



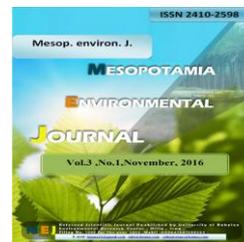
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The effect of manganese poisoning on the histological structure of testis in male Swiss mice

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Abstract

The current study aims to investigate the effect of manganese poisoning on the histological structure of testis. The experimental were conducted on (15) male adult Swiss mice of Balb/ C strain ,which were randomly divided into three groups between control and treatment groups. The animals had been given manganese chloride orally in concentration (50,100) ppm for 30 days ,while the control group have been given distal water for same time period. The animals were killed after (48) hours from finished of treatment ,the animals are weighted and the testis was removed ,weighted ,processed for histological study. The results reveal significant differences when comparison between the experimental and control groups as follow :

- Non significant decrease in body weight with concentration which used in current study .
- A high significant decrease($p<0,01$) in testis weight with increase $MnCl_2$ concentration.
- A high significant decrease($p<0,01$)in seminiferous tubules diameter in group treatment with (50) ppm while no- significant decrease in group treatment with (100)ppm.
- significant decrease at different levels in cell of spermatogenesis (Spermatogonia ,Spermatocyte ,Spermatide) as well as sertoli cell.
- There is histological changes include damage in walls and cells of tubules , odema ,blood vessels and degeneration of spermatogenesis.

Introduction

Minerals are generally directly affect the male reproductive system either when one of the members of the genital tract is affected, or indirectly when the element interferes with the work of endocrine system associated closely linked to the nervous system with the work of the endocrine hormones that are affected with any defect that affects the central nervous system[1], Since the reproductive system efficiency in the completion of the most important operation carried out by a process of the spermatogenesis but depends on the interaction between the nervous and endocrine system[2].

Manganese trace elements essential for natural evolution and the body's natural functions and complete physiological processes in mammals, as it is linked to and regulates many enzymes, where he works, for example, as an assistant enzyme (Co-factor) for many enzymes including arginase which is one of the enzymes necessary for the production of urea [3,4]. This metal is a component of the extensive use in the industry elements it enters the industry stains, steel, tires and be a key in the pesticide industry and added to the fuel to improve its quality therefore be present in the smoke emitted from automobile engines and vapors volatile factories which use manganese as one of the elements in their products is the famous fever industrial fumes [5], manganese permission exists in the aquatic environment or the surrounding air and the human or animal exposure to poisoning him very possible [6]. Despite the importance of life to the metal, but the human exposure to him, and in small quantities outweigh the needs of the body leads to poisoning him and deposition in some important vital organs and that perhaps the most important of the brain, leading to interference in the work of the enzymes and biomolecules in the central nervous system and the appearance of symptoms similar to syndrome Parkinsonism-like disease called (manganism) [7]. Reports published by the American Organization and the World Health Organization [8] that the poisoning manganese led to the failure of the System of reproductive, and the occurrence of weakness and sexual sterility among workers exposed to manganese in addition to giving birth to deformed babies [9] and when conducting analyzes of semen for those workers observed some of the changes occurring in the biological characteristics of semen which prolong the period Liquefaction, decrease in size and appearance of cases of malformation of sperm as well as decrease the preparation sperm cells and low viability [10].

In studies conducted on experimental animals confirmed the researcher [11], that the treatment of Swiss mice manganese led to a decrease in highly significant in fertility while noting researcher (Laskey) and his group (1982) that treated rats manganese through food cause fertility decreases due to the decrease Ion iron (Fe) and not to the evolution of the male members of subsequent generations as well as the occurrence of hormonal imbalances in hormone levels testosterone and follicle stimulating FSH. So it became a study of pollution in this ingredient one health concerns today, particularly on the reproductive system especially the male with the increasing cases of infertility, particularly cases of non infertility unexplained, and as a result for that it is coming idea of the study, which aimed to determine the effect of poisoning manganese on histocompatibility testicular structure and process of the (spermatogenesis) in male white mice.

Materials and methods of work

Laboratory animals

This study used a Swiss white mice *Mus musculus* of Bulb / C . strain on experience (15) a mice male adult age (50-75) days, the mice of weights (25-35) g, were obtained from the collage of Science Advisory Office _ the University of Babylon .The animals are put in a special metal cages prepared for the purpose of breeding animals in the air-conditioned room ranged temperature between 22-27 and under constant lighting 12 light _ 12 hours of darkness capacity system. The animals were given water and standard food pellet that have been processed from the collage of Science _ University of Babylon and continuously according to the requirement of animales. left the animals before starting the experiment for two weeks for the purpose of acclimation.

Material used to study

Manganese

The study included the preparation tow concentration (50 ,100) ppm of chloride manganese (MnCl₂) of high-purity factory by East Anglia Chemical Company from the Department of Chemists / collage of science for woman/ University of Babylon.

Experimental Design

The experiment was designed to study the effect of manganese chloride as follows:

- Study the effect of manganese chloride in body weight.
- Study the effect of manganese chloride testis weight.
- Study the effect of manganese chloride in diameters of seminiferous tubule rate.
- Study the effect of manganese chloride in cells for the spermatogenesis (spermatogonia cells , primary and secondary spermatocyte , spermatide and sertoli cells) .
- Study the effect of manganese chloride in histopathological changes .

Treatment

I attended the concentrations used for the study (50 ,100) ppm of salt solution for manganese chloride by the following equation :

$$W.t (gm) = \frac{p.p.m \times V(ml) \times m.wt (MnCl_2)}{10 \times At.wt (Mn)}$$

It is dissolves 0.16, 0.08 grams of a substance manganese chloride in distilled water and he finished size to the liter for concentrations (50.100) ppm respectively (used concentrations depending on What came in Research Pannapakam and his groups [13], which used high concentrations of manganese while designed this research to determine the effect manganese at low concentrations, the first group (control group) given distilled water orally, while the second group was given drinking water containing a concentration of 50 ppm of the salt solution of chloride manganese, while the third group was given the focus (100) ppm of salt solution of chloride manganese 9and continued to give these concentrations for a period of 30 days.

Animals killing

The animal sacrifice after the end of treatment duration to 48 hours, where I explained the animals after drugged textured chlorofom product by the British BBC company and using sharp scissors has been opened abdominal cavity and eradicated testes for the purpose of histological study, and then weigh the testes using a sensitive balance of the type of Sartorius.

Preparing histological sections

Histological sections and solutions for preparing attended histocompatibility depending on the policies included in the [14] .

Examination of histological slides

Examined the slides using a compound microscope and recorded different measurements for diameters tubule seminiferous using the exact scale of the lens-kind ocular micrometer calibrated scale flour for the theater stage micrometer. For the purpose of clarifying the results of the study were taken some photographs of some of histological sections using a microscope photograph of the type Olympus model DB2- N180 is a Japanese origin provider with a calculator type LG Flatron screen. The study histological structure of the testicle that prepare (4) slides for each animal, and it was selected tow slides of them which used to measured cell spermatogenesis were measured in (20) tubule each section. The study also included the percentage of seminiferous tubules affected account, according to the method described by Balash and his group [15] then the percentage of damage is calculated according to the following equation:

$$\text{Percentage of damage seminiferous tubules} = \frac{\text{Number of damage tubules}}{\text{Total number of seminiferous tubules}} \times 100\%$$

Statistical analysis

Results of the study were analyzed statistically using the statistical program SPSS version 16, according to follow randomized complete design and test the slightest way teams moral L.S.D to see significant results and the results are presented mean \pm S.E .

The results

Changes in body weight

The results showed a decrease for not rise to the level of significance in the weights of manganese chloride-treated animals compared with the control group and the decline towards a greater concentration .figure(1).

Changes in testis weight

Results of the study showed the presence of high significant at a level (P <0.01) in the treatment animals compared to the control group, had decreases increases aswith increasing concentration .figure .(2).

Changes in diameters of seminiferous tubule

It showed a statistical analysis of the results of the study occurrence of high decline ($P < 0.01$) in the treatment animals of 50 ppm, while the decline was not significant in the treatment group at (100) ppm .figure (3)

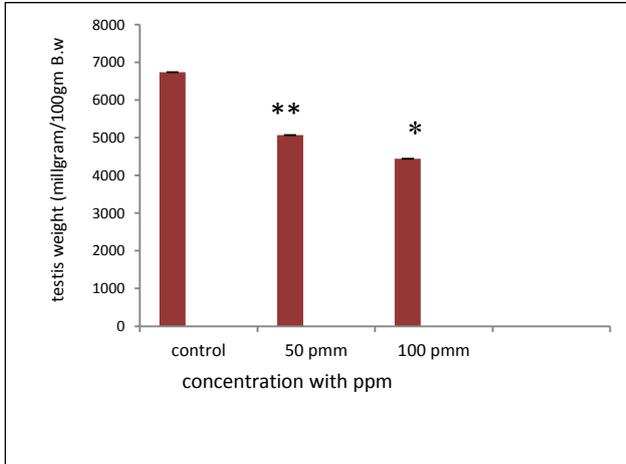


Figure (2) :the changes in testis weight (milligram/ 100gm of B.w.) for treatment groups
 ** high significant different at level ($p < 0.01$) L.S.D = 149.304

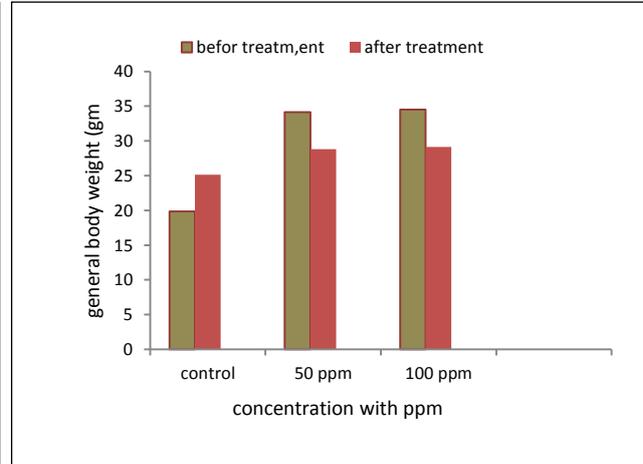


Figure (1) :the changes in general body weight (gm for treatment groups of different concentration of MnCl2 solution

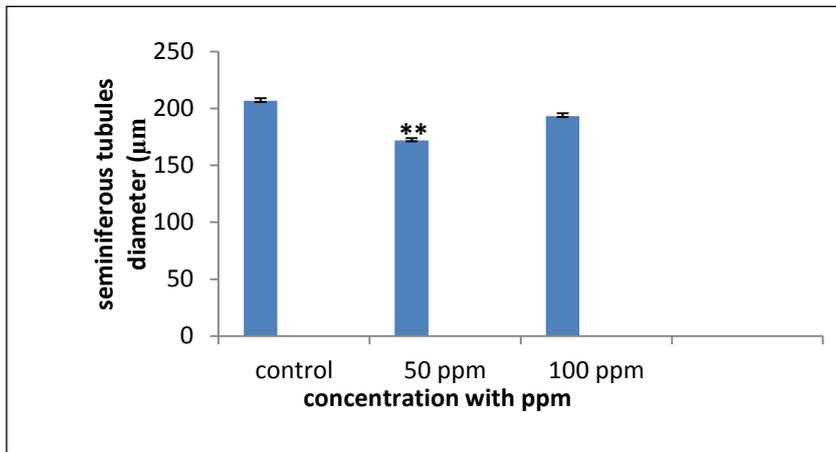


Figure (3) :the changes in seminiferous tubules diameter(µm) for treatment groups of different concentration of MnCl2 solution .
 ** high significant different at level ($p < 0.01$) L.S.D = 16.191

Changes in numbers of cells for spermatogenesis

The results of the study for a highly significant decrease at the level of ($p < 0.01$) in the number of spermatogonia in all treatment groups manganese compared with the control group .figure (4) .while microscopic examination show significant decrease at the level of ($p < 0.05$) in the primary and secondary spermatocyte in the treatment group of 50 ppm, while the decline was highly significant at the level of ($p < 0.01$) in the treatment group at 100 ppm. Form (5). The number of spermatozoa , there was a highly significant decrease at the level of ($p < 0.01$) in all treatment groups manganese form (6). Manganese treatment also caused a highly significant decrease at the level of ($p < 0.01$) in the number of sertoli cells in all treatment groups compared with the control group. figure (7.)

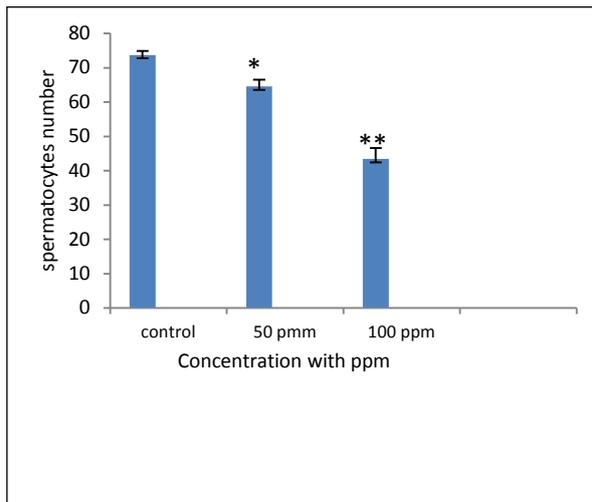


Figure (5) :the changes in number of spermatocytes cells.
 *high significant different at level ($p < 0.05$) L.S.D(0.05) = 7.628
 ** high significant different at level ($p < 0.01$) L.S.D(0.01) = 11.477

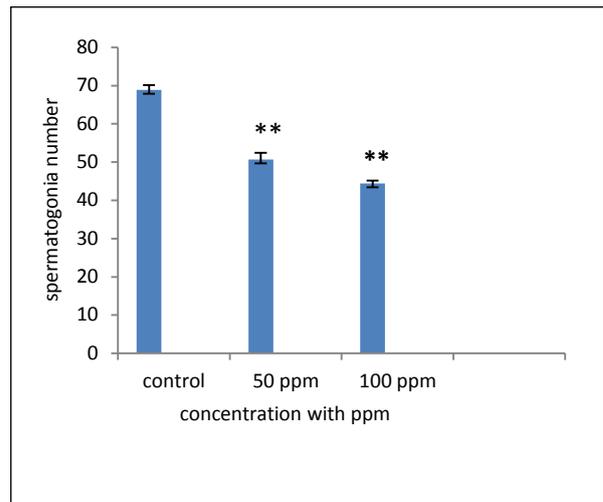


Figure (4) :the changes in number of spermatogonia cells for treatment groups
 ** high significant different at level ($p < 0.01$) L.S.D = 2.062

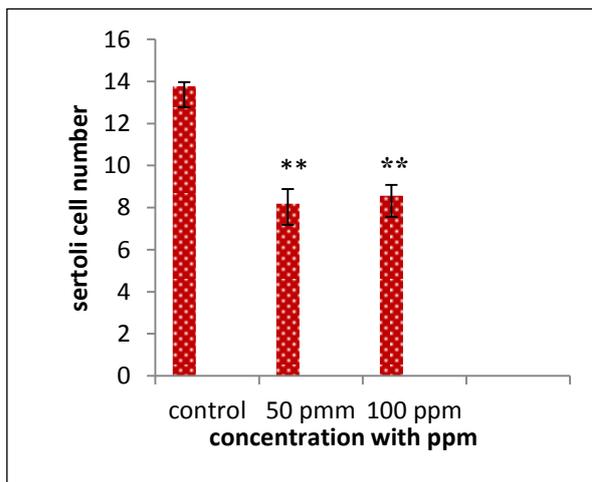


Figure (7) :the changes in number of sertoli cells for treatment groups ** high significant different at level ($p < 0.01$) L.S.D= 2.382

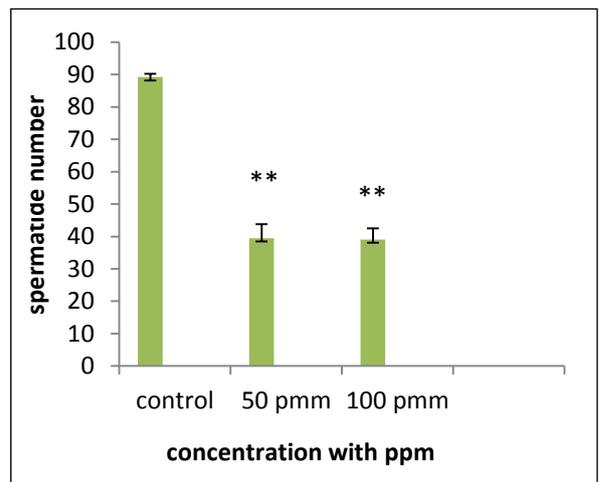


Figure (6) :the changes in number of spermatide cells for treatment groups ** high significant different at level ($p < 0.01$) L.S.D= 13.372

The percentage of affected tubule

the microscopic examination of tubules show founding damage in seminiferous tubules which it is increase with increase concentration . figure (8) .

Histopathological study

The microscopic examination of testis tissue for animals treated with distilled water that promised as control group the appearance of the tubule naturally in terms of the presence of sperm, the effectiveness of the division, the nature of the blood vessels and tissue interstitial. figure (A1 & A2). The treatment groups with manganese has caused histopathological changes where noted degeneration process spermatogenesis generally empty tubule of sperm (so he was in some empty full) and the occurrence of rupture and degenerative and lysis in epithelial tissue lining the tubulesfigure (B1,B2) , decrease in Lydig cell figure (C1) vascular congestion and odema incidence and prevalence of edematous fluid with a clearing spacesfigure (C2).

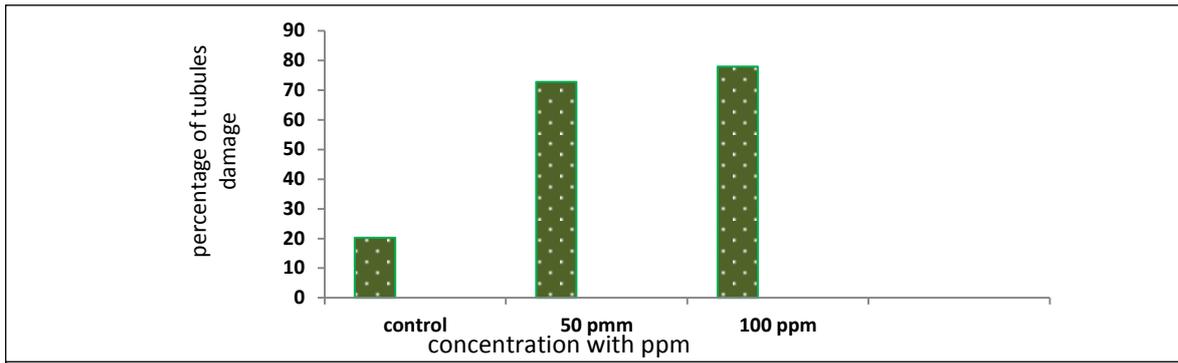


Figure (8) :the percentage of seminiferous tubules damage for treatment groups of different concentration of Mncl2 solution .

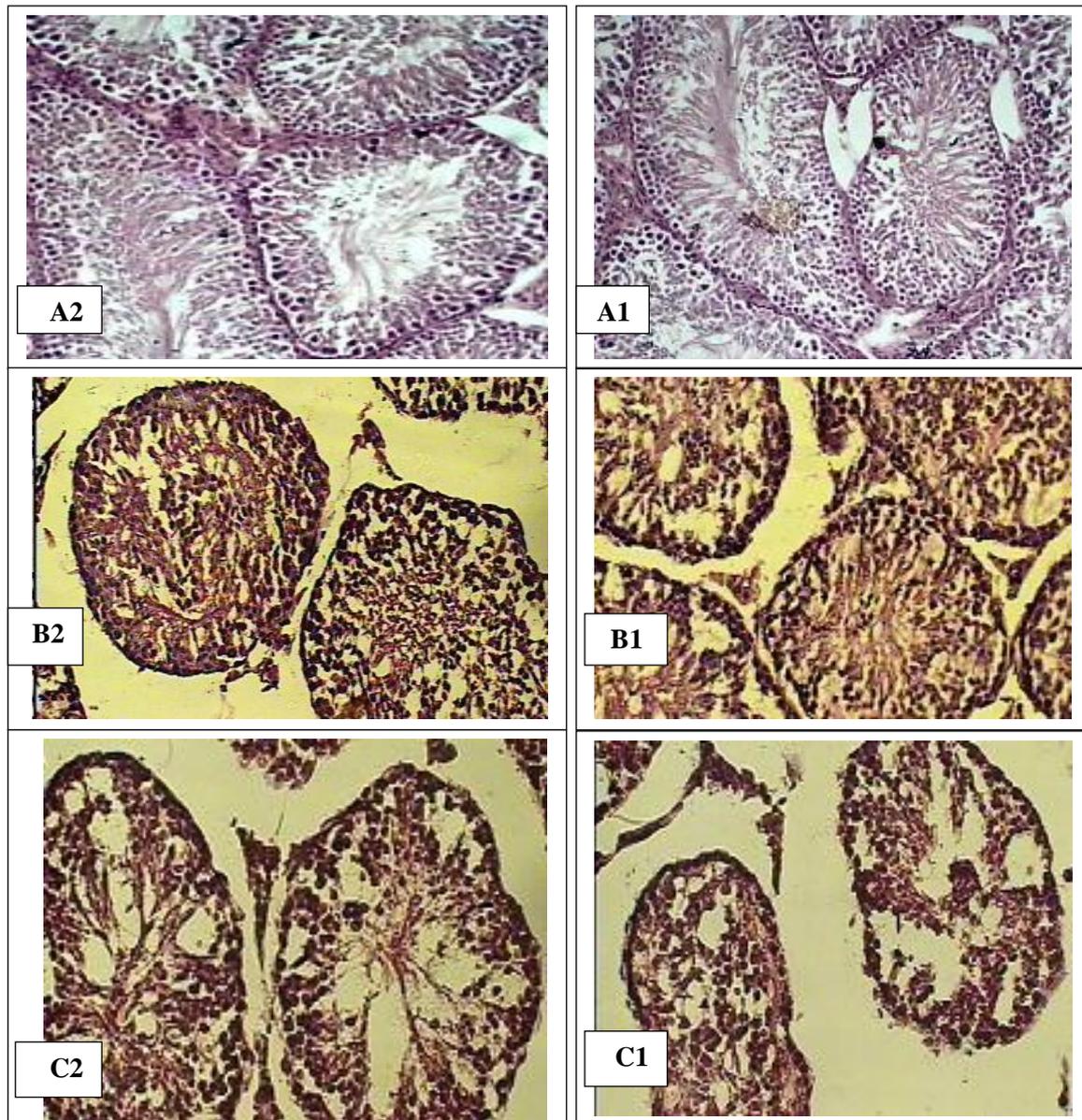


Figure (9) Photomicrograph of testis tissue stained with H.E. (200X): (A1 & A2) Normal Testicular tissue, (B1 &B2) manganese -treated mice testis tissue at (50)ppm show occurrence lysis ,degeneration of spermatogenesis and rupture of tubules . (C1 & C2) testis tissue of mice treated with manganese at (100)ppm notes decrease in Lydig cell , vascular congestion and odema incidence and stopping of spermatogenesis in fully.

Changes in body weight

The results of the study showing increase was not significant in body weight in the animals treatment with manganese compared with the control group. The results of the study agree with the findings of (Zaidi) and his group in 2005 [7] that the treatment of male and female white mice of manganese through food has led to a decrease weights, perhaps return the reason for the decline to the negative impact of manganese on the central nervous system which reduces appetite and food consumption were reflected this effects on animal behavior was observed lack of activity as well as inactivity and introversion.

On the other hand, he has said the researcher (Badiei) and his group [14] that the treatment of sheep of manganese cause of low thyroid effectiveness of thyroid gland and decrease the level of hormones in blood, which leads to increased protein and fat intake and carbohydrates and thus may be the cause of the decline in body weight.

Changes in testicular weight

Manganese treatment led to obtain a highly significant decrease in the general testicular weight, compared with the control group and intensified the decline with increased concentration and results of the study are agreement with the findings of other studies of which (Laskey) and his group (1982). This decline has been back to inhibitory effect of manganese in hormone testicular testosterone level, as studies have confirmed that the treated rats with manganese led to a highly significant drop in hormone testosterone and Weights reproductive organs, In addition, the changes in the tissues of the testes as lysis in some cells and damage to the blood vessels and the components of seminal tubule as well as the absence of sperm from tubule. This was confirmed by histological examination for testicular samples of animals treated at the results of the current study were reflected on the general testis weight.

Histopathological changes

Changes in diameters of seminiferous tubule

Studies and research confirmed that LH or interstitial cell stimulating hormone ICSH significant impact in control on diameters of seminiferous tubule as the injection of LH can cause a significant increase in diameters of tubule [15]. In this study, treatment of manganese cause a highly significant ($P < 0.01$) reduction in diameters of seminiferous tubule was in the treatment group of 50 ppm, while the decline was not significant in the treatment group at 100 ppm and perhaps the reason for this decrease to the change in the level of the hormone LH as a study of the researcher (Cheng) and his group [16] manganese that inhibits the production of LH when injected in male mice. On the other hand we can say that degeneration in the spermatogenesis process (this It has been confirmed results of the current study) and the spread of the edematous fluid interfaces and pressure on the walls of seminiferous tubule and empty the contents of the cell may lead to a decline in the rate of tubule diameter.

The changes in number of cells of spermatogenesis

Microscopic examination and statistical analysis of the results of the current study, for obvious changes in number of cells for spermatogenesis, which (Spermatogonia, primary and secondary spermatocytes and spermatide as well as sertoli cell), as notes for a highly significant decrease in the number spermatogonia and may be the reason for this drop to the toxic effects of manganese on the hypothalamus in the secretion of Gn-RH hormones that stimulate the secretion and formation of hormones stimulating the gonads (LH, FSH), as pointed the researcher McLachlan and his group [17] to decrease the hormones stimulating the gonads leads to a decrease number of spermatogonia. Also it the results of the study is confirmed there is significant decrease at the level of ($P < 0.05$) in the number of spermatocyte (primary and secondary) in the treatment group of 50 ppm, while the decline was highly significant at a level ($P < 0.01$) in the treatment group at 100 ppm, and This decline is identical to discourage happening in the spermatogenesis and noted researcher [4] that the treatment of Fish of manganese led to the degeneration of the spermatogenesis process. The reason in this disorder in the spermatogenesis to a lack of the hormone Testosterone he first charge of this vital process in males, where the importance of this hormone studies have shown in the completion of this process because he

confirmed the researcher (Maclachlan) and his group [18] in his research that the decrease androgens by 20 % would stifle spermatogenesis process.

The results of the study Obtain a highly significant decrease Spermatide This decrease reflects inhibition happening in spermatogenesis due to which is due to stop the division process of Meiosis due to lack of hormone testosterone testicular, plus so decreases made in the sertoli cells (of results The current study) lead to a decrease of the sperm counts due to lack of food processing provided sertoli cell to spermatide. Also it confirmed the results of the study for high significant decrease at ($P < 0.01$) in the number of sertoli cells and attributed the reason for this decrease is that manganese causes what looks like of Apoptotic - like death apoptosis in the cells and secretory tissues due to lack of food processing as a result of the defect occurs in the function mytochonderia causing confusion the action of enzymes in cellular metabolism [19].

Histopathological changes

Showed microscopic examination of section of tissue testes treatment with chloride manganese results clearhisto-pathological changes, as was observed for degeneration of spermatogenesis process in general in all the totals treatment compared with the control group may be due this to disorder happening in the levels of hormones directed Gn-RH (FSH, LH) and hormone testosterone7testicular who promise influential in this important physiological process [20] indicated that studies the manganese from the elements, which has direct effects on the central nervous system as he crosses the blood - brain barrier, where collected in the hypothalamusInterfereswith spermatogenesis process by affecting the nerves in the hypothalamus (as part of the nervesystem) and responsible for secretion Gonadesreleasing hormonesfactors (LH - RH, FSH-RH) [3]. He also appeared in some of the tubule separation of basement membrane (Detachment) with a rupture in some parts of the tubule (Disruption) emerged cases of necrosis and Degeneration cells and possibly attributed the reason for this to the effect of manganese in the level of glutathion (GSH), as studies have shown that manganese is working on the inhibition of enzymes containing a set sulfates in testicular or thiol (-SH) Since glutathion of composite of thiol and manganese able to correlated withthoil group will hinder the work of antioxidant compounds and which also glutathion peroxidase enzyme (GSHPX), leading to the result of these histological changes occur[21]. Treatment and caused the occurrence of Odema and spread liquid edematous and this is compatible with the sentiments researcher Francis & Forsyth (1995) when the treatment of male monkeys with manganese The reason for this to the effect of manganese in the enzyme ATPase & Mg-ATPase causing disorder in ion distribution inside and outside the plasma and get odema and spread edematous fluid in interstitial space .

In Conclusion In spite of the positive effects of manganese on physiological processes, but the exposure for it at low concentrations cause an imbalance in the work of the reproductive tract in males through the degeneration of spermatogenesis process and the occurrence of changes in histological structure of testis .

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