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The Comparative Effect of Copper Oxide Nanoparticles and Copper Sulfate on Reproductive Hormones and Sperm Parameters in Mature Male Albino Mice

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ABSTRACT

Copper is one of the transitory elements that living beings require; any variations in the elements' levels may have an impact on the reproductive system. The aim of this study was to compare how this material influences the male reproductive organ by changing reproductive hormone levels and sperms morphology in male albino mice after 14 days of oral gavages treatment of 25 and 35 mg/kg of CuO NPs and $CuSO_4.5H_2O$. In comparison to the control (untreated mice), all of the animal treatments revealed a decrease in the sexual hormone levels and increased abnormality in sperm morphology and count. Both in terms of concentrations, mice given copper sulfate had a greater effect on hormones and abnormality than mice given copper oxide nanoparticles. The current study showed a comparison of CuONPs and $CuSO_4.5H_2O$ in terms of their capacity to cause acute systemic toxicity in response to oral insults.

Key words : Copper oxide nanoparticles, copper sulfate, male reproductive function, sperm morphology, fertilizes hormones

INTRODUCTION

Environmental contaminants (metals and nanometals) have an effect on male reproductive potential. These contaminants deteriorate semen parameters, DNA integrity, hormone biosynthesis, gene expression and epigenetic modifications by interfering with Leydig and Sertoli cell function (López-Botella *et al.*, 2021). The concentration and viability of sperm usually used as a biomarker of chemical-induced testo-toxicity (Ryu *et al.*, 2019).

Nanomaterials have been more widely used in recent years in a variety of fields. Copper oxide nanoparticles (CuONPs) were among the first engineered nanoparticles to be used as industrial catalysts in manufacturing processes, and they are widely used in semiconductor devices, gas sensors, batteries, solar energy converters microelectronics, and heat transfer fluids as well as skin products and face masks (Grigore *et al.,* 2016). CuONPs are also used in textiles, paints, plastics and food containers (Safaei and Taran, 2018) due to its antimicrobial properties. Copper sulphate pentahydrate ($CuSO_4.5H_2O$) is another copper compound with numerous uses and applications in life, as it is widely used in agriculture, pesticides, the leather industry, and as a precipitant in the event of heavy metal poisoning (Lasiene *et al.*, 2016).

The use of copper sulphate or nano copper oxide in both occupational and environmental settings increases the likelihood of exposure via inhalation, oral, and dermal penetration (Tee *et al.*, 2019). It affects many vital processes after entering the body, including spermatogenesis, which affects hormone secretion Follicle-stimulating hormone (FSH), luteinizing hormone (LH) and testosterone (TSS). Khushboo *et al.* (2018) found a decrease in sex hormone levels after 45 days of exposing rats to 1 and 2 mg/kg bwt/day of copper nanoparticles. Additionally, a study by Tara *et al.* (2018) reported that the oral administration of copper sulphate to rats at a concentration of 200 mg/kg resulted in a reduction in the weights of the animals and studied organs, as well as a decrease in the levels of male hormones (TSS).

There is little information on the influence of

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copper and copper oxide nanoparticles on male sex hormone levels. Therefore, the goal of this study was to investigate how $CuSO_4$.5H2O and CuONPs affected the role of male reproductive system by evaluating sex hormones levels (FSH, LH and TSS) and sperm quality and quantity.

MATERIALS AND METHODS

The research was performed on 30 mature male albino mice, weighting (25-30 g) and 10-12 weeks old, obtained from the National Center for Control and Pharmaceutical Research in Baghdad, Iraq and housed in the animal house of the biological department, College of Education for Pure Sciences, Ibn Al-Haitham. Animals were fed a standard commercial pelleted diet and tap water, and they were kept in a 12 : 12 h light : dark cycle at a room temperature of 22-24°C. Mice were kept in cages with woodchip bedding for a week prior to the experiment use.
Animals were randomly divided into five groups of six animals each.

- G I–Control group, animals received distill water.
- G II–Animals received 25 mg/kg bw/ day of 50 nm CuoNPs orally for 14 days.
- G III–Animals received 35 mg/kg bw/ day of 50 nm CuoNPs orally for 14 days.
- G IV–Animals received 25 mg/kg bw/ day of $CuSO_4$ orally for 14 days.
- G V–Animals received 35 mg/kg bw/ day $CuSO_4$ orally for14 days.

Copper oxide nanoparticles (CuONPS) were obtained from Sky Spring Nanomaterials – USA (Particle size of 40 nm and purity of 99%). CuONPs. Copper sulfate pentahydrate $CuSO_4$.5(H_2O) from sky spring nanomaterials –USA, testosterone mouse ELISA (MBS494055). Mouse luteinizing hormone (LH) ELISA Kit MBS04130 (, and mouse FSH (Follicle Stimulating Hormone) ELISA Kit (MBS2507988) were obtained from MyBioSource, Inc. San Diego, CA, USA.
Following the sacrifice of animals, blood was collected in sterile tubes through cardiac puncture. The blood samples were kept in room temperature after being poured into a 1.5 ml micro tube. Then centrifuged for 5 min at 2500 rpm and stored at -20°C for further experiments.
Sperms from epididymis tail were studded, briefly, after epididymis tail was resected, cut, crushed and mixed with 5 ml of saline solution. In a clean glass slide, a drop of tail solution was added and then a drop of eosin dye was placed and mixed the tail smear was left to dry and then fixed with DPX to study sperm abnormality (head, tail, middle piece of sperm and the cytoplasmic droplet site and all slides were examined under light microscopy with a magnification of 40x.
SPSS software (version 24) was used for statistical analysis. All data were presented as means±standard error (SE.). The differences between initial animal body weight before and after the end of treatment were determined using the *t*-test. Other parameters were analyzed using One-Way Analysis of Variance (ANOVA) with least significant difference. When the probability value was less than 0.05, the value was considered statistically significant.

RESULTS AND DISCUSSION

The results showed significant decrease in the percentage of live sperm and the concentration of sperm in the epididymis tails of mice treated with concentrations (25 and 35 mg/kg) of $CuSO_4.5H_2O$ and CuONPs after 14 days compared to the control group. When comparing the groups with copper sulfate at both the concentrations to the groups treated with copper nano oxide at both the concentrations, the decline was greater (Table 1).
The percentage of sperm abnormalities in the epididymis tails of mice treated with concentrations (25 and 35 mg/kg) of $CuSO_4.5H_2O$ and CuONPs showed statistical increase ($P \leq 0.05$) after 14 days in contrast to the control group (Table 1). With increasing concentrations of $CuSO_4.5H_2O$ at both the concentrations, the proportion of sperm deformities increased when compared to the CuONPs groups, moreover, the groups treated with copper sulfate at a concentration of 25 mg/kg showed a more pronounced decline (Table 1). Sperm abnormalities involved sperm head absence, tail absence, tail warp, tail curvature, median piece convolution and cytoplasmic droplets appearance in the median piece (Fig. 1).

Table 1. Changes in the percentage of sperm live, sperm abnormalities and concentration of sperms in epididymis tail of albino mice treated with $CuSO_4.5H_2O$ and CuONPs for 14 days

Treatment groups	Number	Duration of exposure (days)	Sperm live (%)	Concentration of sperm 10^3*	Spermatic abnormalities in epididymis tail (%)
Control	6	14	39.8±3.8a	115.8±334.6a	8.8±0.6a
25 mg/kg $CuSO_4.5H_2O$	6	14	10.9±2.2b*	5458.3±1124.3b*	58.3±4.9b
25 mg/kg CuO NPs	6	14	27.1±2.8b	13.5±1409.3b	37.1±1.6b*
35 mg/kg $CuSO_4.5H_2O$	6	14	19.6±4.4b	10930.5±2064.7b	63.3±2.7b
35 mg/kg CuO NPs	6	14	20.5±3.5b	10291.6±1790.5b	35.5±1.6b*

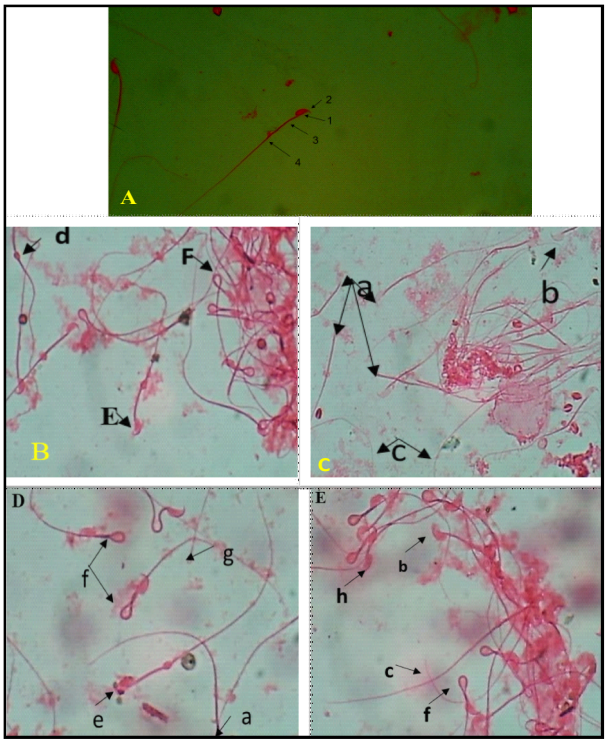

Fig. 1. (A) Control group showed normal sperm in albino mice, (1) Sperm head, (2) Spine of the head, (3) Median piece, (4) Sperm tail, (B) Abnormal sperm in mice treated with 25 mg/kg of $CuSO_4.5H_2O$ for 14 days, (C) Abnormal sperm in mice treated with 35 mg/kg of $CuSO_4.5H_2O$ for 14 days, (D) Abnormal sperm in mice treated with 25 mg/kg of CUONPs for 14 days and (E) Abnormal sperm in mice treated with 35 mg/kg of CUONPs for 14 days. Abnormalities represented by (a) head loss, (b) head enlargement, (c) torsion of the tail, (d) presence of the cytoplasmic drop, (e) loss of the spine, (f) torsion of the median segment and (g) microcephaly.

Values represent mean±standard error. Different vertical letters indicated significant change (P≤0.05) between treatments. *Significant change (P≤0.05) between concentrations.

The results of the study showed a statistically significant decrease in the testosterone hormone (TSS), follicle stimulating hormone (FSH) and luteinizing hormone (LH) compared to the control group as shown in Table 2.

Values represent means±standard error. Different vertical small letters indicated statistically significant difference between treatments (P≤0.05). Similar vertical small letters indicated that the difference was not statistically significant (P≤0.05).

Copper sulfate and copper oxide nanoparticles, according to our findings, can both impair sperm parameters such as motility and morphology, as well as affect hormones level (FSH, LH and TSS) in male mice. Overall, the findings imply that toxic effects posed by copper sulfate were higher than nano at the same concentrations and times utilized in this investigation. The testicular damage, spermatogenesis suppression and sperm proportion are strongly linked with reactive oxygen species (ROS). Physiologically, normal sperm function requires a minimal level of ROS, and sperm function reduced with ROS levels and exceeded the physiological level. Increased ROS production caused phospholipid peroxidation in mitochondria of spermatozoa (Kowalczyk, 2022). Increase in the level of malondialdehyed (MDA) in homogenous tissue of the testes (data not shown), notably for groups treated with copper sulfate compared to nano oxide groups. Moreover, DNA damage caused by oxidative stress in sperm resulted in a decrease in Leydig cells and TSS synthesis (Guo *et al.*, 2021). Copper exposure caused morphological and physiological changes, as shown in a study by Parlak *et al.* (2021) who found that orally administrated of 500 mg/kg of Cu for 14 days increased fat oxidation in the testes and decreased antioxidant concentrations, affecting sperm quality and increasing abnormalities for spermatogenesis and

Table 2. Changes in hormone levels in albino mice treated after 14 days with CuONPs and CuSO4 at 25 and 35 mg/kg

Treatments	No. of animals	Duration of exposure (days)	Hormone levels (ng/ml)		
			TSS	FSH	LH
Control	6	-	0.140± 0.049a	16.615±1.315a	22.158±1.055a
25 mg/kg CuO NPs	6	14	0.040± 0.007b	6.135±0.910b	7.584±1.256b
25 mg/kg CuSO4	6	14	0.072±0.024a	10.135±1.320c	16.845±2.193c
35 mg/kg CuO NPs	6	14	0.036± 0.009b	9.198±1.074b	7.928±1.005b
35 mg/kg CuSO4	6	14	0.018±0.002b	7.194±0.747c	3.779±1.518b

suppressing spermatogenesis. Immunosuppression was also reported after high dose/chronic dose of copper compounds due to the damage of cells of immune system organs by arrest G0/G1 and apoptotic via P73 pathway of liver cells (Keswani *et al.*, 2015).

Further, differences in dissolution played an important role in the toxicological differences between CuONPs and copper sulphate (El-Bialy *et al.,* 2020). Cu ingestion at high doses had been reported to influence cellular and immunological responses. Higher dosages of copper reduced serum gonadotrophins and testosterone either by acting with pituitary receptors which resulted in pituitary secretion of FSH and LH, as well as testosterone release from Leydig cells, reduced following copper treatment. This was because nanoparticles disrupted the Steroidogenic Acute Regulatory protein (STAR), a cholesterol transporter that regulated cholesterol transport to the inner mitochondrial membrane and stimulated steroid hormone synthesis (Manna *et al.*, 2016). Additionally, higher dosages of copper limited the action of these enzymes, which reduced steroidogenesis and blood testosterone levels.

Other suggested mechanism could be due to the NPs being given at low concentrations, which could have a protective effect due to their antioxidant functions, whereas excessive dosages could be toxic. Thus, more research is needed to determine if the influence responses to CuONPs found in this study are reversible and long term effects, considering the possibility of short time delay in the onset of CuO NPs pathologies.

CONCLUSION

Exposure to CuONPS and $CuSO_4.5$ (H_2O) impaired sperm morphology and decreased the level of reproductive hormones (FSH, LH and TSS). The effects were stronger in animals given copper sulfate at a concentration of 35 mg/kg, indicating that the substance had a toxic effect on the reproductive system and aqueous copper sulfate had the ability to penetrate biological barriers, and accumulate in tissues, and cause cell death and oxidative stress.

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