Original article

Effects of Mercury Chloride on Sperm Parameters and Pathological Changes in Kidney of Mouse

Habiba A. El Jaafari[1]*, Amr M.Fathalla[1] and Suhera Aburawi[2]

[1]Department of Zoology, Faculty of Science, University of Tripoli, Tripoli-Libya.
[2]Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, University of Tripoli, Tripoli-Libya.

Received 20 October 2015/ Accepted 25 November 2015

Abstract

Mercury chloride ($HgCl_2$) is a toxic substance increasingly being recognized as a potential environmental pollutant. In this study, sperm parameters have been studied to relate the effects of $HgCl_2$ on mice fertility and to evaluate the toxicity of $HgCl_2$ on kidney. The kidney is well documented as the target organ for $HgCl_2$. Forty Swiss albino male mice, eight to ten -week-old and weighing 28 - 30 g. Animals were divided into four groups, each group contain ten animals and injected intraperitoneally weekly with 0, 0.5, 1.0 and 2.0 mg mercury chloride /kg body weight for eight weeks respectively. Sperms of vas deferens were evaluated with respect to sperm count, motility, and morphology. Total sperm count and motility were significantly decreased in dose dependent manner of Mercury chloride, while sperm abnormality increased consistently at different doses of mercurial treatment over a period of eight weeks. Maximum sperm abnormality among the treated groups was noted in the group given 2 mg/kg body weight. The study revealed that Mercury chloride has a potency to alter sperm parameters. Also, histological alterations were also observed in the Kidney.

Key words: Mercury Chloride, Sperm, Mouse, Kidney

Introduction

There are a serious rise in the environment of toxic chemicals and heavy metals. The accumulation of heavy metals in the body of organisms causes significant health risks. Mercury is one of the fairly harmful heavy metals for lives (Gul *et al*. 2004) and it has extremely toxic characteristic for organisms (Sharma *et al*. 2005). Mercury is a naturally occurring metal in organic (methyl mercury, ethyl mercury and phenyl mercury) and inorganic forms (mercury chloride) which possess different toxicity (Trasande *et al*. 2005). The potential health effects of mercury have been a matter of concern because of potential wide human exposure consequent to its wide spread use.

It distributed throughout the environmental by both natural sources and human activities and easily accumulated in the animal tissues. Most of the mercury in the atmosphere is elemental mercury vapor and inorganic form, which may be deposited in water, soil and sediments. Mercury vapor is highly lipophilic and is efficiently absorbed through the lungs and oral mucosa (Crinnion 2000). Mercury is widely used in refinery, plastic and paints antiseptic, scientific instruments, photography, fuel combustion, wastewater treatment facilities, paper mills and medical and agriculture field.

This metal easily penetrates into tissues such as kidney, lung, blood stream, connective tissue, brain, adrenal and other endocrine glands (ATSDR 1999; Joseph *et al*.2001; Jagadeesan and Sankarsamipillai 2007) and it may be caused damages in all these tissues. Reproductive hazards from metal exposure in males are one of the fastest growing areas of concern in toxicology today. Exposure to different heavy metals causes irreversible toxic insult to male reproductive system. Heavy metals produce cellular impairments at structural and functional level in male reproductive system. The effect of heavy metals, such as lead, mercury, cadmium, chromium and arsenic on male reproduction has been studied in details in various experimental species.

Metals could interfere with the gametogenic cells or Leydig cell or spermatozoa directly in semen. These effects may results in reduced fertility or associated with pregnancy wastage, congenital malformation associated with genetic diseases. Potential toxicity of Metals caused alteration in sperm morphology, count, motilityas well as biochemical disruptions of enzymes and hormones (Jagadeesan and Sankarsamipillai 2007). Therefore, the present study aims to evaluate the effects of mercuric chloride on sperm parameters and histopathology on kidneys.

Materials and methods

Animals and Diet

Forty Swiss albino male mice 8 to 10 weeks of age weighing 28-30 g were used for this study. These mice were inbred in the animal house of the Zoology Department/ Faculty of Science / University of Tripoli. The mice were housed in plastic cages containing wooden flakes and had free access to water and standard diet (ad libitum).

Experimental design

Experiment was conducted for eight weeks. Animals were divided into four groups, each group contain ten animals. Group I was received an equivalent amount of

***Corresponding Author:** Habiba A. El Jaafari, Department of Zoology, Faculty of Science, University of Tripoli, Tripoli-Libya, Tele: 0927227431, E. mail: habibaali350@gmail.com

distilled water and served as control. Group II, Group III and Group IV were injected intraperitoneally with 0.5, 1.0 and 2.0 mg mercury chloride /kg body weight / weekly respectively.

Seminal Fluid collection

Groups of treated and untreated mice were killed after 24 hours from the last injection by cervical dislocation. Sperm of each mouse were obtained by squeeze the vasa deferentia gently into 1ml normal saline in small dish. The specimen was mixed gently by a special dropper to distribute the seminal fluid. Sperm suspension was incubated for 15 minutes at 32 ^{o}C to allow sperm separation (El Jaafari 1995).

Determination of sperm count:

Sperm counts were made using the method by Sakamoto and Hashimoto (1986) modified by El Jaafari (1995). The sperm were counted by charging both chambers of improved Neubauer Hemocytometer with sperm suspension. Sperm count expressed as million per millileters.

Examination of sperm morphological

For sperm morphology test, two smears were made from each mouse, and allowed to dry in air. Smears were stained with 1% eosin Y in water for 10 minutes. From each mouse 500 sperms were examined at 400 magnifications for morphological abnormalities. Results were expressed as percentage of abnormal sperm (Otitoloju *et al*. 2010).

Determination of sperm motility

Sperms from both treated and untreated mice were examined according to Ficsor and Ginsberg (1980) by using the improved Neubauer hemocytometer (American optical Co., Buffalo. N. Y). Numbers of motile and non-motile sperms of treated and untreated mice were counted under the X40.

Histological study

For histological examination, specimens from kidneys tissue were taken immediately after sacrificing mice, and fixed in 10% formalin solution. The fixed specimens were then trimmed, washed, dehydrated, and embedded in paraffin. Sections of 5 µm thickness were cut with a manual microtome, stained with Hematoxylin and Eosin (H&E). The stained sections were examined under the microscope and the different cell types were carefully studied and photographed (Bancroft and Gamble 2002).

Statistical analysis

The statistical analysis was conducted using SPSS (Software packing version 18). Treatment was compared by applying one way ANOVA. Post hoc test (LSD) was performed. The difference was considered significant at $p<0.05$.

Results

The effect of mercuric chloride ($HgCl_2$) on sperm motility, count and abnormality is shown in (Table 1). The results indicated that the administration with $HgCl_2$ caused a significant ($p\geq0.05$) decrease in both sperm counts and sperm motility with associated significant ($p\geq0.05$) increase in the percentage of abnormal sperms in dose dependent manner as compared with the control group. Sperm morphology test of treated mouse with different doses of mercuric chloride showed different sperm phenotype abnormalities such as Club-shaped head, Banana-like head, bent head, fused head, two tails, lasso-like and folded mid piece.

Table 1: Toxic effect of mercuric chloride on sperm count, motility and morphology of mouse.

Treatments	Sperm count (10^6/ml)	Number of motile sperm(10^6/ml)	Number of sperm with abnormal morphology
Control	26.15 ± 0.33	10.87 ± 0.35 (41.56%)	11.00 ± 1.72 (2.2%)
Mercuric chloride (0.5mg/kg)	20.9 ± 0.29*****	7.00 ± 0.37***** (33.49%)	63.37 ± 3.47***** (12.67%)
Mercuric chloride (1.0mg/kg)	17.52 ± 0.62*	3.62 ± 0.49***** (20.66%)	115.62 ±5.04***** (23.12%)
Mercuric chloride (2.0mg/kg)	13.45 ± 0.43*	3.62 ± 0.49***** (26.91%)	171.62 ± 10.28***** (34.32%)

Parameters= mean± SE; *****, significantly different compared to control mice at $p\leq0.05$.
Numbers in parenthesis represent the percentage value.

Histopathological observations:

$HgCl_2$ administration caused prominent histopathological damage in kidneys compared with those of control group mice (Figure 1). The kidney sections from the control group preserved histological structures with normal appearance of glomerulii, proximal and distal convoluted tubules and collecting tubules (Figure 1.A). In contrast, the kidney sections of $HgCl_2$ treated mice with 0.5mg/kg and 1.0 mg/kg showed significant areas of tubular dilation with hemorrhage and degeneration of renal corpuscles, inflammation of renal epithelium, congestion of renal lumen and cloudy swelling (Figure 1.B and Figure 1.C).

The kidney treated mouse with 2.0 mg/kg showed hydropic degeneration of renal epithelial tubules (Figure 1.D).

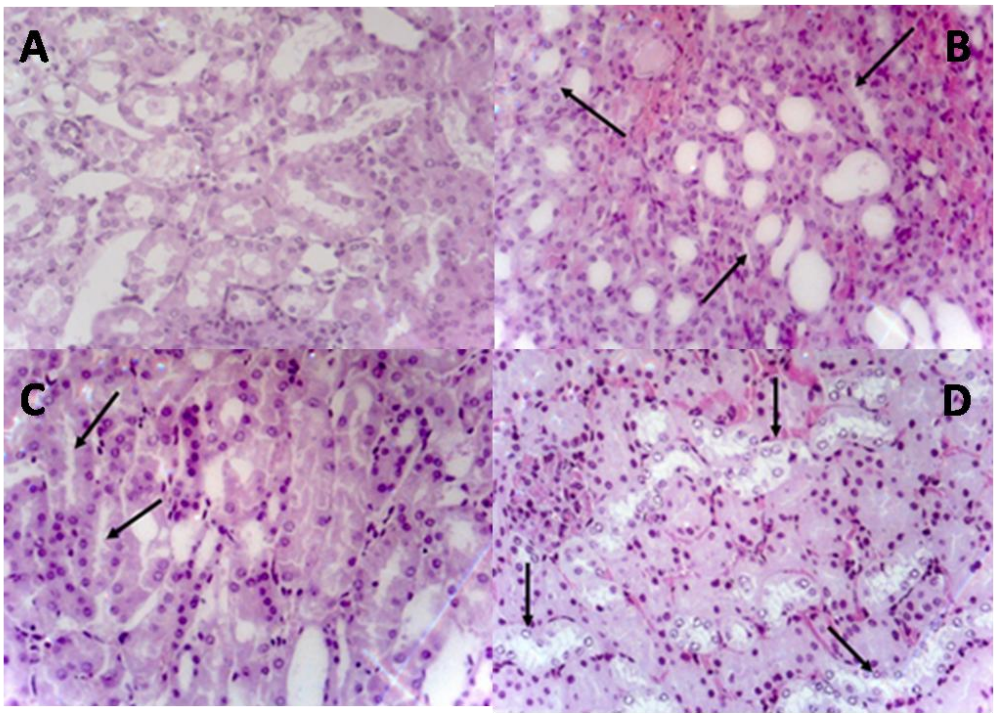

Figure 1: Histopathological changes in kidney of mouse: **(A)** photomicrographof untreated mouse kidney (control), (B) Photomicrograph of treated mouse kidney with 0.5 mg/kg $Hgcl_2$ showed renal tubular dilations with hemorrhage and degeneration of renal corpuscles, inflammation of renal epithelial and congestion of renal lumen, Cloudy swelling, (C) Photomicrograph of treated mouse kidney with 1.0 mg/kg $Hgcl_2$, (D) Photomicrograph of treated mouse kidney with 2.0 mg/kg $Hgcl_2$ showing hydropic degeneration of renal epithelial tubules.

Discussion

The mercuric chloride toxic effect is due to its ability to forms organomercury complexes with proteins (Lorshieder *et al*.1995). It has great affinity for thiol-groups of Biomolecules, such as glutathione (GSH) and sulfhydryl proteins, which may contribute to its toxicity (Hansen *et al*.2006). In the present study the administration of different doses (0.5, 1.0 and 2.0 mg/kg) of $HgCl_2$ caused significant ($p \leq 0.5$) reduction in both sperm count and sperm motility with associated significant increase in the percentage of abnormal sperms as compared with the control groups, which assure with those results obtained by other investigators (Otitoloju *et al*. 2010 and Rao and Sharma, 2001).

These changes may be attributed to impairment of sperm maturation and secretory functions of epididymal cells which might be due to oxidative stress or insufficiency of androgens. It was also in agreement with Khan *et al*. (2004) which reported that mercuric chloride produced adverse effects on reproductive performance of mice even though the route of administration was different. Moreover, it was consistent with the investigation of Fossato da silva *et al*.(2011). Which relate the disturbance in sperm parameter (increase in sperm abnormal shape and decrease in both sperm motility and total sperm count) to the effects of mercuric chloride on testicular spermatogenic and steroidogenic functions in experimental animals.

It is well known that mercuric chloride is one of the pro-oxidants that induce oxidative stress which cause lipid peroxidation and membrane damage leading to loss sperm motility (Vachhrajani *et al*.1988). Mercury can concentrate in the kidneys and testes, leads to kidney failure and infertility through the effects on epididymis and interfere in spermatogenesis (Eto *et al*.1997), these facts might be the explanation for the decrements in sperm count, motility and morphology in this study.

Histopathological examination revealed that mice administered with different doses of mercuric chloride shows disturbance in structural integrity and cloudy swelling compared with control group, it is well known that the mercuric chloride accumulate more in renal epithelium, so the endothelial damage by reactive oxygen species could be the main sources for producing large scale hemorrhages in kidney ,liver ,brain and heart. Degenerative changes in the renal tubules epithelium of kidneys may be due to deposit of mercuric chloride in the kidneys, this results was in agreement with other investigators (Oda *et al*.2012 and Gado *et al*.2014). The pathological changes in the kidney tissue include interstitial oedema, tubular dilation, and sloughing of individual epithelial cells. Also administration of mercury chloride dose 2.0 mg /kg body weight causes hydropic degeneration in renal epithelium.

Conclusion:

On the basis of sperm parameters, and kidney pathological feature, our finding suggest that exposure to mercuric chloride in male Swiss albino mice causes severe tissue damage in all segments of kidneys and has different effects on sperm parameter in short time. It becomes clear that mercuric chloride exhibit considerable toxic effect on most vital organs. So there are urgent needs to develop preventive measures to contain mercuric chloride toxicity.

References

ATSDR (Agency for Toxic Substances and Disease Registry): Toxicological profile for mercury. (1999). Atlanta: Department of Health and Human Services, *public health Service.*

Bancroft JD, Gamble M (2002). Theory and practice of histological techniques 5th ed. Philadelphia: Churchill Livingstone, pp.125-138.

Crinnion WJ (2000). Environmental medicine, part three: long-term effects of chronic low dose mercury exposure. *Altern Rev Med.*5:209-223.

Eljaafari HA (1995). Mutational effect of Acrylamide on mouse testes and sperm parameters. *M.Sc. Thesis,* Zoology department, Faculty of Science , University of Tripoli. Tripoli- Libya.

Eto K, Yasutake A, Miyamoto K, Tokunaga H and Otsuka Y (1997). Chronic effects of methylmercury in rats.II. Pathological aspects. Tohoku *J Exp Med.*182:197-205.

Ficsor Gand Ginsberg LC(1980). The effect of Hydroxyurea and Mitomycin C on sperm motility in mice. *Mutation Res*.70:383-387.

Fossato da Silva DA, Teixeira CT, Scarno WR, Favareto AA, Fernandez CDB, Grotto D, Barbosa JrF and Kempinas WDG (2011). Effects of methyl mercury on male reproductive functions in Wistar rats. *Reprod Toxicol*.31:431-439.

Gado A, AbdelNasser I, Meshaal R, Majed MA, Fahad IA, Faheid AA and Khalid AA (2014). Effect of Carnitine against mercuric chloride- induced Nephrotoxicity. *J Am Sci*.10(3):44-48.

Gul A, Yilmaz M and Selvi M (2004). The study of the toxic effects of mercury-II- chloride to chub Leuciscus cephalus. *G.U.J.Sci.*17:53-58.

Hansen J. Zhang H, Hones DP (2006). Differential oxidation of thioredoxin-1 thioredoxin -2, and glutathione by metal ions. *Free Radic Biol Med.*40:138-145.

Jagadeesan G, Sankarsami Pillai S (2007). Hepatoprotective effects of taurine against mercury induced toxicity in rats. *J Environ. Biol.*28:753-756.

Joseph Mercola DO and Klinghardt MD (2001). Mercury toxicity and systemic elimination agents. *J Nutr Environ Med*. 11:53-62.

Khan AT, Atkinson A, Grahan TC, Thompson SJ, Ali S and Shireen KF (2004). Effects of inorganic mercury on reproductive performance of mice. *Food Chem Toxicol* 42:571-577.

Lorschieder FL, Vimy, MJ and Summers AO (1995). Mercury exposure from silver tooth filling: emerging evidence questions a traditional dental paradigm. *FASEB J.* 9:504-508.

Oda SS, El-Ashmawy IM (2012). Protective effect of Silymarin on Mercury - induced Acute Nephro Hepatotoxicity in Rats. *Global Veterinarria* 9(4):376-383.

Otitoloju AA, Otubanjo OA and Osunkalu VO (2010). Preliminary study on the Induction of sperm head abnormalities in mice, *Mus musculus*, exposed to Radiofrequency Radiations from global System for mobile Communication Base Stations. *Bull Environ Contam Toxicol* (84):51-54.

Rao MV and Sharma PS (2001). Protective effect of vitamin E against mercuric chloride reproductive toxicity in male mice. *Reprod Toxicol*. 15(6):705-712.

Sakamoto J and Hashimoto (1986). Reproductive toxicity of acrylamide and related compounds in mice: Effect on fertility and sperm morphology. *Arch Toxicol.*59:201-205.

Sharma MK, Patni R, Kumar M and Kumar A (2005). Modification of mercury induced biochemical alterations in blood of Swiss albino mice by Spirulina fusiformis. *Environ Toxicol har.*20:289-296.

Stacchitti A, Borsani E, Rodella L, Rezzani R, Bianchi R and Lavazza A (2003). Dose-dependent mercuric chloride tubular injury in rat kidney. *Ultrastructural Pathol.* 27(4), pp.253-259.

Trasande BL, Landrigan PJ and Schechter C (2005). Public health and economic consequences of methyl mercury toxicity to developing. *Environ Health Persp*.113:590-596.

Vachhrajani K, Makhija S,Chinoy N, Chowdhury A (1988). Structural and functional alterations in testis of rats after mercuric chloride treatment. *J Reprod Bio Compara Endocrinol.* 8:97-104.