury intoxication. Toxic effect of HgCl\textsubscript{2} on hematopoietic system may be responsible for changing the haematological parameters.

Similar to present findings, alteration of hematological parameters were reported by several workers [23, 24, 27-30]. The reduction of all hematological parameters (total erythrocyte count, total leucocyte count, hemoglobin content and packed cell volume) was reported earlier [23, 24, 27, 30]. However, total leucocyte count and packed cell volume were increased in mercury poisoning [29]. The reasons of such difference might be due to different experimental conditions.

The visceral organs were apparently normal in the rats of group A (control group) and group E. There was presence of slight congestion in the spleen, kidney and heart and pinpoint hemorrhage in the liver of rat of group C and group D. On the other hand, highly congested kidney and slight hemorrhage were found in heart and intestine. Necrosis in stomach were also found in group B. Similar results have been reported by other workers [23, 24, 31], who also found the similar type of findings in mercury poisoning. However, in this study, we found that combination of iron and vitamin B\textsubscript{6} subsided the toxic effect of mercury in the major vital organs.

The toxic effect of mercuric chloride alone is clear; however, it is possible to recover the effect of mercury toxicity by using iron and vitamin B\textsubscript{6} together.

CONCLUSION

Mercury is a highly toxic agent causing serious damage to living tissue with various toxic signs, decreased body weight and pathological changes in different vital organs of the body. However, the combined administration of iron and vitamin B\textsubscript{6} were good choice for restoring mercury induce hematological alteration. The
findings of the present research study provide basic information about the detrimental effect of mercury toxicity in rats that suggest very important issue to human and livestock in the heavily mercury-contaminated areas of Bangladesh and other countries of the world. Further study is recommended to find out the more information in details with regard to mercury toxicity and its prevention and control strategies in livestock and human beings.

ACKNOWLEDGEMENTS

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