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Immunohistochemical Malondialdehyde Antibodies Changes of the Adult Mice Testes Affected by Prenatal Manganese Chloride Exposure

Hayder J. Mubarak[1] *PhD*, **Nameer F. Gaeab**[2] *MSc*, **Hussein A. Jarullah**[1] *PhD*

[1]Dept. of Human Anatomy, College of Medicine, Al-Nahrain University, Baghdad, Iraq, [2]Dept. of Human Anatomy, College of Medicine, Diala University, Iraq

Abstract

Background The harmful effect of manganese chloride on postnatal spermatogenesis was evidently concluded in previous experimental researches, however, the molecular changes related to this effect of manganese chloride needs further elaboration.

Objective To investigate the toxic effect of prenatal manganese chloride exposure on adult mice testes using malondialdehyde (MDA) antibodies as an immunohistochemical marker.

Methods In this study, 30 pregnant mice were divided into control and experimental groups. The experimental animals were given 0.1 ml of manganese chloride solution (8000 mg/Liter concentration) orally during the first 17 days of pregnancy. The control group of pregnant mice was given 0.1 ml of distilled water orally rather than the solution of manganese chloride. Paraffin sections of the offspring mice testes were stained for general histological features and for anti-MDA immunohistochemical evaluation. The Aperio Image Scope v.9 software was used to evaluate the immunohistochemical reaction.

Results Sections of testes from mice of the experimental group showed distorted morphology and organization of the stages of sperm development with distorted histological criteria of the interstitial tissue. Results from mice testes revealed statistically significant variability of anti-malondialdehyde (MDA) immunohistochemical expression in the experimental group compared to that of the control group.

Conclusion Manganese chloride induced lipid peroxidation as part of its toxic effect. This lipid peroxidation caused cellular injury leading to apoptosis and autophagy.

Keywords Testes, development, manganese chloride, mice, toxicity, immnuohistochemistry, apoptosis

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List of abbreviation: MDA = Malondialdehyde

Introduction

The reproductive toxicity of manganese had been proved to affect the testes. The exposure to manganese affected the levels of malondialdehyde (MDA) in the testicular tissue and increased the number of apoptotic cells, and caused obvious histopathological changes in the testes [(1)]. Among these heavy metals, the normal testicular functions depend on zinc, manganese, and selenium [(2)].

Manganese is the second of the most common ten metals on the surface of earth; it forms more than hundred compounds [(3)]. Manganese is important in the metabolism of

carbohydrates, lipids, and proteins, and it acts as a co-factor for many enzymes [4].

Manganese chloride ($MnCl_2$) is a solid substance that is soluble in water forming an acidic solution (pH 4) [5]. Manganese is absorbed in the intestine [6], and it is not metabolized inside the boby [7]. Manganese is excreted with feces, bile, milk, sweat, and urine [8].

MDA is a natural material formed in all mammalian cells as a product of lipid peroxidation. MDA is a highly reactive byproduct of polyunsaturated fatty acid peroxidation and arachidonic acid metabolism, it can combine with many functional groups on proteins, lipoproteins, and DNA [9].

The toxicity of manganese was reported to be mainly affected the central nervous system, mainly the hypothalamus [10]. Also, the toxicity of manganese was suggested to affect the gastric mucosa [11], and affected the hemoglobin synthesis [12], and affecting testicular development [13].

The aim of this study is to investigate the effect of exposure to $MnCl_2$ sub-lethal dose during prenatal development of the mice testes using MDA antibody as an immunohistochemical marker.

Methods

In this study 30 healthy pregnant female adult Swiss albino mice (Mus musculus) were used, these mice were approximately 6 weeks of age and weighing 20-25 g. The animals were taken from the animal house of the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies / Al-Nahrain University. These animals were maintained under uniform conditions of natural photoperiod (12 hours light/dark cycle), and temperature (24-32 °C). The animals had free access to standard diet and water.

Detection of the vaginal plug was used as an indication of fertilization and pregnancy. The animals were divided into two groups with 15 mice in each group; the control group and the experimental group. The control group of pregnant female mice was given 0.1 ml of distilled water orally.

The experimental group was given a sub-lethal dose of 8000 mg/L $MgCl_2$ solution [13]. This solution was administered orally to the pregnant mice through oro-gastric intubation (polyethylene catheter fitted to a 1ml hypodermic syringe). The amount of $MgCl_2$ solution given each time was 0.1 ml (0.8 mg) every morning at 24 hours' intervals during the first 17 days of pregnancy.

After birth, the offspring received breast feeding from their mothers for three weeks. Then after, 30 male mice from the offspring were isolated in special cages and grown to reach the 6 weeks of age in the same animal house. The adult male offspring was sacrificed by cervical dislocation and scrotal incisions were done to obtain the testes.

Testicular tissues were processed for paraffin sectioning for histological examination according to the routine methodology [14].

Paraffin sections of the testicular tissue were used for immunohistochemical staining. The MDA antibodies were provided from Abcam (code no. ab6463). They are rabbit polyclonal antibodies containing small molecules of synthetic malondialdehyde conjugated to bovine serum albumin. The immunohistochemistry detection kit is called Expose Mouse and Rabbit Specific HRP/DAB Detection IHC Kit from Abcam (code no. ab80436).

Aperio Image Scope version 9 software was used for the evaluation of MDA antibodies immunohistochemical reaction. This image analysis software involves counting the number of strong positive pixels to evaluate the immunohistochemical stain. The list of positive pixel count algorithm includes parameters obtained from the application of this software to quantify the amount of a specific stain present in a scanned slide image. These parameters when first selected have been pre-configured for brown color quantification. Pixels which are stained, but do not fall into the positive-color specification, are considered

negative stained pixels. Analysis of variance (ANOVA) has been used for statistical evaluation of the mean values of MDA immuno-histochemical reactivity.

Results

All female mice treated with the $MgCl_2$ completed their pregnancy successfully.

Histological changes of the testes in the control group:

The examination of the mice testicular sections from the control group showed normal histology with normal arrangement of spermatogenic cells; these cells were arranged in a radial pattern from the outer basement membrane to the lumen of seminiferous tubules (Figure 1). The cells of these tubules were bound together in a chain like configuration.

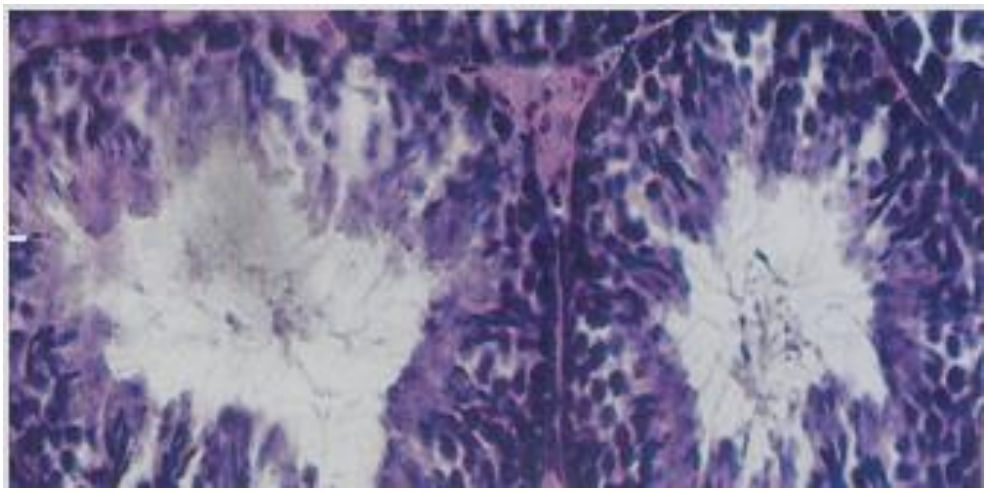

Figure 1. Mice testicular paraffin sections from the control group stained by hematoxylin and eosin showing normal testicular histology (X400)

Histological changes of the testes in the experimental group:

Testicular sections of the mice from the experimental group showed wide intercelluar spaces between the cells of the seminiferous tube compared to the control group. The nuclei of these germinal epithelial cells in the experimental group were more condensed, and the fully developed spermatozoa were less in number at the luminal part of the tubules.

The seminiferous tubules of the treated group exhibited distorted morphology and organization of the chain like serial stages of sperm development (Figure 2).

Anti-MDA immunohistochemical changes in the testes of the experimental groups (Figures 3 & 4):

The evaluation of the counted mean values of MDA immuno-histochemical reactivity obtained by the application of the Aperio Image Scope software in the testicular tissue of mice in the experimental group revealed statistically significant variability compared to those of the control group ($p<0.001$). The counting of the mean value of the number of strong positive pixels was higher in the experimental group (2700.174±325.35), compared to that of the control group (2060±325.35).

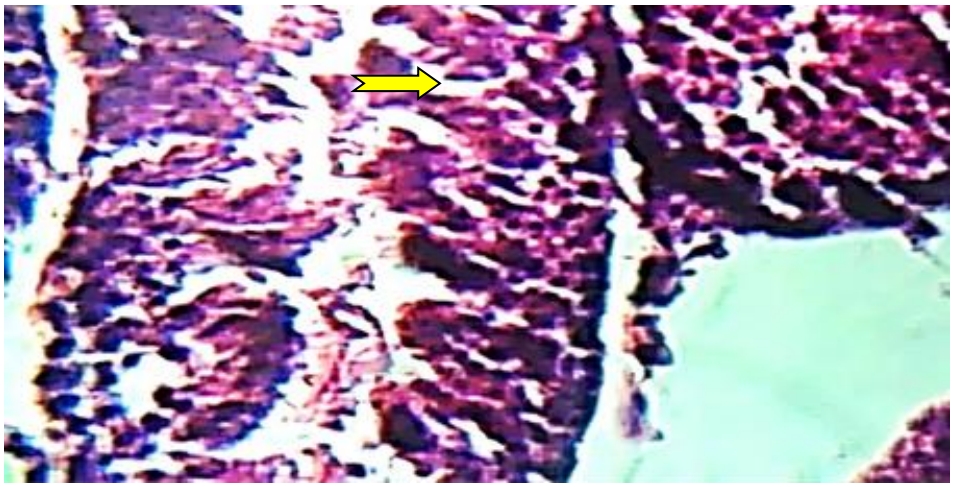

Figure 2. Mice testicular paraffin sections from the experimental group stained by hematoxylin and eosin showing tissue clefts (arrow) with distortion of spermatogenetic cells chain (X400)

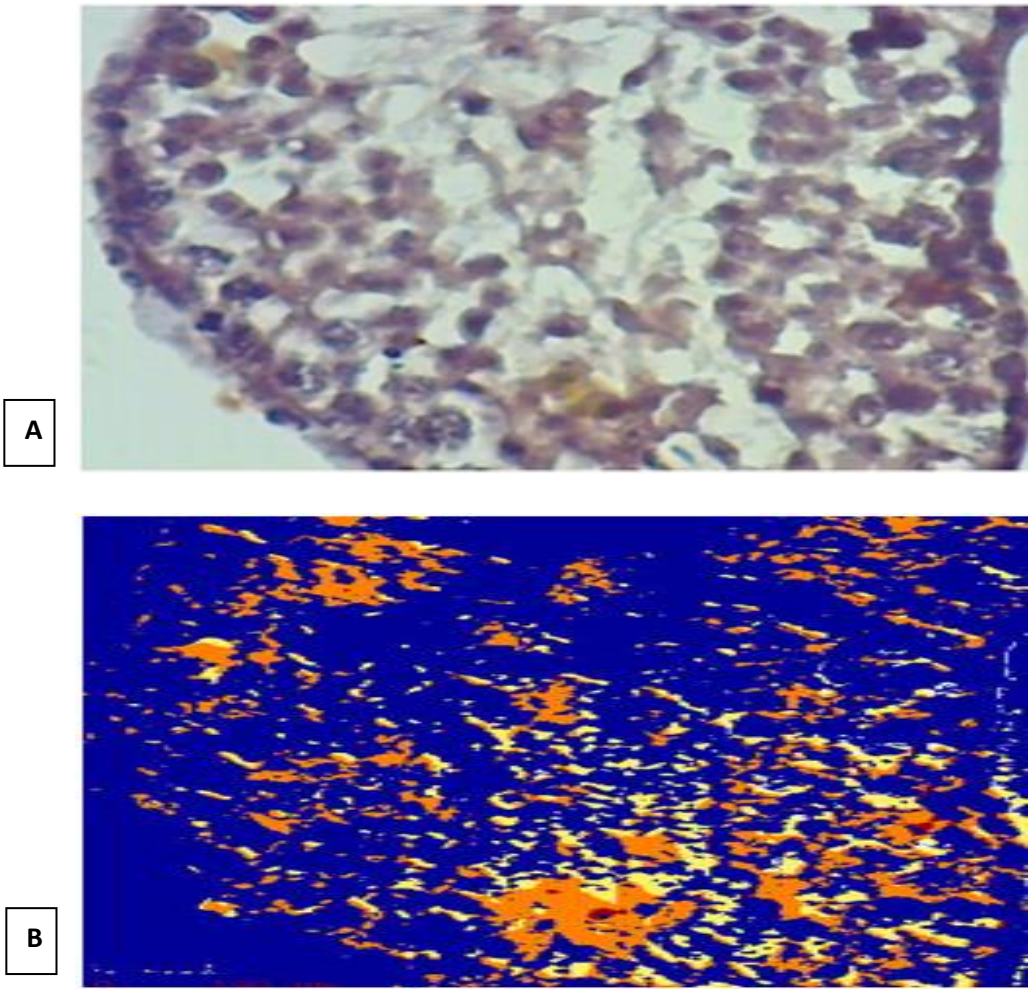

Figure 3. (A) Anti-MDA immunohistochemial changes in the mouse testis from the control group (400X). (B) The snap shoot as analyzed by Aperio Positive Pixel Count Algorithm software. The positive brown color appeared red in color in the markup image of the Aprio image scope software

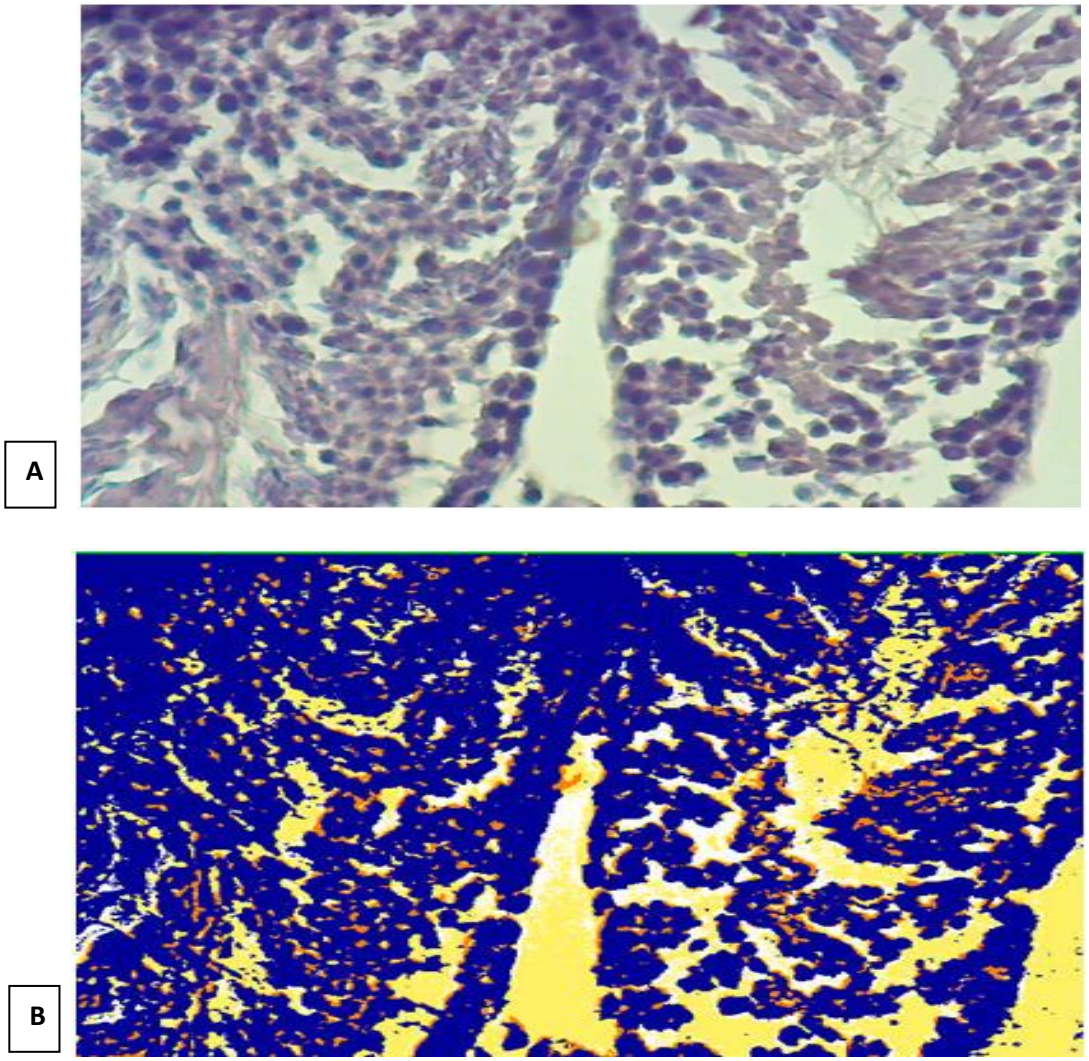

Figure 4. (A) Anti-MDA immunohistochemial changes in the mouse testis from the experimental group (X400). (B) The snap shoot as analyzed by Aperio Positive Pixel Count Algorithm software. The positive brown color appeared red in color in the markup image of the Aprio image scope software

Discussion

The harmful effect of $MgCl_2$ on postnatal spermatogenesis was evidently concluded in previous experimental researches [13].

Manganese compounds proved to decrease testicular weight and to produce necrosis of the seminiferous tubules after oral administration to adult experimental animals [13].

In support to the results of this study, it was reported that manganese compounds produce delay in postnatal sexual maturation in male mice associated with histological changes in the testes including congestion and seminiferous tubular necrosis [15,16]. The postnatal toxicity of manganese on testicular tissue was not investigated in this study as the doses of manganese chloride were administered only during the first 17 days of pregnancy.

The destructive effect of manganese compounds was suggested to be related to the substantial damage of the luteinizing hormone (LH) receptors on the surface of the Leydig cells resulting in loss of the function of testosterone secretion by these cells [17]. Testosterone has significant effect in stimulating Sertoli cells to

produce androgen binding protein which maintains the process of spermatogenesis [18].

Anti-MDA immunohistochemical reactivity shown in our results proved that $MgCl_2$ induced lipid peroxidation. The lipid peroxidation after cellular injury leads to apoptosis and autophagy. The cellular membranes, because of their high lipid content, are especially susceptible to damage because lipid peroxidation reactions can alter the structure and function of critical membrane lipids leading to cell injury and cell death [19].

This study concluded that prenatal oral administration of $MgCl_2$ produces postnatal necrosis of the germinal epithelium in the seminiferous tubules. Anti-MDA immunohistochemical reactivity proved the role of lipid peroxidation leading to apoptosis.

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Author contribution

Fathel: performing the laboratory research work. Dr. Jarullah: performing production of the results. Dr. Mubarak: performing the interpretation of the results.

Conflict of interest

The author discloses no any financial and personal relationships with other people or organizations that inappropriately influence (bias) our work.

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Correspondence to Dr. Hayder J. Mubarak
E-mail: hayder_67_67@yahoo.com
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