International Journal of Natural Sciences Research
2014 Vol. 2, No. 2, pp. 12-19
ISSN(e): 2311-4746
ISSN(p): 2311-7435

THE IMPACT OF CHRONIC EXPOSURE TO MANGANESE ON TESTICULAIRE TISSUE AND SPERM PARAMETERS IN RAT WISTAR

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ABSTRACT

Aim: To assess the effect of chronic manganese chloride exposure on testis and sperm parameters. Methods: Twenty four male rats, 6 months old, were divided in 2 groups. 12 rats in the lot exposed to manganese, were watered by water containing manganese chloride tetrahydrate (MnCl2 4H2O) at dose of 4.79 mg.ml^{-1} for 12 weeks. And the group of control male rats received distilled water in the same conditions. The effects on the testicular histology, sperm concentration and percentage of abnormal sperm morphology were observed.

Results: After a chronic exposure, microscopic examination of the testes showed degeneration of the seminiferous tubules and the gremlin cell. Seminal parameters indicated a decrease in the sperm levels (21.3 10^{6} ±0.96 10^{6} cells / ml) compared to the control group (62.70 10^{6} ± 3.22 10^{6} cells / ml) and a rise of morphological abnormalities (66.1 % ±2.93 %) compared to the control rats (10.83 % ±1.30 %).

Conclusion: The present study demonstrated that long-term manganese chloride exposure may have a direct deleterious effect on fertility and reproduction functions of male rats.

Keywords: Manganese, Testis, Male rats, Sperm concentration, Sperm morphology, Testicular histology, Fertility, Reproduction.

1. INTRODUCTION

Manganese (Mn) is a naturally occurring element present not only in the earth's crust, but also in many foods. Mn is anessential trace element, and under most conditions, humans consume sufficient amounts of Mn in their diet [1, 2]. In small quantities, it is an important element for humans, playing many roles for normal mammalian physiology [3]. It is a trace metal required for normal lipid, protein, and carbohydrate metabolism, playing a key role in numerous enzyme families including oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases [4]. However industrial use of Mn and Mn containing compounds (for example, in the production of paint pigments, dry cell batteries, glass and ceramics as well as mining of Mn ores and welding of

mild steel) may expose workers to excessive amounts of this chemical [5]. High dose of Mn seems to cause serious neurotoxicity, immunotoxicity and developmental toxicity, particularly in male [6]. It is also known that Chronic exposure to this metal can cause alterations in development as well as reproductive dysfunction [7]. In human occupational exposure to Mn^{+2} decreased libido and impotency, and may result in lowered sperm count and semen quality [8, 9]. Ahmed, et al. [5] , postulated that ingestion of high doses of $MnCl_2$ by male and female mice had adverse effects on fertility and reproduction. MnSO4 adversely affected semen quality index and sperm viability in broiler breeder semen in vitro [10]. It was reported that chronic administration of $Mncl_2$ at low doses to female rats resulted in increased serum levels of puberty-related hormones such as luteinizing hormone (LH), follicle-stimulating hormone (FSH), and estradiol, and advanced the time of vaginal opening [11].On the basis of these data we sum proposed to study the impact of manganese on male reproductive system by analyzing seminal parameter and testicular histology.

2. MATERIALS AND METHODS

2.1. Animals and Dosing Procedure

Experiments were carried out on 24 « Wistar » forty adult male, aged of 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% protéins). The rats were distributed into two groups of twelve rats. The first group is the control group (T) receiving distilled water and the second group (M) is the lot of rats exposed to manganese, receiving oral manganese chloride tetrahydrate (MnCl2 4H2O) at the dose of 4.79 mg Mn.l[1] [12].

2.2. Fertility Test

Just before the end of the experiment the animal of different groups were housed with female rats of the same age in order to test their fertility. After a period of seven days of cohabitation, the females have been separated from the males. The number of females gravid and the birth rate is unregistering.

2.3. Histological Study

At the end of the experiment, the animals were sacrificed. The testicles are carefully removed, rinsed with cold saline, dried and weighed. Histological study was performed according to standard techniques, after fixation in fixative (formalin 1/10), paraffin embedding and staining with hematoxylin-eosin.

2.4. Spermatozoa Count

Testis and epididymis each group of rats cut with scissors and homogenized 10 to 20 ml of 0.9% NaCl containing 0.05% Triton X-100. The homogenates are placed in the refrigerator at 4 ° C for one hour. 400 ml of homogenates for each rat are diluted in 0.9% NaCl containing 0.05%

Triton X-100 and 500 ml of Trypan blue to 4%. The resulting mixture, 10µl of sample is placed on a Thoma cell [13].

2.5. Sperm Morphology

A drop of spermatozoal suspension was mounted between the slide and the cover slide. Each sample was examined at 40X magnification. At least 200 spermatozoa were observed for the calculation of percentage of the total numbers of spermatozoa. The percentage of abnormal sperm morphology was calculated from the following formula:

%= (Abnormal sperm / total sperm count) ×100

Abnormal sperm morphology the sperm cells were categorized based on the presence of one or more abnormal features such as tail defects (short, irregular, coiled or multiple tails); neck and middle piece defects (distended, irregular, bent middle piece, abnormally thin middle piece); and head defects (round head, small or large size, double or detached head) [14].

2.6. Statistical Analysis

The mean ± S.D. Values were calculated for each group to determine the significance of intergroup difference. Each parameter was analyzed separately using two-way ANOVA analysis of variance. To find the difference between the groups Student't' test was used. P values <0.05 were considered to be significant.

3. RESULTS

3.1. Effect of Manganese on the Fertility of Male Rats

The results in Table 1 indicate the existence of a dose effect relationship between dose and fertility in rats. We noted that females coupled with intoxicated males have a reduced pregnancy rate (33.3 %), compared to females mated with control rats (75%).

Table-1. Effect of manganese on the fertility of male rats.

Groups	Number of pregnant females, (%)	Number of newborns
Group (T)	9/12, (75%)	10±2
Group (M)	4/12, (33.3%)	4±1

3.2. Body Weight and Testis Weights

The results in Table 2 show a significant diminition body weight and Testis weights of manganese-exposed groups (M) compared to the control group of rats (T).

Table-2. Body weight and organ weights of the different experimental groups.

	Initial body Weight (g)	Final body weight (g)	Body weight difference (g)	Testis weight (g)
Group T	281.33±3.01	376.67±3.75	95.33±4.05	3.32± 0.15
Group M	280.29±6.35	327.50±6.95	47.17±9.33 ***	2.66±0.20**

Results are expressed as mean ± S.D

*P<0.05, significantly different compared to control value (Student's t-test).

**P<0.01, significantly different compared to control value (Student's t-test).

*** P <0.001 significantly different compared to control value (Student's t-test).

3.3. Sperm Analysis

The results in Table 3 show a significant decrease in the number of sperm of manganese-exposed groups compared to the control group. The percentage of abnormal spermatozoa was markedly increased in the animals exposed to daily concetration of manganese chloride (66.17±2.93) compared with the control animals (10.83±1.30) given distilled water.

Table-3. Semen Parameters of the different experimental groups.

	groups	
Parameters	**MN**	**T**
Spem concentration (10^6)/ml	21.3±0.96 ***	62.70±3.22
Mophlogy (abnormal %)	66.17±2.93***	10.83±1.30

Results are expressed as mean ± S.D

*P<0.05, significantly different compared to control value (Student's t-test).

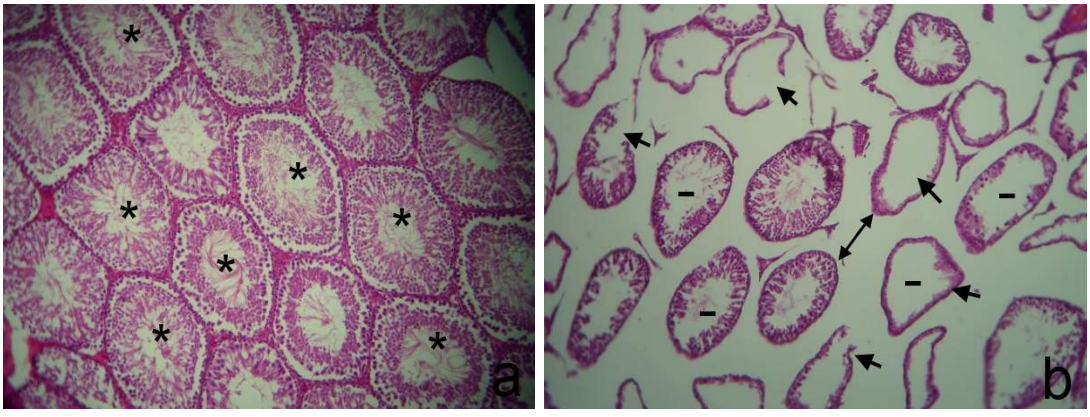
**P<0.01, significantly different compared to control value (Student's t-test).

*** P <0.001 significantly different compared to control value (Student's t-test).

3.4. Histological Study

Histological study in the testicles reveals normal architecture in the control animals (T). Figure 1-a showed that seminiferous tubules were richly populated and gave healthy appearance. All the cells of the spermatogenic series such as spermatogonia, spermatocyte, spermatids and spermatozoa, even sertoli cells could be identified in the tubules. Lumen could easily be delineated in almost all the tubules and majority of them were occupied by mature spermatozoïdes. While the analysis of histological sections of the testes of manganese poisoning group (M) Figure 1-b shows that these stages are affected. Among the disturbances reported: degeneration of the seminiferous tubules, a total absence of sperm and / or a low sperm count, with large interstitial spaces and lack of cells Lydig around basement membranes.

Fig-1. Are Haematoxylin stained sections of rats testis. X40. **a**: Normal architecture and seminiferous tubes filled with sperm(*) in the control group (T) (animals received distilled water). **b**: (M) expose to manganese (4.79 mgMn /ml during 12 weeks) testis showing degeneration of the seminiferous tubules (arrow), a total absence of sperm and / or a low sperm count (-), with large interstitial spaces and lack of cells Lydig (arrow double sense).



4. DISCUSSION

The effect of long-term ingestion of manganese chloride tetrahydrate was investigated on fertility of male. However after a period of 12 weeks, manganese exposure induced a significant decrease in body weight and weight of testis in rats exposed to Mn compared with control rats, which is consistent with the work undertaken by Ajibade, et al. [15], indicating a significant reduction in organ weights of rats exposed to oral concentrations of 5, 15 to 25 mg Mn / kg. And a dose-dependent decrease in body weight gain was found in rats exposed to 10 and 20 mg Mn / kg for 30 days [16].

Further, crosses between males exposed to manganese chloride and control male rats with healthy females, highlight the impact of manganese poisoning on the fertility of male rats. The result of the coupling showed a decrease of implantation sites per litter and pregnancy rates and the number of births. These results are consistent with those made by Ahmed, et al. [5], which show that the manganese induced in mice decreased fertility in males and decreased number of implantations in the uterus. Domenec, et al. [17], indicate that exposure of mice to manganese concentration of 8 and 16 mg / kg / day. Causes Foetotoxicity, consisting mainly of a reduction in fetal weight and an increased incidence of morphological abnormalities. Otherwise, in our results. Decrease in the number of pregnant females is caused by the inability of a male to fertilized, because of various defects in sperm morphology and / or decrease in the numbers of sperm. Confirmed by Barber, et al. [10], who report that $MnSO_4$ adversely affected semen quality index and sperm viability in broiler breeder semen in vitro. It was also demonstrated after a clinical study of 200 person, the correlation between blood manganese concentration and semen variables, that ambient exposure to manganese levels is negatively associated with reduction in sperm motility and concentration [18].

In addition, we also suggest that the increased of abnormal sperm in intoxicated rats may be related to the effect of manganese on the Sertoli cells, given their important role in the differentiation of sperm and spermatogenesis. The high percentage of anomalies can be explained by the cytotoxicity of manganese during spermiogenesis (transformation of the spermatid into sperm) during which form the head, the middle part and the flagellum. Other work found an increase in the expression of caspase-3 mRNA in germ cells after having exposed to manganese. This has led to an increase in apoptosis in spermatogenic cells. and decreased expression of vimentin Sertoli cell in rats, which might be one of the most important molecular mechanisms of reproductive toxicity of manganese [19].

To confirm this suggestion, histological techniques were used. The results clearly indicate a testicular damage characterized by remarkable disturbances at the seminiferous tubules where the different stages of spermatogenesis were affected. This is confirmed by Ponnapakkam, et al. [20], who shown that subcutaneous administration of Mn chloride for a period of three weeks cause testicular damage in Sprague-Dawley rats. They reported that Mn causes degeneration of the seminiferous epithelium, a decrease of number of spermatids and spermatocytes in the seminiferous tubules. The results of the study showed that the male reproductive system is a target of Mn exposure. However, the achievement of Sertoli cells may also be explained by the disruption of the functioning of Leydig cells, which have a direct role in the regulation of spermatogenesis by producing hormones specifically testosterone. Indeed our observations of histological sections have indicated low activity and a decrease in interstitial cells (composed of cells Lydig). This is consistent with the work of Jana, et al. [21], who suggest that decreased serum testosterone is due to inhibition of testicular steroidogenic enzymes such as delta 5,3 beta-hydroxysteroid dehydrogenase (Δ5, 3β -HSD) and 17 beta-hydroxysteroid dehydrogenase (17β-HSD) responsible for the synthesis of testosterone. In another hand, in vitro studies conducted by Cheng, et al. [22], on the Leydig cells, indicate that exposure to manganese from 2 to 4 hours disrupts steroidogenesis in Leydig cell by decreasing the expression of StAR protein (steroidogenic acute regulatory protein), while exposition 24 to 48 hours cause of adverse effects on both StAR protein and P450 as well as the enzymatic activity of 3b-HSD.

To conclude, the data presented in this work indicate that long-term exposure to manganese chloride by adult male causes some adverse effects on both fertility and reproduction.

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