

Testicular histomorphometry and endocrine alterations at puberty in offsprings from mercury treated rats

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Abstract

This study was conducted to evaluate the effects of different doses of mercury on fetal testis. In this investigation 20 adult female rats were divided in four groups. All animals became pregnant by natural mating. Mercuric oxide was induced in three groups by different doses (0.5, 1 and 2 mg/Kg/day) at 10 terminal days of pregnancy. After parturition, male offspring were reared for 60 days. At the end of the experiment (60 days after birth), the body weights of neonates were measured (after birth and also 60 days after birth) and the testes were isolated from the offspring of all rats, also the blood serum was isolated and used for endocrine analysis. Various histological parameters were determined using histological techniques. These parameters were included the thickness of testicular capsule (μm), the number of seminiferous tubules ($/\text{mm}^2$), the number of Sertoli, leydig and spermatogonia cells ($/\text{mm}^2$), and the seminiferous tubules' diameter (μm). Results revealed decreases in the LH, FSH and testosterone hormones, and body weight in the experimental groups. The diameters of the seminiferous tubules, and the numbers of the spermatogonia, sertoli cells and leydig cells have decreased significantly in experimental group by 2 mg/Kg/day dose. Mercury exposure exhibited deleterious effects on male gonads during fetal life by alterations in the hormones and testicular cell numbers and this disorder remains persistent during the post-neonatal period.

Keywords: Male hormones, Mercury, Offspring, Rat, Testis

1. Introduction

Prevalence of mercury intoxication by food and environmental sources has risen globally worldwide. Pollution of soil and water by natural phenomena such as volcanoes and industrial activities, pollution of marine food resources by mercury in the water and preservative compounds of vaccines are possible causes of mercury intoxication (1). Fish is the main route of exposure to mercury, which mainly accumulates in the large predators (2). Dental amalgam in pregnant mothers is also another source of mercury exposure for developing fetus and newborn (3). Mercury has been recognized as an industrial hazard that adversely affects male reproductive systems of humans and animals including fertility (4, 5), additionally, higher blood

mercury concentration is associated with male infertility, as higher seafood consumption is associated with elevated blood mercury concentrations in infertile population (6). Some studies have demonstrated the effects of mercury on several testicular reproductive activities such as reduction of sperm motility and male fertilizing ability (7). Numerous studies have been performed to recognize the effects of different mercury compounds on male gonad development, as well as its ultra structural effects on the testis. Moussa et al. (2011) and Fossato Da Silva et al. (2011) have utilized methylmercury (MeHg) (8,9), and Ramalingam and Vimaladevi (2002) and Boujbiha et al. (2009) have treated with mercuric chloride (HgCl_2) (10,4) in their investigations. So this study was conducted to evaluate the effects of different doses of mercuric oxide on testicular development of female rat offsprings and to investigate histomorphometrical

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alterations in the testicle at day 60 after birth, due to this mercury compound.

2. Materials and methods

2.1 Animals

Twenty adult female Sprague Dawley rats (200–230 g and 4–5 months old) were housed in an air-conditioned room ($22 \pm 2^\circ\text{C}$) and supplied with standard pellet food and tap water *ad libitum* (11). The animals were cared and treated in accordance with the guidelines for laboratory animals established by the National Institute of Health as well as by the local ethical committee.

2.2 Experimental design

Animals were divided into four equal groups, three experimental groups (T1, T2 and T3) and one normal or control group (C). Female oestrus stage animals from the four groups were caged with male rats for mating (1 male/1female ratio). Mating was confirmed by vaginal smear observation (12). Since in Sprague Dawley rats genital system forms at day 13 of pregnancy (13), on days 10–20, Mercury was administrated by oral gavage of red Mercuric oxide (Fluka, Switzerland). in three experimental groups by doses of 0.5, 1 and 2 mg/Kg/day for groups T1, T2 and T3, respectively. These doses were determined according to the previous studies (5,10,14).

After parturition, male offspring of each group were isolated and the weights of them were measured, then they reared in similar conditions in an animal house for 60 days. At the end of the experiment, they were anesthetized with diethyl ether and killed by whole blood collection through a heart puncture. Blood serum was isolated and used for endocrine analysis and the weights of the offspring were measured. The testes were then isolated from the offspring of all rats.

All tissue samples were fixed in 10% buffered formalin fixative and subsequently embedded in paraffin. The sections (5 μm thickness) were stained with H&E and Green Masson's trichrome techniques (15). For histomorphological and histomorphometric study, the sections were observed under a light microscope, and the following factors were evaluated in the testes of both control and experimental groups:

- 1) The thickness of testicular capsule (μm)
- 2) The number of seminiferous tubules ($/\text{mm}^2$)
- 3) The number of leydig cells ($/\text{mm}^2$)
- 4) The average number of sertoli and spermatogonia cells in the seminiferous tubules
- 5) The diameter of the seminiferous tubules (μm)

The thickness of the testicular capsule and diameter of seminiferous tubules were measured at $\times 100$ magnification using the Olysia software and an Olympus BX51 light microscope. Additionally, seminiferous tubules were counted at $\times 40$ magnifications, using a 441- intersection grid placed in the ocular of the light microscope (Olympus BX51). Ten sections were chosen at random, from each testis, and the number of round or nearly round seminiferous tubules in a square millimeter (mm^2) was obtained.

The numbers of leydig cells per unit (mm^2) and the spermatogonia and sertoli cells were determined at $\times 1000$ magnification, using a 441- intersection grid placed in the ocular of a light microscope (Olympus BX51). Ten sections were randomly chosen for each testis. The total number of spermatogonia and sertoli cells per seminiferous tubule were determined from the corrected counts of spermatogonia cell nuclei and sertoli cell nucleoli per seminiferous tubule cross section (16).

Serum testosterone, FSH and LH levels were measured by the radioimmunoassay (RIA) technique and commercial kit (Immunotech-Radiova, Prague, Czech Republic).

2.3 Statistical analysis

Morphometric data are presented as the mean $\pm$ SD, and analysis of particularly morphometric data was performed with Student's *t* and ANOVA tests, using the SPSS program.

3. Results

Findings have demonstrated that the body weights of neonates from the experimental groups have decreased at birthday compared to that of control, which has been significantly decreased in the group T3 ($3.12 \pm 0.4\text{g}$) compared to the control ($3.95 \pm 0.44\text{g}$), whereas these decreases were not significant in groups T1 ($3.57 \pm 0.5\text{g}$) and T2 ($3.34 \pm 0.45\text{g}$). Additionally, it has been determined that the offspring's body weight of the experimental groups have decreased compared to control group at day 60 after birth (Figure 1). The mean body weight of the group T3 offspring showed a significant ($p < 0.05$) decrease compared to control ($121.3 \pm 5.2\text{ g}$ vs. $134.9 \pm 6.3\text{ g}$).

The levels of serum FSH, LH and testosterone in the experimental groups were lower than in the control group. As shown in figure 2, FSH, LH and testosterone values have decreased significantly ($p < 0.05$) in the groups T2 and T3 compared to those in controls.

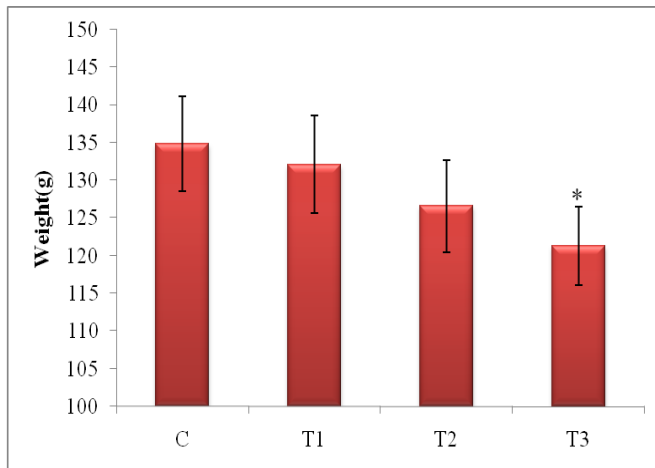


Figure 1: Comparison of the body weight of offspring from mothers of experimental groups (T1, T2, T3) and control group (C) after birth. Values represent mean $\pm$ SD. *represents a significant difference at $p<0.05$.

Table 1 demonstrates different parameters of testes from the offspring of the mercury treated mothers by 3 different doses (groups T1, T2 and T3) and control mothers (group C) at day 60 after birth. The number of seminiferous tubules and the thickness of the testicular capsule have not shown significant alterations in experimental groups compared to the control group. The diameter of seminiferous tubule decreased in experimental groups compared to that in control; this reduction was significant ($p<0.05$) in group T3 compared to control group. The average

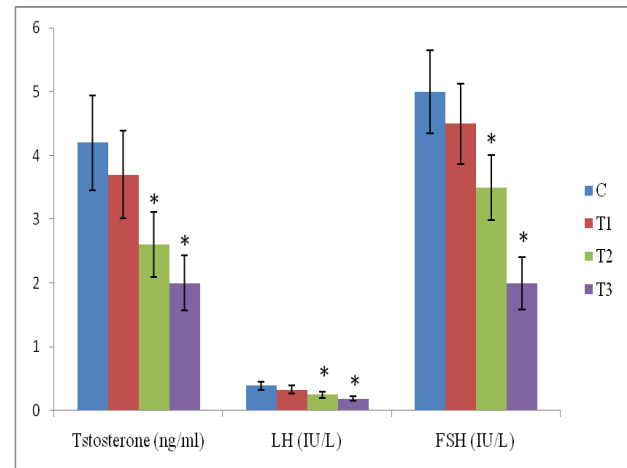


Figure 2: Comparison of testosterone, LH and FSH levels in offspring of female rats of experimental groups (T1, T2, T3) and control group (C) after birth. Values represent mean $\pm$ SD. Asterisk (*) represents a significant difference at $p<0.05$.

number of spermatogonia cells decreased in experimental groups compared to that of control; as in group T3, this decrease was significant ($p<0.05$). The average number of sertoli cells decreased in experimental groups compared to control and was significant in group T3 ($p<0.05$). The number of leydig cells decreased in experimental groups compared to that in control, and this reduction was significant ($p<0.05$) in group T3 compared to the control group.

Groups	C	T1	T2	T3
NST (/mm ²)	10.44 $\pm$ 0.77	10.42 $\pm$ 0.83	10.37 $\pm$ 0.75	10.1 $\pm$ 0.82
DST (μ m)	252.8 $\pm$ 16.5	263.4 $\pm$ 17.3	248.2 $\pm$ 16.1	235.5 $\pm$ 17.8*
TTC (μ m)	22.5 $\pm$ 2.8	22.4 $\pm$ 3.1	22.3 $\pm$ 2.9	22.2 $\pm$ 3
NSp (/ST)	70.3 $\pm$ 9.1	65.3 $\pm$ 10.1	57.7 $\pm$ 9.9	49.2 $\pm$ 9.7*
NSe (/ST)	20.7 $\pm$ 2.1	19.5 $\pm$ 2.8	18.2 $\pm$ 2.4	16.1 $\pm$ 1.6*
NLe (/mm ²)	979.5 $\pm$ 80.2	953.9 $\pm$ 77.5	907.3 $\pm$ 79.2	827.1 $\pm$ 83.6*

Table 1: Comparison of the testicular parameters of control (C) and experimental groups (T1, T2 and T3). NST: Number of seminiferous tubules, DST: Diameter of seminiferous tubule, TTC: Thickness of testicular capsule, NSp: Number of spermatogonia cells, NSe: Number of sertoli cells, NLe: Number of leydig cells. Values represent mean $\pm$ SD. *represents a significant difference at $p<0.05$.

4. Discussions

Studies have ascertained that mercury penetrates the placental barrier and accumulates in the fetus after exposure of pregnant animals to mercury (17). Accordingly, mercury may transfer to the fetuses

from mercury polluted mothers. As the results showed, the body weights of offspring decreased in the experimental groups compared to the control group at the birthday and 60 days after birth. Body

weight loss due to mercury exposure is a typical phenomenon in adult rats and is caused by anorexia (18). Higher mercury concentration in fetal blood has also been associated with an increased risk of being born small for gestational age in length (2).

As results demonstrated, the levels of serum FSH, LH and testosterone decreased in the experimental groups. AS Ramalingam et al. (2003) demonstrated mercury treatment results in decrease in the levels of LH and FSH in adult albino rats. This change in the FSH and LH levels may be caused by the dysfunction of pituitary-testicular axis (14). Additionally, Moussa et al. (2011) have reported that mercury cause a fall in the plasma testosterone in the contaminated animals, which seems to be in relation with the decrease in the secretion of testosterone (8).

As findings show the numbers of spermatogonia, sertoli and leydig cells decreased, additionally, the diameter of the seminiferous tubules reduced in the experimental groups on account of mercury consumption. It has been determined that the male gonad seems to have a special affinity for mercury, and it has been shown that the testicular toxicities of mercury materials impair spermatogenesis and steroidogenesis in a number of laboratory animal species (19). Homma-Takeda et al. (2001) have suggested that methylmercury impairs spermatogenesis by germ cell deletion via cell and stage specific apoptosis (20).

As it has been determined previously, mercury utilization results in LH and FSH reduction (14), also it is generally accepted that both gonadotropins LH and FSH are necessary for initiation and maintenance of spermatogenesis; accordingly, reduction of gonadotropins would decrease the spermatogenesis and reduce sperm counts (21).

It has been determined that mercury chloride may induce oxidative damages and histopathological alterations in the testis (22). Boujbiha et al. (2009) have suggested that an increase in free radical formation relative to loss of antioxidant defense system after mercury exposure may render testis more susceptible to oxidative damage leading to their functional inactivation (4). Geier et al. (2009) have shown that utilizing mercury at low concentrations is able to induce mitochondrial dysfunction, reduced cellular oxidative reduction activity, cell death, and cell degeneration (23).

Ernst et al. (1991) have reported that administration of mercury in the drinking water have resulted in intracellular accumulations of mercury in the interstitial Leydig cells as well as in the Sertoli cells of the seminiferous tubules (24), additionally, Monsees et al. (2000) have been explained that

Sertoli cell, which play an essential role in spermatogenesis, might be a main target for mercury contamination (25).

It has been demonstrated that mercury may induce some alterations in the testes as decrease the volume of germinal epithelium, increase relative volume of interstitium and increased apoptosis occurrence suggest damages interstitium and reveals occurrence of edemas (26). As Penna et al. (2009) have demonstrated, sub-lethal concentrations of mercury contribute to functional alterations of spermatogenesis with arrest at spermatocyte stage, hypo-spermatogenesis and possibly impaired steroidogenesis which together could affect male fertility (27).

5. Conclusion

We conclude that fetal testes may be affected by mercury exposure of their pregnant mothers, through reduction in the diameter of seminiferous tubules, and numbers of the cells in the testes such as spermatogonia, sertoli cells and leydig cells. These alterations may remain after birth and affect the male reproductive essential activities.

Acknowledgment

This work has been funded by Islamic Azad University, Yasooj Branch.

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