Original Article
Toxicology International, *Sept-Dec 2016 / Vol-23 / Issue-3, 212-220*
ISSN (Print) : 0971-6580
ISSN (Online) : 0976-5131
DOI: 10.22506/ti/2016/v23/i3/146713

Manganese Chloride Induced Hepatic and Renal Toxicity in Wistar Rats

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Abstract

Manganese (Mn) is an essential trace metal and acts as cofactor in many cellular enzymes. But excessive concentration of the Mn is potentially toxic resulting in several disorders. The present study was designed to evaluate toxic effects of manganese chloride ($MnCl_2.4H_2O$) exposure on marker biochemical parameters, antioxidant defense system and histomorphology of liver and kidney in rats. Adult male Wistar rats were randomly divided into three groups. In group A, the rats were treated with vehicle (0.5 ml normal saline) and served as control. The rats in group B and C were exposed to $MnCl_2$ (50 and 100 mg/kg b.wt./day, p.o. respectively) for 120 days. The results showed significant elevation in cholesterol and triglyceride contents in liver while glycogen concentration showed significant depletion. Serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin, urea and creatinine were significantly increased by $MnCl_2$ treatment. The present data also indicated that $MnCl_2$ significantly increased lipid peroxidation (LPO) and concomitantly decreased the levels of glutathione (GSH), ascorbic acid and superoxide dismutase (SOD) activity in both liver and kidney extracts compared to control. Histopathological study of the liver of $MnCl_2$ exposed rats showed dose based degenerative and atrophic changes, infiltration of mononuclear cells and dilation of sinusoids. Sections of kidney also showed dilation of glomeruli and degenerative changes in the tubules. Taken together these data suggest that $MnCl_2$ exposure has adverse impact on the morphology and functions of both liver and kidney in rats.

Keywords: Histopathology, Kidney, Lipid Peroxidation, Liver, Manganese Chloride

Received: 18-04-2016 **Revised:** 07-07-2016 **Accepted:** 12-08-2016

1. Introduction

Manganese (Mn) is a naturally occurring essential trace element that is found throughout the body and is involved in several key processes[1]. Manganese acts as a cofactor in many cellular enzymes, like arginase, superoxide dismutase (SOD), pyruvate carboxylase, alkaline phosphatase, and glutamine synthetase[2]. Mn deficiency in humans appears to be rare, because Mn is present in many common foods. However, wide industrial use of Mn or Mn compounds in production of steel, paints, dry cell batteries, glass ceramics, certain pesticides, fertilizers, in leather and textile industries and as antiknock agent in gasoline as well as mining and smelting operations of Mn ores may cause exposure of workers to excessive amount of this metal[3,4].

Excessive accumulation of Mn in brain, liver, kidney, pancreas and other tissues due to impaired transporter failure of hepatic detoxification mechanism is known to have deleterious effect on cell function and integrity[5]. It has been reported that manganese can accumulate in mitochondrial matrix and disrupt mitochondrial functions[6,7]. Excess Mn^{2+} has been reported to be toxic by enhancing formation of reactive oxygen species and oxidation byproducts of catecholamines[8]. Additionally, Mn^{2+} also induces disruption of the homeostasis of Fe, Ca and trace minerals[9,10].

Experimental studies have shown that high doses of Mn cause serious neurological and cardiovascular toxicities[11,12]. It is also known that chronic exposure of Mn causes alteration in development as well as impairment of reproductive function[13-16].

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Liver and kidney are important organs of metabolism, detoxification, storage and excretion of nutrients, therapeutic drugs, xenobiotics and their metabolites and are especially vulnerable to damage. Some animal studies have shown that chronic parenteral administration of Mn or its compounds results in accumulation of Mn in liver, kidney and other tissues[17,18]. However, there is limited information available about Mn induced hepatic and renal toxicity[19-21], despite the fact that these are the major organs regulating Mn homeostasis in the body. Looking to these facts the need for better understanding of the adverse effects of Mn on liver and kidney morphology and functions is becoming more important due to increased release of manganese into environment. Therefore, the present investigation was undertaken to study the toxic effects of sub chronic exposure of $MnCl_2$ on hepatic and renal histoarchitecture and functions in male Wistar rat.

2. Materials and Methods

Chemicals: Manganese chloride ($MnCl_2.4H_2O$) was purchased from Ranbaxy Fine Chemicals Limited, New Delhi, India. All other chemicals used in the study were of analytical grade. $MnCl_2$ solution was prepared by dissolving it in sterile normal saline.

Animals: Colony bred adult male Wistar rats weighing 160– 180 g were used in the present study. The animals were housed in polypropylene cages under well regulated light/ dark (12h:12h) cycle at standard temperature (22±1˚C) and provided rat chow (Aashirwad Food Industries, Chandigarh) and tap water *ad libitum*. The animals were maintained as per guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) regulations. The study was approved by Institutional Ethical Committee, Department of Zoology, University of Rajasthan, Jaipur, India.

Experimental Design: Twenty four male rats were randomly divided into three groups of 8 rats each. Group A (vehicle control) received normal saline (0.5 ml/rat/day, p.o.) for 120 days. Group B and C received $MnCl_2$ dissolved in normal saline (0.5 ml/rat, p.o.) at the dose level of 50 and 100 mg/kg b.wt./day, respectively for 120 days. At the end of the experiment (120 days), all the overnight fasted animals were sacrificed under ether anesthesia. Blood was taken up by cardiac puncture. Liver, kidneys and other vital organs were quickly removed, washed thoroughly in ice-cold physiological saline [0.9% (w/v) NaCl], blotted and weighed. Blood was centrifuged after clotting, and the serum was separated and stored at -20˚C for biochemical analysis. A part of the liver and kidney tissues were fixed in Bouin's fixative for histopathological study and the remaining parts were frozen at -70˚C for tissue biochemistry.

Body and Organ Weights: Initial and final body weight of each animal was recorded and relative weights (/100g b.wt.) of liver and kidneys of each animal were calculated.

Biochemical Parameters: Serum samples were analyzed for total cholesterol, sugar, alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, urea and creatinine by using standard kits following the manufacturer's instructions (Accurex Biomedical Pvt. Ltd., india).

Tissue Biochemical Parameters: Estimation of total cholesterol[21], triglycerides[22] and glycogen[23] contents were made in frozen liver tissue samples.

Lipid Peroxidation and Antioxidant Defense Parameters: Lipid peroxidation was measured as thiobarbituric acid reactive substances (TBARS) in the liver and kidney tissues according to the method of Ohkawa, Ohishi, and Yagi[25]. Estimation of Superoxide dismutase (SOD) activity[26], glutathione (GSH)[27] and ascorbic acid[28] levels were also made in liver and kidney tissue samples. The protein content was spectrophotometrically estimated in both tissues using Folin reagent and bovine serum albumin as the standard[29].

Histopathology: Both liver and kidney tissue samples were dehydrated by a graded ethanol series and embedded in paraffin. The paraffin blocks were cut into 6 µm thick sections and stained with hematoxylin and eosin. Sections were examined using a Nikon light microscope (Nikon Eclipse E400) at X400. Histopathological alterations from the normal structure were recorded. At least four fields from each section of the slides of both tissues were

assessed for the appearance of the degenerative effects and this was compared with control sections.

Statistical Analysis: All the values were presented as mean± SEM for all experiments. The statistical analysis was performed by one way ANOVA using IBM SPSS statistics 20 software. Post hoc tests were performed using Tuckey HSD test for the comparison between the treatment groups and control group. Differences between two means were considered significant if P≤0.05.

3. Results

3.1 Body Weight and Relative Weights of Liver and Kidney

Changes in body weight and relative weights of liver and kidney are shown in Table 1. During the study no mortality was observed in any group. During the experiment an increase in the body weight of all the rats was recorded. However, the body weight gain observed in both $MnCl_2$ treated groups was significantly less when compared to the control group. Relative weight of liver exhibited a significant increase in both treated groups [B (p≤0.05) and C (p≤0.01)] as compared to control, whereas no significant change was observed in the relative weight of kidney in both $MnCl_2$ exposed groups.

3.2 Serum Biochemistry

Levels of serum marker parameters of hepatic and renal function tests are summarized in Table 2. Compared to control, the serum levels of AST, ALT, ALP, total bilirubin and cholesterol were significantly elevated in both $MnCl_2$ treated groups while sugar level was increased significantly only in group C (p≤0.05). Serum urea and creatinine levels showed significant increase in both $MnCl_2$ treated groups (group B (p≤0.05) and group C (p≤0.01)) when compared to control group.

3.3 Tissue Biochemistry

Tissue biochemical results are shown in Table 3. As compared to control group, total cholesterol and triglycerides concentrations in liver showed significant elevation in both $MnCl_2$ treated groups [group B (P≤0.05) and group C (P≤0.01)]. Whereas, the glycogen content in liver showed significant depletion in both groups [B (p≤0.05) and C (p≤0.01)] when compared to control.

Table 1. Effect of $MnCl_2$ exposure on body weight and relative liver and kidney weight of rats

Treatments	Body weight (g)		Liver weight	Kidney weight
	initial	final	(mg/100 g b. wt.)	(mg/100 g b. wt.)
Group A (control)	170.25± 2.68	259.62± 3.60 (52.56%)	2933.30± 42.34	610.96± 12.02
Group B ($MnCl_2$ 50 mg)	171.50± 2.34	231.75 ± 6.82[a] (35.71%)	3515.77 ± 150.00[a]	652.38 ± 18.47[ns]
Group C ($MnCl_2$ 100 mg)	170.87± 1.44	225.62 ± 8.25[b] (32.52%)	3427.04 ± 107.16[b]	663.43± 25.20[ns]

Values are mean ± SEM of eight rats. Levels of significance: a p≤0.05; b p≤0.01, MnCl2 treated rats compared with Group A control rats

Table 2. Effect of $MnCl_2$ exposure on various serum biochemical parameters in rats

Treatments	Cholesterol (mg/dL)	Sugar (mg/dL)	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	Bilirubin (mg/dL)	Urea (mg/dL)	Creatinine (mg/dL)
Group A (control)	78.35± 1.64	92.62± 3.28	34.90± 1.10	62.24± 1.72	71.5± 2.56	.41± .02	28.94± 1.12	.60± .03
Group B ($MnCl_2$ 50 mg)	87.16± 2.20[a]	99.25± 4.69[ns]	39.38± 1.23[a]	71.05± .83[a]	83.4± 3.54[a]	.53± .04[a]	36.36± 2.22[a]	.69± .03[a]
Group C ($MnCl_2$ 100 mg)	89.13± 2.76[b]	106.37± 3.31[a]	40.79± 1.38[b]	73.89± 2.50[b]	86.6± 3.56[b]	.56± .02[b]	38.45± 2.50[b]	.72± .02[b]

Values are mean ± SEM of eight rats. Levels of significance: ns non significant; a p≤0.05; b p≤0.01, MnCl2 treated rats compared with Group A control rats

Table 3. Effect of $MnCl_2$ exposure on liver tissue biochemical parameters in rats

Treatments	Cholesterol (mg/g)	Triglycerides (mg/g)	Glycogen (mg/g)
Group A (control)	8.24±.16	8.02± .11	5.49± .10
Group B ($MnCl_2$ 50 mg)	8.97± .22[a]	8.44±.11[a]	5.00± .15[a]
Group C ($MnCl_2$ 100 mg)	9.23± .23[b]	8.58 ± .13[b]	4.84± .16[b]

Values are mean ± SEM of eight rats. Levels of significance: a p≤0.05; b p≤0.01, MnCl2 treated rats compared with Group A control rats

3.4 Lipid Peroxidation and Antioxidant Marker Parameters

The effects of manganese chloride exposure on thiobarbituric acid reactive substances –TBARS (byproduct of lipid peroxidation) and selected antioxidant defense marker parameters in liver and kidney are presented in Fig 1 (a,b,c,d). In $MnCl_2$ treated rats, TBARS levels showed dose dependent significant increase in both, liver and kidney in group B ($p \leq 0.05$ in both liver and kidney) and group C ($p \leq 0.001$ in liver; $p \leq 0.01$ in kidney), while the levels of reduced glutathione (GSH), ascorbic acid and the activity of total superoxide dismutase (SOD) were significantly declined in both $MnCl_2$ treated groups as compared to control group.

3.5 Histopathology

Light microscopic observation of the liver tissue of control rats showed a normal histoarchitecture as indicated by normal hepatocytes radiating from the central vein with prominent nuclei and characteristic cord like arrangement separated by sinusoids (Fig 2). Exposure of rats with $MnCl_2$ at 50 mg/kg b. wt. dose showed more or less normal lobular pattern with mild degree of degenerative changes in hepatocytes and infiltration of mononuclear cells (Fig 3). Rats exposed to high dose of $MnCl_2$ (100 mg/kg b.wt.) showed marked degenerative and atrophic changes in liver such as vacuolization, necrosis in the parenchymal cells around the central vein, dilation of sinusoids and infiltration of mononuclear cells (Fig 4).

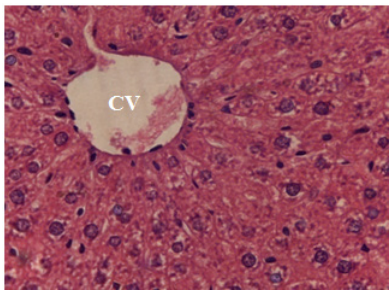


Figure 2. Photomicrograph of the liver of control rat showing normal hepatocytes radiating from the central vein(CV). H&E, X400.

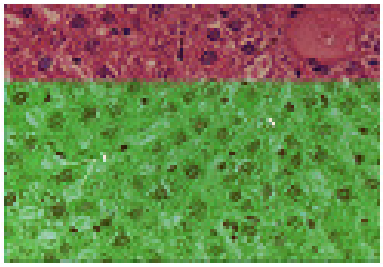


Figure 3. Photomicrograph of the liver of $MnCl_2$ (50 mg/kg b.wt.) treated rats showing mild degenerative and necrotic changes(N) and vacuolization (V). H&E, X400.

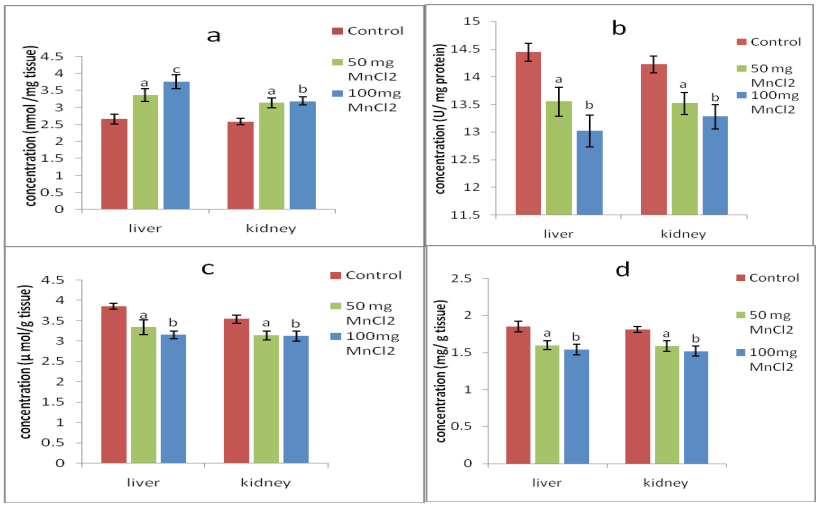


Figure 1. Effect of $MnCl_2$ exposure on oxidative status indicators in liver and kidney. (a) lipid peroxidation (b) superoxide dismutase(SOD) activity, (c) glutathione (GSH) level and (d) ascorbic acid level. Values are expressed in mean ± SD of n=8. $^{a}p \leq 0.05$; $^{b}p \leq 0.01$; $^{c}p \leq 0.001$, compared with control group.

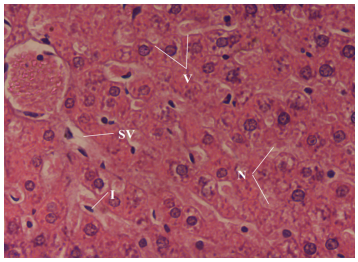


Figure 4. Photomicrograph of liver of $MnCl_2$ (100mg/ kg b.wt.) treated rats showing degenerative and necrotic changes(N), sinusoids widening (SW), vacuolization (V) and infiltration of mononuclear cells(I). H&E, X400.

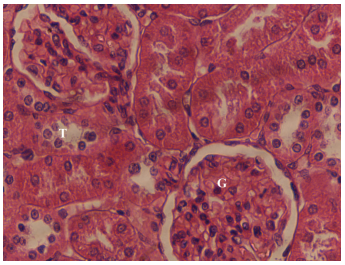


Figure 5. Photomicrograph of the kidney of control rat showing normal glomerular (G) and tubular (T) morphology. H&E, X 400.

The histopathological picture of the kidney of control rats exhibited normal structure of the cortex and medulla (Fig 5). Slight tubular necrosis and glomerular dilation were observed in the focal areas of renal tissue of 50 mg/ kg b.wt dose group animals (Fig 6). Hypertrophy and degeneration of epithelial cells of proximal renal tubules with infiltration of mononuclear cells and dilation of glomeruli were evident in most of the areas of kidney in animals treated with higher dose of $Mncl_2$ (100 mg/kg b.wt.) (Fig 7).

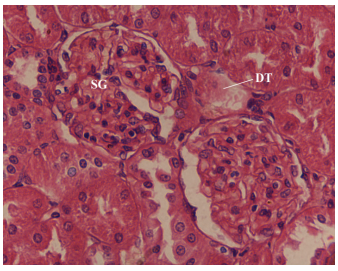


Figure 6. Photomicrograph of kidney of $MnCl_2$ (50 mg/kg b.wt.) treated rats showing tubular degeneration (DT) and swollen glomeruli (SG). H&G, X400.

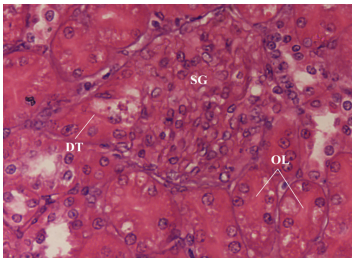


Figure 7. Photomicrograph of kidney of $MnCl_2$ (100 mg/ kg b.wt.) treated rats showing swollen glomeruli (SG) loss of glomerulus space (curved line), severe tubular degeneration (DT) and obstructed lumen (OL). H&E, X400.

4. Discussion

Manganese (Mn) is an essential trace element which plays important role in antioxidant defense and acts as cofactor in many enzymes. Due to heavy industrial use, mining and smelting operations a large population is chronically exposed to this metal. Exposure to high concentration of manganese may be detrimental to human health[3]. However, the effect of long term exposure to Mn or Mn compounds on liver and kidney are poorly understood. To the best of our knowledge, no study has been carried out on the effect of subchronic exposure of Mn on lipid peroxidation and antioxidant defense markers. The present study was undertaken to study the toxic effects of an oral intake of $MnCl_2.4H_2O$ (50 mg/ kg and 100 mg/ kg b.wt.) on the liver and kidney structure and functions in male Wistar rats.

Body and organ weight are important indicators of the toxic effect of any exogenous compound on the body. In the present study, $MnCl_2$ exposed rats registered a less gain in body weight as compared with the control. This decrease in gain of body weight might be associated with lack of food consumption occurring due to loss of appetite or gastrointestinal disturbances[30,19]. Results of our study showed significant increase of relative liver weight in $MnCl_2$ treated rats. This increase in liver weight might be due to increased accumulation of fat in the hepatocytes and/or increased blood contents as a result of the sinusoid dilation[31]. Similar increase in liver weight has also been observed by Huang et al.[20] in rats after exposure with $MnCl_2$. In contrast, to these results, Zheng, Zhou, and Fu[7] did not observe any effect on the relative weight of liver in male rats after i.p. injection of $MnCl_2$ for 6 weeks. This

discrepancy in results might be due to short duration of the study. The results of the present study did not show any alteration in relative weight of kidney in $MnCl_2$ exposed rats.

Liver plays significant role in carbohydrate, lipid and protein metabolism. Significant elevation in serum and hepatic cholesterol and hepatic triglycerides levels in $MnCl_2$ treated rats observed in the present study could be due to disturbances of lipid metabolism resulting in accumulation of these lipids in liver[32]. Glycogen is chief storage form of carbohydrate in the body. A significant decline in hepatic glycogen level and elevation of blood glucose level in $MnCl_2$ administered rats observed in the current study suggest adverse impact of $MnCl_2$ on carbohydrate metabolism[33]. It has been reported that Mn also accumulates in pancreas and is associated with a decrease in the serum insulin and glycogen levels, which in turn disturbed carbohydrate homeostasis and cause hyperglycemia in rats[34]. Additionally, the effect of Mn on blood glucose may be attributed to the inhibition of many glycolytic and Krebs's cycle enzymes[35].

Serum enzymes, such as AST, ALT and ALP are sensitive biomarkers of liver function and are originally present in higher concentration in hepatocyte cytoplasm[36]. When there is hepatocellular damage, these enzymes leak into blood circulation[37]. Results of the serum biochemical study showed that $MnCl_2$ exposure in rats resulted in significant increase in AST, ALT and ALP activities than those of control group, suggesting hepatocellular damage and malfunction[38]. These results are in agreement with earlier studies where similar increase in serum AST, ALT[32,19] and ALP activities[19,20] have been reported in $MnCl_2$ exposed animals. In the present study, a marked increase in serum total bilirubin level was observed in Mn treated rats. This increase in serum bilirubin level might be due to hepatotoxicity, structural and functional changes in bile canaliculi leading to cholestasis[39].

Serum urea and creatinine levels are markers of kidney functions. Blood urea is derived from normal metabolism of proteins and is excreted in the urine. Elevated level of urea usually indicates glomerular damage[40]. Meanwhile creatinine which is a waste product from muscle creatine is commonly measured as an index of glomerular function[41]. Increased levels of serum urea and creatinine observed in the present study in $MnCl_2$ exposed rats indicate kidney damage. Histopathological study of the kidney also supports these findings.

It is well known that Mn has a variety of oxidative states[42]. Mn is generally believed to exert cellular toxicity via a number of mechanisms, including the induction of free radical production, direct or indirect formation of reactive oxygen species (ROS)[43]. These free radicals can cause lipid peroxidation[20]. Lipid peroxidation is one of the main manifestations of oxidative damage which plays an important role in the toxicity of many xenobiotics[44]. It alters the chemical characteristics and physical organization of cellular membranes, leading to functional loss[45]. Our data confirmed that chronic intoxication with Mn causes a significant increase in the generation of lipid peroxides (TBARS) in both liver and kidneys of rats. This increase in the levels of TBARS indicates that production of free radicals exceed the capacity of detoxification mechanisms. These results are in agreement with previous studies, which have also reported a significant increase in lipid peroxidation in liver of $MnCl_2$ exposed rats[20,46].

Superoxide dismutase (SOD) is believed to play a major role in the first line of antioxidant defense by catalyzing the dismutation of superoxide anion radicals, into hydrogen peroxide and molecular oxygen. In present study, a significant decrease in the activity of SOD in both liver and kidney tissues of $MnCl_2$ treated rats was observed. Huang et al.[47] also reported a significant decline in SOD activity after long term administration of $MnCl_2$ in rats. The observed reduction in SOD activity might be due to excess utilization of SOD in catalyzing dismutation of superoxide anion radicals. Reduced glutathione (GSH), is a crucial component of the antioxidant defense mechanism, playing principle role in balancing the intracellular redox status by not only acting as an antioxidant but also participating in many metabolic processes[48]. GSH homeostasis in healthy liver tissue is maintained by the balance between biosynthesis, uptake and export[49]. Our study showed a significant depletion of GSH content in both liver and kidney of $MnCl_2$ challenged rats. These results are consistent with earlier reports where similar depletion in GSH content have been reported in liver of $MnCl_2$ exposed rats[46,47]. Ascorbic acid is an excellent hydrophilic antioxidant; it readily scavenges ROS and peroxyl radicals[50]. Decreased level of GSH and ascorbic acid observed in the present study might be correlated with the increased utilization of these antioxidants in deactivation of the increased level of reactive oxygen species.

Histomorphological observations showed that oral administration of $MnCl_2$ to rats caused severe hepatotoxicity, characterized by gross hepatocellular

necrosis, nuclear picnosis, Karyolysis, vacuolization, mononuclear cell infiltration and dilation of sinusoids. Similar histopathological alterations in liver of rats after challenging with $MnCl_2$ have been reported by some workers[20,46]. Sections of kidney tissue from $MnCl_2$ exposed rats revealed signs of tubular necrosis, interstitial fibrosis and dilation of large number of glomeruli. Similar to our findings, marked vacuolization of the cortical convoluted tubules and coagulative tubular necrosis were detected in rats administered to 100 ppm $MnCl_2$ for 3 and 6 months respectively[21]. Glomerular epithelial hypertrophy and tubular necrosis have also been reported in rats intoxicated with other heavy metals[51,52,53]. The observed hepatic and renal toxicity might be due to accumulation of manganese in these tissues[17,18]. Accumulation of Mn in liver can result in disruption of cellular energy metabolism and antioxidant defense system potentially[7] leading to cellular necrosis and/or apoptosis[52]. The observed histopathological changes in liver and kidney of Mn exposed rats are supported by results of the biochemical parameters.

On the basis of the results of the present study it can be concluded that the sub-chronic exposure with $MnCl_2$ causes oxidative stress leading to hepatic and renal damage in rats, thus could pose a serious risk for health. Therefore, further studies are suggested to better understand the mechanism of Mn induced hepatic and renal toxicity.

5. Acknowledgements

The authors are highly thankful to the Head of Department for providing necessary facilities. The first author also acknowledges University Grants Commission, New Delhi for awarding BSR Fellowship.

6. Conflict of Interest

None Declared.

7. References

1. Keen CL, Ensunsa JL, Watson MH, Baly DM, Donovan SM, Monaco MH et al. Nutritional aspects of manganese from experimental studies. Neurotoxicol 1999; 20: 213-23.
2. Saric M. Manganese. In: Friberg L, Nordberg GF and Vouk V, editors. Handbook on the toxicology of metals. 2nd edn. Amsterdam: Elsevier Science Publishers; 1986. pp 354-86
3. Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological Profile for Manganese. Atlanta: US Department of Health and Human Services, Public Health Service; 2000.
4. Sanatamaria AB. Manganese exposure, essentiality and toxicity. Indian J Med Res 2008; 128: 484-500.
5. Butterworth RF. Metal toxicity liver disease and neurodegeneration. Neurotox Res 2010; 18: 100-5.
6. Liccione JJ and Maines MD. Selective vulnerability of glutathione metabolism and cellular defense mechanisms in rat striatum to manganese. J Pharmacol Exp Ther 1988; 247: 156-61.
7. Zhang S, Zhou Z and Fu J. Effect of manganese chloride exposure on liver and brain mitochondria function in rats. Environ Res 2003; 93:149-57.
8. Parenti M, Rusconi K, Cappabianca W, Parati EA and Groppetti A. Role of dopamine in manganese toxicity. Brain Res 1988; 473: 236-40.
9. Zheng W, Zhao Q, Slavkovich V, Aschner M and Graziano JH. Alteration of iron homeostasis following chronic exposure to manganese in rats. Brain Res 1999; 833: 125-32.
10. Chen MT, Cheng GW, Lin CC, Chen BH and Huang YL. Effects of acute manganese chloride exposure on lipid peroxidation and alteration of trace metals in rat brain. Biol Trace Elem Res 2006; 110: 163-78.
11. Crossgrove J and W Zheng. Manganese toxicity upon overexposure. NMR in Biomed 2004; 17: 544-53.
12. Jiang Y and Zheng W. Cardiovascular toxicities upon manganese exposure. Cardiovasc Toxicol 2005; 5: 345-54.
13. Laskey JW, Rehnberg JF and Hein JF. Effects of chronic manganese exposure on selected reproductive parameters. J Toxicol Environ Health1982; 9: 677-87.
14. Sanchez DJ, Domingo JL, Llobet JM and Keen CL. Maternal and developmental toxicity of manganese in the mouse. Toxicol Lett 1993; 69: 45-52.
15. Wirth JJ, Rossano MG, Daly DC, Puscheck E, Potter RC and Diamond MP. Manganese exposure is negatively associated with human sperm motility and concentration. Epidemiology 2007; 18: 270-73.
16. Chandel M and Jain GC. Toxic effects of transition metals on male reproductive system: a review. J Environ Occup Sci 2014; 3: 204-13.
17. Shukla GS and Chandra SV. Concurrent exposure to lead, manganese, and cadmium and their distribution to various brain regions, liver, kidney, and testis of growing rats. Arch Environ Con Tox 1987; 16: 303-10.
18. Dorman DC, Struve MF, Marshall MW, Parkinson CU, James RA and Wong BA. Tissue manganese concentrations in young male rhesus monkeys following subchronic manganese sulfate inhalation. Toxicol Sci 2006; 92: 201-10.
19. Khan KN, Andress JM and Smith PF. Toxicity of subacute intravenous manganese chloride administration in beagle dogs. Toxicol Pathol 1997; 25: 344-50.

20. Huang P, Chen C, Wang H, Li G, Jing H, Han Y et al. Manganese effects in the liver following subacute or subchronic manganese chloride exposure in rats. Ecotoxicol Environ Saf 2011; 74: 615-622.
21. Nuha and AL-Harbi Y. Histological alteration induced by manganese chloride in the male testes, epididymis and kidney of the albino rats. IOSR-JPBS 2014; 3: 12-9.
22. Zlatkis A, Zak B and Boyal AJ. A new method for direct determination of cholesterol. J Clin Med 1953; 41: 486-9.
23. Gottfried SP and Rosenberg B. Improved manual spectrophotometric procedure for determination of serum triglycerides. Clin Chem 1973; 19: 1077-8.
24. Montogomery R. Determination of glycogen. Arch Biochem Biophys 1957; 67: 378-89.
25. Ohkawa H, Ohishi N and Yagi K. Assay for lipid peroxide formation in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979; 95: 351-8.
26. Marklund S and Marklund G. Involvement of superoxide radical in the auto oxidation pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem 1974; 7: 469-74.
27. Moron MJ, Depieare JW and Mannivik B. Leaves of glutathione reductase and glutathione transferase activities in rat lung and liver. Biochem Biophys Act 1979; 582: 67-8.
28. Roe JH and Kuether CA. Determination of ascorbic acid in whole blood and urine through the 2,4-DNPH derivative of dehydroascorbic acid. J Biol Chem 1943; 147: 399-407.
29. Lowry OH, Oser MJ and Randoll RJ. Protein measurement with the folin phenol reagents. J Biol Chem 1951; 193: 265-75.
30. Misselwitz B, Mhhler A and Weinmann H. The toxicologic risk for using manganese complexes? A literature survey of existing data through several medical specialities. Invest Radiol 1995; 30: 611-620.
31. Pandey G, Jain GC and Mathur N. The effect of sub chronic exposure to ammonium molybdate on hematological and hepatic parameters in albino rats. AJPBR 2012; 2: 136-42.
32. Klimis-Tavantzis DJ, Taylor PN and Wolinsky I. Manganese, Lipid Metabolism and Atherosclerosis. In: Klimis-Tavantzis DJ, editor. Manganese in Health and Disease. CRC press inc; 1994. pp: 87-100.
33. Nath K and Kumar N. Toxicity of manganese and its impact on some aspects of carbohydrate metabolism of a freshwater teleost, *Colisa fasciatus*. Sci Total Environ 1987; 76: 257-62.
34. Baly DL, Lo¨nnerdal B and Keen CL. Effects of high doses of manganese on carbohydrate homeostasis. Toxicol Lett 1985; 25: 95-102.
35. Malthankar GV, White BK, Bhushan A, Daniels CK, Rodnick KJ and Lai JC. Differential lowering by manganese treatment of activities of glycolytic and tricarboxylic acid (TCA) cycle enzymes investigated in neuroblastoma and astrocytoma cells is associated with manganese-induced cell death. Neurochem Res 2004; 29: 709-17.
36. Sherlock S and Dolley JS. Assessment of liver function. In: Dooley JS, Lok ASF, Burroughs AK and Heathcote EJ, editor. Sherlock's diseases of the liver and biliary syste. 12th edition. UK: Wiley Blackwell Publication; 2011. pp 20-35.
37. Sallie R, Tredger JM and Williams R. Drugs and the liver. Part 1: Liver testing function. Biopharm Drug Dipos 1991; 12: 251-9.
38. Salehi F, Krewski D, Mergler D, Normandin L, Kennedy G, Philippe, S et al. Bioaccumulation and locomotor effects of manganese phosphate/sulfate mixture in sprague-dawley rats following subchronic (90 days) inhalation exposure. Toxicol Appl Pharmacol 2003; 191: 264–71.
39. Ayotte P and Plaa GL. Biliary excretion in Sprague-Dawley and Gunn rats during manganese-bilirubin-induced cholestasis. Hepatology 1988; 8: 1069-78.
40. El-Shenawy SMA and Hassan NS. Comparative evaluation of the protective effect of selenium and garlic against liver and kidney damage induced by mercury chloride in the rats. Pharmacol Rep 2008; 60: 190-208.
41. Rosner MH and Bolton WK. Renal function testing. Am J Kidney Dis 2006; 47: 174-83.
42. Syed EA, Helen MD, Glenn DN, George WL and William JS. Manganese induced reactive oxygen species: comparison between Mn^{+2} and Mn^{+3}. Neurodegeneration 1995; 4: 329-34.
43. Milatovic D, Gupta RC, Yu Y, Zaja-Milatovic S, and Aschner M. Protective effects of antioxidants and anti-inflammatory agents against manganese-induced oxidative damage and neuronal injury. Toxicol Appl Pharmacol 2011; 256: 219-26.
44. Stohs SJ and Bagchi D. Oxidative mechanism in the toxicity of metal ions. Free Radic Biol Med 1995; 18: 321-36.
45. Poli G, Schaur RJ, Siems WG and Leonarduzzi G. 4-Hydroxynonenal: A membrane lipid oxidation product of medicinal interest. Med Res Rev 2008; 28: 569-631.
46. Lebda MA, El-Neweshy MS and El-Sayed YS. Neurohepatic toxicity of subacute manganese chloride exposure and potential chemoprotective effects of lycopene. NeuroToxicol 2012; 33: 98-110.
47. Huang P, Li G, Chen C, Wang H, Han Y, Zhang S, et al. Differential toxicity of Mn^{2+} and Mn^{3+} to rat liver tissues: oxidative damage membrane fluidity and histopathological changes. Exp Toxicol Pathol 2012; 64: 197-203.
48. Lushchak VI. Glutathione homeostasis and functions: potential targets for medical interventions J Amino Acids 2012; Article ID 736837.
49. Wu G, Fang YZ, Yang S, Lupton JR and Turner ND. Glutathione metabolism and its implications for health. J Nutr 2004; 134:489-92.
50. Kumar A, Rahal A, Zoheb SM, Prakash A and Mandil R. Antioxidant role of ascorbic acid on oxidative stress induced by sub-acute exposure of lead and cypermethrin in erythrocytes of wistar rats. Toxicol Environ Chem 2015; 96: 1248-59.
51. Brzoska MM, Moniuszko-Jakoniuk J, Pilat-Marcinkiewicz B and Sawicki B. Liver and kidney function and histology in rats exposed to cadmium and ethanol. Alcohol Alcohol 2003; 38: 2-10.
52. Missoun F, Slimani M and Aoues A. Toxic effect of lead on

kidney function in rat Wistar. Afr J Biochem Res 2010; 4: 21-7.
53. Agarwal R, Goel SK and Behari JR. Detoxification and antioxidant effects of curcumin in rats experimentally exposed to mercury. J Appl Toxicol 2010; 30: 457-68.
54. Orrenius S, Gogvadze V and Zhivotovsky B. Mitochondrial oxidative stress: implications for cell death. Annu Rev Pharmacol Toxicol 2007; 47: 143-183.