The toxic effect of nickel chloride (II) and potassium dichromate (VI) on the activity of reproductive system in male mice

Abeer cheaid yousif AL-fatlawi

Faculty of applied medical sciences, university of Karbala, Iraq.

Abstract:

Increase distribution of heavy metal and its compound in the environment, especially through anthropogenic and natural activity, raises increasing concern for toxicological effects. The present study was based on the fact that Ni and Cr elements are important as the environmental factor produce the male genital system abnormalities. 35 male mice (10 weeks old) were randomly divided into seven groups 5 animals for each group, group 1 served as control received tap water, group 2,3 and 4 received (20, 40, and 60 mg/kg of $Nicl_2$ respectively), while group 5,6 and 7 received (20, 60, and 100 mg/kg of $K_2Cr_2O_7$ respectively). The results showed a high significant decrease ($P \leq 0.0001$) in the sperm count of male mice for intermediate and high dose treated with $Nicl_2$ (II) as compared with control group, while no any significant differences between the lowest doses as compared with control group. The result showed a high significant difference ($P \leq 0.0001$) in the percentage of sperm abnormalities for intermediate and high dose treated with $Nicl_2$ (II) as compared with control group. Also the result showed a high significant difference ($P \leq 0.0001$) in the sperm count for all groups treated with different doses of $K_2Cr_2O_7$ (VI) as compared with control group. About percentage of sperm abnormalities the result showed a high significant difference ($P \leq 0.0001$) in the percentage of sperm abnormalities for intermediate and high dose treated as compared with control group.

Key words: potassium dichromate, nickel chloride and testicular toxicity,

Introduction

Natural environmental factors as well as different anthropogenic elements and many other sources toughly influence in the reproductive system for both humans and animals (Fergusson, 1990). Heavy metals consider an important group of ecophysiological influence among these sources, these elements causes many defects on the semen fluid and they are divided into several groups:

1. Essential elements: it have high physiological role such as (Ca. K, Na, Mg and Fe).
2. Less toxic elements: (we can note the relatively narrow ranges of tolerance of these elements, with concentration in "excess" and "deficiency", which is toxic for semen) such as Cu, Zn, Mn, Ni, Cr and Co.
3. High toxic heavy metals; even a trace amount of these elements in semen is very dangerous and harmful such as Pb and Cd, and Hg (Marzec *et al.*, 2012).

The toxicity of heavy metals may cause many pathological and physiological dysfunctions of organs (Al-Harrby *et al.*, 2011), in which heavy metals have classical testicular toxicant such as elicit apoptosis of germ cells, damage of spermatogonia and spermatocytes, activated of reactive oxygen species (ROS) and form free radicals, alteration and damages mouse sperm DNA (Obregon and Hartley 2008). Nickel and chrome elements are natural components found in the crust of earth, they cannot be degraded or destroyed, these elements are very dangerous at high concentration and can be transferred by water, air and human food chain (Abdul-Wahab and Marikar, 2011; Pandey and Maduri, 2014).

These elements are produce mainly by anthropogenic source like tannery facilities, chromate production, stainless steel welding, chrome pigment production, inks, leather tanning, chrome plating, paints, plastics, fungicides, the ceramic and glass industry, in photography, chrome alloy , corrosion control nickel- cadmium batteries, coins, jewelry, plate and screw used for connecting bones in orthopedics surgery and in the manufacture of artificial organs, nickel-chrome plate (Samir *et al.*, 2012; WHO,2007).

Many recent studies have indicated an increasing prevalence of various abnormalities and disorder of the reproductive system in human and animal males, there is growing concern about the considerable decrease in sperm density and denaturation of sperms (Viskum *et al.*, 1999; Lerda, 1992). Exposure to heavy metals is a risk factor in the assessment of spermatogenesis. Marzec *et al.*,(2012) heavy metals is suspected as one of the environmental sources of pollution liable to decrease the sperm quality; Rabbits receiving the dose of AlCl3 at 34 mg/kg body weight displayed decreased sperm concentration, sperm motility and ejaculate volume (Yousef *et al.*, 2005). As well as laboratory mice injected with 1 mg/kg of CrO3 causes increased rates of sperm abnormalities decreased sperm counts and percentages of motile sperm (Acharya *et al.*, 2006). Additionally, increased percentages of morphologically abnormal spermatozoa were found in men occupationally exposed to Cr (VI) (Oliveira *et al.*, 2010). Other study showed that Cr (VI) considered the major risk factor on the growing and adults testis (Ernst, 1990; Saxena *et al.*, 1990).

The tissues of testis are target organ for a metal that causes free radical by induce oxidative damage (Acharya *et al.*, 2004). Massanyi *et al.*, (2007) sowed disorder effects in the structure and function of the seminiferous tubule at the site of production spermatozoa in male mice when received different doses of nickel chloride.

Material and methods:

Animals and doses preparation: Healthy mature Albino Mice were used in this study. Animals were bred in the animal house of pharmacy College, Karbala university under control temperature, 22±2 Cº, at (12) hours light and (12) hours dark. The mice were housed in plastic cages measuring 30×12×11 cm. The experiment starts from February to March 2014. Thirty five mature mice (20-25g) (10 weeks old) were divided randomly into 7 groups each with 5 mice. Group 1 served as control received tap water, group 2 received (20 mg/kg of $Nicl_2$), groups 3 and 4 received (40 and 60 mg/kg of $NiCl_2$) while group 5 received (20 mg/kg of $K_2Cr_2O_7$), groups 6 and 7received (60 and 100 mg/kg of $K_2Cr_2O_7$) all groups received doses orally for 6 weeks then doses calculate according to the body weights by methods of (Suckow *et al.*, 2001).

Testicular Sperms Counts:

Mice testis was cutting in to the very small pieces by using scissor and razor, until seminiferous tubules were cut to so small pieces where no intact tubular can be seen in order to release the sperm (homogenizer completely). Then sample was transfer to test tube contain 0.2 ml of sperm count solution and 9.8 ml of formal saline. After mixed very well sperms were counted by haemocytometer slide under (10X) object lens of light microscope. The sperm was calculated by using the following equation:

Sperm counts=N/80 X 400 X 10 X 10 X 1000.
Where values in equation represents:
80=counted small field of haemocytometer.
400=total area of small fields
10=depth of slide.
10=dilution rate.
1000=to convert sperm number in 1ml, Seed *et al*., (1996).

Sperm counts solution:

Sperm count solution was prepared as following: Add 0.2ml eosin stain solution to 9.8ml formal saline.

1. Eosin stains solution prepared by dissolved 1gm eosin in 100ml of sodium citrate solution (6gm sodium citrate dissolved in 200ml D.W).
2. Formal saline prepared by adding 10 ml of 40% formalin to 90 ml normal saline (Seed *et al.*, 1996).

Finally dividing the total number of sperms on the total weight of the testis (Sakamoto and Hashimoto, 1986).

The percentage of sperm abnormalities:

The testis was immediately cut transversely into two parts and fresh smear was taken by allowing the cut surface of the testis to touch a clean and warm slide ($37c^o$) then added 1-2 drops of physiological saline, 1-2 drops of eosin-necrosin stain in the same temperature after that mixed together for half minutes. And took the tip of the second slide is part of the mix pulls at a sharp angle and gently the first slide. Prepared slides were then air dried before being examined for the detected percentage of abnormalities sperms by accounted in a hundred sperm within the microscope field according following equation: (Hemavathi and Rahiman, 1993).

Percentage of Sperm abnormality % =number of abnormal sperms/total number of sperms *100

Abnormal Sperm Morphology

Statistical analysis:

Data were analyzed using SAS View 512+ Software (Abacus Concept, Inc. Calabasas, CA, USA). Differences among groups were measured using one way analysis of variance (ANOVA) followed by the least significant differences. The results were expressed as means ±SEM and differences were considered statistically significant at ($p \leq 0.0001$) for both sperm concentration and sperm abnormality (AL-Rawi, 2000).

Results:

Sperm concentration and percentage of abnormality

The result of present study in the table (1) showed very high a significant ($p<0.0001$) decrease in the concentration of sperm for both groups treated with Nickel chloride ($NiCl_2$) (II) at doses 40 and 60 mg/kg of body weights as compared with the control group, while no any significant differences between theses doses (40 and 60 mg/kg) itself, as well as no any significant differences between the lowest dose as compared with the control group, on other hand as no any significant differences between the lowest dose and intermediate dose.

The same table showed very high a significant difference ($p<0.0001$) in the percentage of sperm abnormalities for all doses treated with $NiCl_2$ as compared with the control group, while no any significant differences between the lowest and intermediate dose, as well as no any significant differences between intermediate and the highest dose.

From table (2) the result demonstrated that very high a significant ($p<0.0001$) decrease in the concentration of sperm for all doses treated with potassium dichromate ($K_2Cr_2O_7$) (VI) as compared with the control group, the result also showed as no any significant differences between the lowest and intermediate, but both these doses have a significant differences ($p<0.0001$) with a highest dose in the concentration of sperm.

Table (2) also revealed no significant differences in the percentage of sperm abnormalities between the lowest dose as compared with the control group, and between the lowest doses as compared with intermediate doses. While high a significant differences ($p<0.0001$) among intermediate and high doses as compared with the control group.

Table (2): The effects of different doses of Nickel chloride on the concentration of sperm and the percentage of sperm abnormalities.

*

Concentration of $NiCl_2$ (mg/kg)	The concentration of sperm 10^6/g	The percentage of sperm abnormalities %
Control	27.620* ± 1.5628 A**	0.06600 ± 0.0081 C
20	23.468 ± 4.6026 AB	0.34800 ± 0.04103 B
40	15.038 ± 1.8269 BC	0.4440 ± 0.0614 AB
60	13.340 ± 2.4697 C	0.5300 ± 0.0796 A
LSD	8.6195	0.1579

Significant difference ($P\leq0.0001$), mean ± stander error, **Different letter refer to significant difference, LSD (least significant differences). While similar letters refer to no any significant difference

Table (2): The effects of different doses of potassium dichromate on the concentration of sperm and the percentage of sperm abnormalities.

Concentration of Cr element	The concentration of sperm	The percentage of sperm abnormalities %
Control	27.620 * ± 1.5628 A**	0.06600 ± 0.0081 C
20	16.618 ± 2.0537 B	0.37200 ± 0.0603 B
60	12.544 ± 2.6541 B	0.48600 ± 0.0422 AB
100	6.704 ± 1.0650 C	0.63200 ± 0.0796 A
LSD	5.7744	0.1631

*

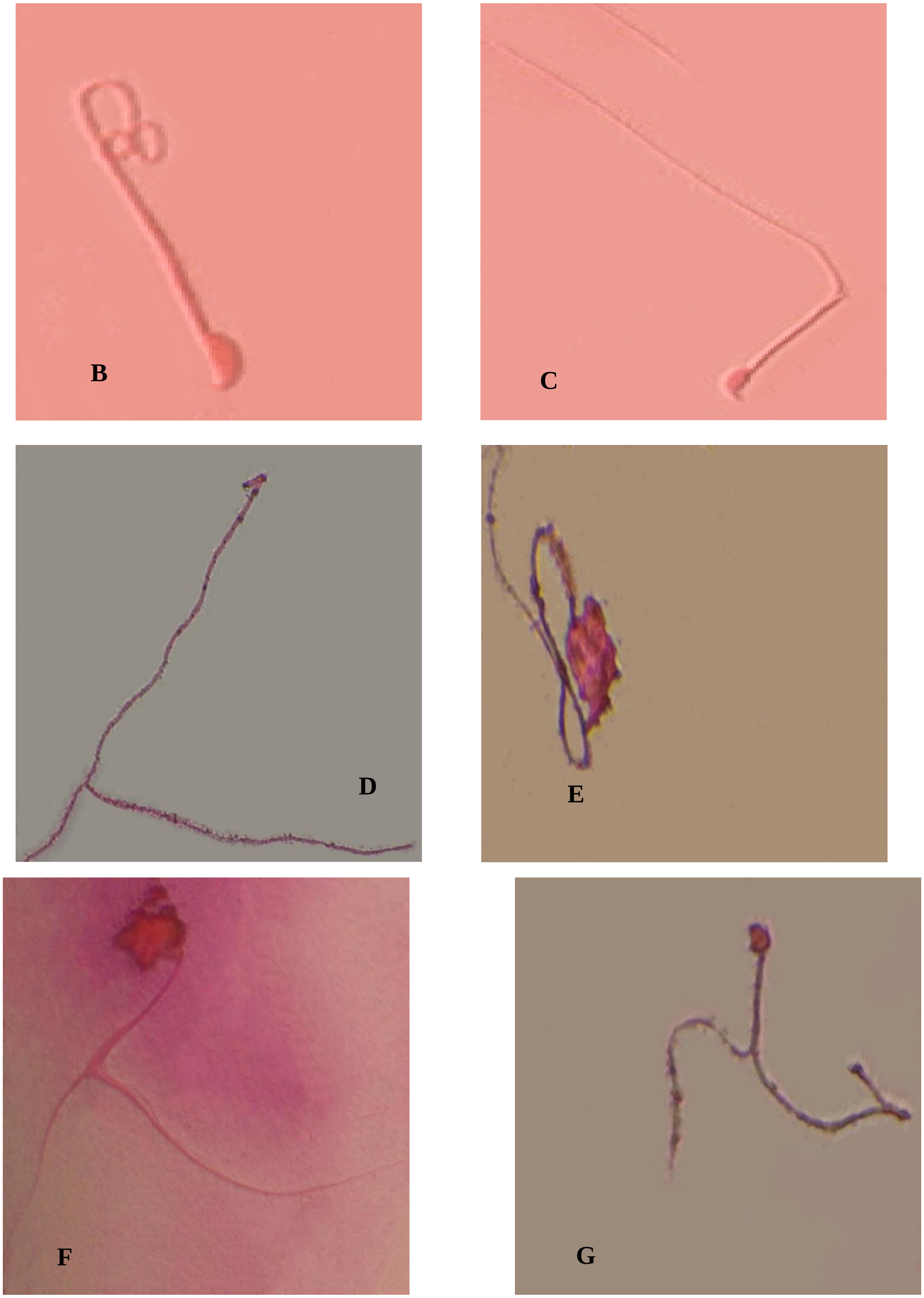
Significant difference (P≤0.0001), mean ± stander error, **Different letter refer to significant difference, LSD (least significant differences). While similar letters refer to no any significant difference.

The morphology of sperms denaturation

The result demonstrated different deformities obtained through images of sperm for male mice treated with different doses of elementals Ni and Cr for (6 weeks), which has been obtained different abnormalities figure of sperms as compared with the control group figure (1), and this abnormalities include the head, middle piece and tail area figure (2).

A

Figure (1) demonstrated normal sperm of male mice


B
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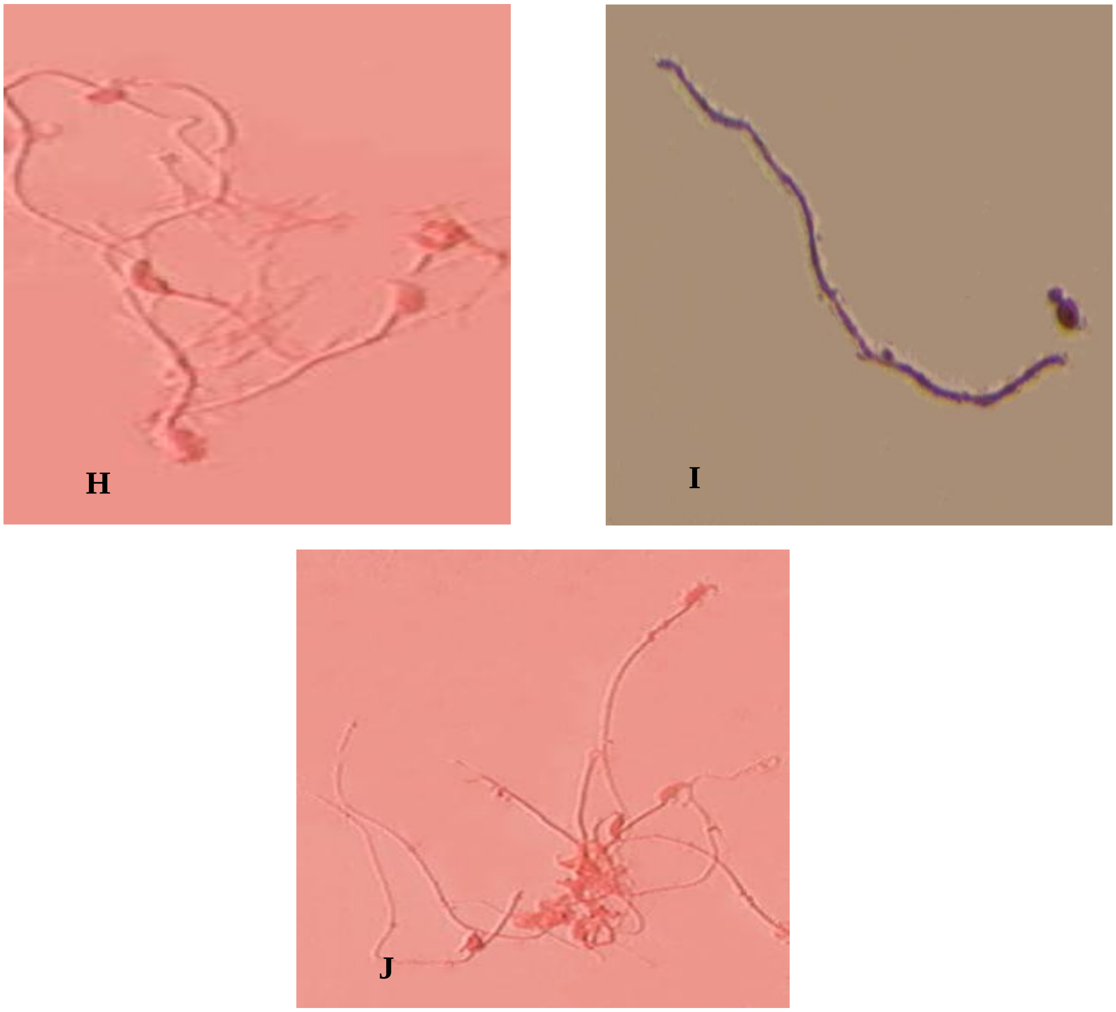

Figure (2): demonstrated denaturation of sperm for male mice treated with different doses of Ni (II) and Cr (VI) elements.

- **A.** Intact sperm with normal head middle piece and tail as control (100X).
- **B.** Denaturation occurred of tail sperm (coiled tail) (200X).
- **C.** Denaturation occurred of head sperm (Tapered head) and denaturation occurred of tail sperm (coiled tail) (100X).
- **D.** Denaturation occurred of head sperm (Amorphous head) and denaturation occurred of tail sperm (Splitting tail) (40X).
- **E.** Denaturation occurred of head sperm (Macrocephalic), denaturation of middle piece and denaturation of tail (coiled) (400X).
- **F.** Denaturation occurred of head sperm (Amorphous head) with double tail (Splitting tail) (400X).
- **G.** Denaturation occurred of head sperm (Amorphous head) and denaturation occurred of tail sperm (Splitting tail) (100X).
- **H.** Adhesion occurs in sperm (100X).

I. Denaturation occurred of sperm (removed head) (100X).
J. Sever adhesion in sperm (100X).

Discussion:

Many result of different study revealed increasing effects of heavy metals on reproductive system in male's mice and causes increasing prevalence of various abnormalities of sperms. The observations and results of the present study demonstrated that nickel and chrome elements causes reduced fertility by reduced the sperm's counts and percentage of normal sperms. The reasons behind reduced the sperm's counts can be attributed to the fact that the metal induced oxidative stress in cells can be partially responsible for the toxic effects of heavy metals by generation reactive oxygen species(ROS) and inhibited antioxidant defenses system (Rafique *et at*., 2009a; Chowdhury,2009b ;Lampiao, 2012). The present study in agreement with what found by (Al-Harrby *et al.*, 2011). Other authors have also described a reduction in epididymal sperm counts and motility in male mice treated with different doses of aluminium nitrate returns to decreased the activity of reproductive hormones including LH, FSH and testosterone also the decreased can be attributed to decrease testicular sperms production due to necrosis of seminiferous tubules and dysfunction of it (Al-Dujaili, 2005) many other results showed the same effect (AL-Taee, 2009). Al-Mosawy, (2004) found decreased spermatozoa counts and motility in rats after oral administration of Cr (VI) and $Alcl_3$. The present study in agreement with Sharma and Garu (2011) who demonstrated that necrosis and atrophy in the tissue of testis of male rat when received lead by orally due to decreased of testis volume, seminiferous tubules diameter and germinal epithelium by damage Sertoli and Leydig Cells. Also the present result in agreement with other result such as obtained by (Li *et al.*, 2013). However the present data in agreement with (Alarc *et al.*,2012) who found significant correlations between the measured concentrations of the three heavy metals (Pb,Cd and Hg) in the same biological fluids with decrease motility , denaturation morphology and decrease sperm concentration.

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