Effect of Long-Term Manganese Intoxication on Selected Biochemical Parameters and Blood Smears of Wistar Rats

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Abstract- Manganese, an environmental pollutant due to human activities, is a public health problem and responsible for several metabolic disturbances. The aim of this study is to assess the manganese chloride chronic exposure effect on selected biochemical parameters and blood smears in albino rats. Chronic exposure to this metal induced an elevation of renal bio-indicators, increase in liver transaminase and a hyper-platelet aggregation observed in blood smears.

Keywords- Manganese; Biochemical Parameters; Hyper-Platelet Aggregation; Blood Smears

I. INTRODUCTION

Manganese (Mn) is a naturally occurring element which is widespread in the environment. Mn is crucial for maintaining the proper function, regulation of many biological processes and therefore an essential nutrient. It is a constituent of many enzymes involved in fat and protein metabolisms, and used by various antioxidant enzymes such as Mn superoxide dismutase (MnSOD) and glutamine synthetase[1,2,3]. In addition, this important element is involved in immune function, regulation of blood sugar, production of cellular energy, reproduction, digestion, bone growth, carbohydrate metabolism and blood clotting [4]. Industrial use of Mn (ie: the production of paint pigments, dry cells batteries, glass and ceramics as well as mining of Mn ores and welding of mild steel) may expose workers to risks of these chemical applications[5]. However, high dose of Mn seems to cause serious neurotoxicity, immunotoxicity and developmental toxicity, particularly in male. It is also known that Chronic exposure to this metal can cause alterations in development as well as reproductive dysfunction[6,7]. The aim of this study is to assess the effect of Manganese chloride chronic exposure on the hematological and biochemical systems of wistar rats.

II. MATERIAL AND METHODS

Experiments were performed on 24 adult male rats, aged for 5 to 6 months and weighing 280±10g. The animals were housed in room with a 12/12-hour light/dark cycle, at 22 ± 2°C and had access to ad libitum water and food (15% proteins). The rats were distributed into two groups of twelve animals. The first group is the control group (T) receiving distilled water and the second group (M) is the experiment lot of rats exposed to manganese, receiving oral manganese chloride tetrahydrate (MnCl2 4H2O) with the dose of 4.79 mg Mn.l-1[9]. After twelve weeks of experimentation, the animals were sacrificed in the morning after fasting for 12 hours, by intraperitonial injection with a solution of chloral (C2H3Cl13O2) at 10%. After incision of the abdomen, blood is collected by cardiac puncture in heparin tubes for biochemical analysis and dry tubes for blood smears. Blood serum urea, creatinine concentration and ASAT and ALAT activity was determined by colorimetric methods with kit Chronolab. Blood smears were performed according to standard techniques, after fixation in (alcohol 70%), staining with May-Grunwald-Giemsa and mounted between the slide and the cover slide.

III. STATISTICAL ANALYSIS

The mean ± SEM values were measured for each group to determine the significance of intergroup difference. Each parameter was analyzed separately using one way analysis of variance (ANOVA). To find the difference between the groups, Student’s t’ test was used. P values <0.05 were considered to be significant.

IV. RESULTS

The administration of the manganese chloride dose, at 4.79 mg/ml, to rats in drinking water for 12 weeks provided as results, an increase of the analyzed biochemical parameter concentrations of experimental group (Exposed to manganese) compared to control group (Table I). The blood smears are used to detect possible hematologic abnormalities and to aid in diagnosis and understanding of the pathophysiologic mechanisms of manganese poisoning. No abnormalities were reported at the blood smear of rat control groups (T) (Fig. 1). However the Observation of the slides of rats exposed to manganese, had
indicated, at low magnification (×40), several clear areas plaques in blood smears (Fig. 2). These areas represent aggregation of platelets, observed at higher magnification (×100) (Fig. 3).

Fig. 1 Blood smear from a control rat was observed at low magnification (×40), staining with May-Grunwald-Giemsa.

Fig. 2 Blood smears of rats exposed to Mn. Arrow shows clear areas at low magnification (×40), staining with May-Grunwald-Giemsa.

Fig. 3 One of the clear areas observed with a large magnification (×100), show a hyper platelet aggregation. Staining with May-Grunwald-Giemsa.
V. DISCUSSION

Manganese is one of the most dangerous occupational and environmental toxins. It accumulates in the human organism mainly in brain, liver and kidneys, where it causes functional changes\(^5\). As a result of long-term exposure to manganese there may come a hepatic dysfunction. In fact, the liver is one of the most important major organs involved in the storage, biotransformation and detoxification of toxic substances, was of interest in heavy metal poisoning\(^6\). This might justify the elevation of ASAT and ALAT concentration in our study. Rivera-Mancia et al.\(^7\), had observed that manganese and hepatic encephalopathy were the most common pathologies associated with the effects of Mn exposure. It has been reported that Mn had a special affinity to mitochondrial and was mainly stored in those tissues with rich mitochondrion. The accumulation of Mn in the brain mitochondria was associated with neurological symptoms of manganism in vivo\(^8,9\). Liver is also rich of mitochondria, which is obviously the target organ of Mn accumulation. Earlier reports explained the pathogenetic mechanism of manganism, which mainly had the influence exerted directly on the internal liver tissue\(^10\). Animal experiments carried out on mice, exposed to Mn, showed Mn accumulation and damage in the liver and kidney\(^11\). It was reported after Mn chronic exposure to high doses there may be a glomerular filtration impairment which may reflect an increase of urea and creatinine concentration in our studies, Lafferty et al.\(^12\), observed by examining the effects of an infusion of MnCl\(_2\) to normal rats, an interaction with the glomerular filtration. In the same conditions, Dudek et al.\(^13\), confirmed the inhibitory effect of Mn on slow calcium channels mainly due to the antagonistic effect of manganese on the calcium ion (Ca\(^{2+}\)) during the process of glomerular filtration. Panapakkam et al.\(^14\), have reported in their histological study that the most striking lesions were observed in the kidney and prostate glands of male animals. Results of this study suggest that male rats were more sensitive to the effects of high levels of manganese given orally than female rats and the genitourinary structures represent target organs of this metal.

To facilitate the understanding of the pathophysiological mechanisms of manganese poisoning, blood smears were performed. Our results showed that chronic treatment with manganese chloride cause significant disruptions at platelet cells inducing hyper-platelet agglutination. Further, various studies reported abnormalities caused by manganese at the other elements of the blood (erythrocyte and leucocyte) by inducing anemia due firstly to a decrease in hemoglobin with an iron deficiency anaemia induced by an antagonistic effect of manganese and iron ions. In another hand, is due to a spinal cord lesion causing an abnormal shape and size of red blood cells resulted in a microcytic anemia. According to the work of Rossander-Hultén et al.,\(^15\); they reveal a competitive inhibition of iron absorption by manganese and concludes that an increase in Mn concentration is likely to have an impact on hemoglobin due mainly to low iron absorption. In the same conditions, Oates et al.\(^16\), revealed that manganese may borrow specific transmembrane receptors iron (Nramp2/DMT1) inserted into the apical membrane of enterocytes by inducing iron deficiency by preventing the absorption of ferric ions. In the same year Conrad et al.\(^17\), showed that tissue level Mn (especially erythropoietic bone marrow) can substitute for iron ions in pairs (Fe+3/ transferrin) which is attached to the transmembrane receptors erythroblast (DMT1) and thus accumulated the spinal level. These results are consistent with those of studies conducted by Earnhardt et al.\(^18\), reported that exposure to manganese chloride causes disruption of hematopoiesis in bone marrow levels.

It can evoke the assumption of achieving oxidative stress state caused by manganese in the bone marrow cells. Knowing that manganese can replace the various trace elements in the same valence in metalloproteins (enzymes) which requires the presence of these trace elements in their activities and subsequently induced disturbances at different metabolisms. Kulmacz et al.\(^19,20\), confirmed this hypothesis by their studies, in vitro on prostaglandin synthetase Fe-PGH (a metallo-enzyme requires iron), that showed Manganese may be substituted for iron ions in this enzyme (Mn-PGH) and conducted the same function but by inducing the production of free radicals in gold peroxidation of arachidonic acid (AA) (prostaglandin metabolism). Also studies of Odenwallner et al.,\(^21\), performed in vitro, suggested the (Mn-PGH) synthase in a complete action of cyclo-oxygenase (COX), but only partial 0.9% of the peroxidase activity of iron (Fe-HMP). However, this present study has demonstrated a direct deleterious effect of Mn on the hematological and biochemical system of rats. So it is necessary to search for compounds being able to limit the consequences of chronic exposure to environmental pollutants as in a large-scale survey in occupational activities.

### CONFLICT OF INTEREST

No conflict of interest. There are no financial and personal relationships with other people or organizations that could unduly influence our work.

**TABLE I. EFFECT OF MANGANESE CHLORIDE ON RAT BLOOD SERUM SELECTED BIOCHEMICAL PARAMETERS.**

<table>
<thead>
<tr>
<th></th>
<th>Control (T)</th>
<th>Manganese (M)</th>
<th>statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea (g/l ± SEM)</td>
<td>0.19±0.001</td>
<td>0.62±0.007</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Creatinine (mg/l ± SEM)</td>
<td>2.92±0.17</td>
<td>8.71±0.29</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>ASAT (U/l ± SEM)</td>
<td>55.49±1.46</td>
<td>77.51±1.53</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>ALAT (U/l ± SEM)</td>
<td>18.76±0.44</td>
<td>42.86±0.95</td>
<td>P&lt;0.001</td>
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