Effects of copper nanoparticles on reproductive organs of male albino rats

Genan A. Al- Bairuty and Mohammed N. Taha

Dept. of Biology / College of Education for Pure Sciences –Ibn Al-Haitham / University of Baghdad / Republic of Iraq

E –mail: galbairuty@yahoo.com

ABSTRACT

Copper nanoparticles (Cu-NPs) are widely used in various industrial and commercial applications and little is known about their potential toxicity on reproductive system. In the present study, the effect of Cu-NPs on the weight of some reproductive system organs and the sperm characteristics of male albino rats were examined. Rats were administrated with 0.5 ml of 20 and 40 mg/kg/BW Cu-NPs intraperitoneally for 3, 6 and 9 days. The results showed statistically significant decrease in the final body weight and increase in the relative sex organs (testis, epididymis, seminal vesicles and prostate gland) with both Cu-NPs treatments compared to control group. A significant decrease was found in the percentage of sperm viability and increase in the percentage of sperm abnormalities and sperm concentration were observed with both Cu-NPs concentrations compared to the control with concentration and time effects.
Thus, the results of this study revealed for the first time that intraperitoneal injection of Cu-NPs has a negative influence on the effectiveness and activity of male reproductive system in albino rats.

Keywords: Copper nanoparticles, Organs weight, Sperm characteristic, Rats, Testes, Epididymes

---

الملخص باللغة العربية

تحظى الجزيئات النانوية (Cu-NPs) بشهرة واسعة من خلال استخداماتها في العديد من الصناعات، كما أن لها تطبيقات تجارية كثيرة، ولكن لا تزال المعلومات حول تأثيرها على الجهاز التناسلي غير كافية.

سعت هذه الدراسة إلى الكشف عن تأثير جسيمات النحاس النانوية (Cu-NPs) على أوزان بعض الأعضاء التناسلية ومواصفات النطف في ذكور الجرذان البيضاء.

تم حقن الجرذان داخل البريتون بمقدار 0.05 مل من تركيزين مختلفين من جسيمات النحاس النانوية (Cu-NPs) 20، 40 ملغم / كليوغرام/ وزن الجسم لمدة 3، 6، 9 أيام. وقد أظهرت النتائج وجود فروقات إحصائية معنوية تمثلت بانخفاض أوزان الجسم النهائية وزيادة في الوزن النسبي للأعضاء التناسلية (الخصي، البرابخ، الحويصلات المنوية، وغدة البروستا ت) لكلا الجرعتين من جسيمات النحاس النانوية مقارنة مع مجموعة السيطرة. من ناحية أخرى، انخفضت النسبة المئوية لحيوية النطف إحصائيا مع زيادة ملحوظة في النسبة المئوية لتشوهات النطف وتركيزها لكلا الجرعتين من Cu-NPs مقارنة مع مجموعة السيطرة ، وكان التغير في هذه النسب متأثراً بالتركيز ومدة التعرض. وقد خلصت الدراسة للمرة الأولى إلى أن حقن الجسيمات النانوية داخل البريتون في الجرذ له تأثير كبير على نشاط وفاعلية الجهاز التناسلي الذكري للجرذ الأبيض.

INTRODUCTION

Nanoparticles are coming into our daily life in excessive quantity. The unique characteristics of nanosubstances, such as size, large surface area, ultrahigh reactivity, and shape effects allow them to produce many specific effects regarding their bulky states (1). Recently, a new form of Cu metal has been engineered containing of nanoscale copper particles (Cu-NPs), which are considered as type of metal-containing nanomaterials (2). Cu-NPs are used in lubricants, polymers/ plastics, textiles, metallic coating, antimicrobial, home appliances, wear resistance and inks, etc (3,4). Therefore, nano copper are likely to distribute in the environment and enter human body via different paths such as effluent, disposal, and consumer products (3) and cause toxicity via pulmonary, oral, nasal, skin or other routes of exposure. Then, nanoparticles can penetrate through cell membrane via blood-brain barrier and blood-testis barrier (5,6) to reach and impact all organs of the body (7). In *In vitro* study, Cu-NPs and CuO-NPs were found to be able to generate oxidative stress (8,9). In fish, Ostaszewska *et al*.(10) found that 0.15 mg L^{-1} of Cu-NPs caused pathological changes in epidermis (irregular structure and pyknotic nuclei), gills (lifting epithelium, fusion of lamellae and epithelial necrosis), and liver (dilation of sinusoid space, pyknotic nuclei and overfilled blood vessels) of *Siberian sturgeon* larvae after 21 days of exposure. Additionally, Al-Bairuty *et al.*(11) found that 20 and 100 $\mu g l^{-1}$ of Cu-NPs caused pathologies in gills, liver, kidney, gut, brain and muscle of rainbow trout after 10 days of exposure. In *in vivo* study, oral administration of Cu-NPs in mice caused injuries in the kidney, liver and spleen (3). However, there are still scarce and controversially evident of effects of Cu-NPs on spermatozoa parameters and other reproductive indices such as organs weight and pathologies. Little is known about the effects of other types of nanoparticles on reproductive organs and sperm characteristic in animals. For example oral administration of 20 µg/kg/day silver nanoparticles (AgNPs) caused disorganization of the germinal epithelium with loss of some spermatogenic cells types (spermatocytes and spermatids) and degeneration, necrosis as well as atrophy of the seminiferous tubules after 90 days of exposure (12). Kim *et al.* (13) found that oral administration of AgNPs in male rats caused decline in body weight, accumulation of NPs and enlargement of testis as well as some hepatocyte toxicity after 90 days of exposure. Oral administration of AgNPs in male albino mice caused decrease in the weights of testis and tunica albuginea, epididymis head and tail, and decline in the percentage of sperm vitality and sperm concentrations as well as an increase in the percentage of sperm abnormalities were observed in both testis and epididymis after 5, 10, 15 days of exposure (14). Ema *et al*. (15) had reviewed that nanoparticles are able to penetrate into reproductive tissue through biological barriers and may damage various cells such as reduce sperm numbers, viability and change cell functions, as well as inhibit the embryo development. However, there is a gap information about the effect of intraperitoneal injection of Cu-NPs on the male reproductive system indices. Therefore, the aim of this study was to investigate the effect of intraperitoneal injection of different doses of Cu-NPs (20 and 40 mg/kg/BW) on body weight, the weight of some organs of male reproductive system, and sperm characteristic in testes and epididymes (head, tail) of albino rats.

MATERIALS AND METHODS

Characterization of copper nanoparticles (Cu-NPs)

The powder of Copper nanoparticles were purchased from Xuzhou Hongwu Nanometer material CO.,LTD. with 20-30 nm size and purity 99.99% (manufacture Hongwunanometer).

Animals and treatments

Male albino rats (197- 200 gms weight and 10-12 weeks old) were obtained from National Center for Control and Pharmaceutical Research /Baghdad/ Iraq and housed in animal house of biological department/ College of Education for Pure Sciences/ Ibn Al-Haitham. Animals were fed with standard commercial pelleted diet and tap water and maintained under a 12:12 h light: dark cycle and room temperature 22–24°C. Rats were kept in polycarbonate cages with woodchip bedding and acclimatized to the environment for 2 weeks prior to experimental use. Animals were randomly divided into 3 groups (n =54). The animals were assigned to each experimental group, with 18 animals per group and 6 per day.

Group 1 - Control group of animals were intraperitoneal injected with 0.5 ml of distilled water once a day for 3, 6, and 9 days (each day have 6 animals).
Group 2 –Experimental group of animals were intraperitoneal injected with 0.5 ml of 20 mg/kg/BW Cu-NPs once a day for 3, 6, and 9 days (each day have 6 animals).
Group 3 – Experimental group of animals were intraperitoneal injected with 0.5 ml of 40 mg/kg/BW Cu-NPs once a day for 3, 6, and 9 days (each day have 6 animals).

Preparation of copper nanoparticles

The low (20mg/kg/BW) and high (40 mg/kg/BW) concentrations of Cu-NPs suspensions were prepared by weighting 0.8 and 1.6 g of Cu-NPs powder and diluted with 100 ml of distilled water, then stirred with magnetic stirrer overnight and next dispersed by ultrasonic vibration for 30 min. Before injection, the low and high concentrations of Cu-NPs were vortexed in order to avoid the aggregation of the particles.

Animal weighting, sample collection and preparation

Before and after the end of 3, 6, and 9 days of the exposure duration, six rats were weighted by using a Mettler pc Balance in order to record Initial and final body weights. Then, rats were anaesthetized (using diethyl ether) and sacrificed for samples collection from each groups.

Organs samples

Testis, epididymis (head and tail), seminal vesicle and prostate gland from each rats were collected and weighed by using a sensitive balance. The coefficient-relative organ weights were calculated as organ wet weight, (gm)/body weight of animal *100%).

Evaluation of sperm viability, morphology and concentration

The spermatozoa were collected from the testis and epididymis (head and tail) and examined forlive and dead sperms, sperm cell concentration and sperm abnormalities according to (16).

Statistical analysis

All data values were giving as mean ± standard error (S.E.). The resulting data of the current study was analyzed using the SPSS software, version 21. Data were tested for treatment, time, and treatment x time interaction effects by using general linear model/multivariate followed by least squares difference post hoc test. When a statistically significant effect was showed by this model, a one way ANOVA was used to assess for simple effects. The differences were considered significant at level $p \leq 0.05$.

RESULTS

Effect of Cu-NPs on body weight and coefficient-relative reproductive organs weight

The results exhibited significantly decrease ($P \leq 0.05$) in the average body weights of animals after 3, 6 and 9 days of injected with 20 and 40 mg/kg Cu-NPs compared to the same animals groups before injected (table 1). Some concentration and time effects were observed in the average body weight after treated with Cu-NPs in this study where there were more significantly decrease in the average body weight of animal injected with 40 mg/kg Cu-NPs for 9 days compared with 20 mg/kg Cu-NPs for 3 and 6 days (table 1). The average body weight of control animals showed a statistically significant increased ($p \leq 0.05$) after injected with distill water for different periods (6, 9) compared to the same control animal before injected (table 1).

Table (1): Initial and final body weight of rats before and after injected with 20 and 40 mg/kg/BW of Cu-NPs for 3, 6 and 9 days

Treatments	Time/day	Initial body weight before treatment (gm)	Final body weight after treatment (gm)
Control	3 days	200.67 ± 2.91	206.5 ± 3.34 a
20 mg/kg Cu-NPs		202.67 ± 3.99	195.83 ± 4.02 a
40 mg/kg Cu-NPs		197.00 ± 3.36	180.00 ± 3.80 *b
Control	6 days	200.66 ± 1.81	214.00 ± 2.47 *a
20 mg/kg Cu-NPs		197.00 ± 2.69	181.83 ± 2.81 *b
40 mg/kg Cu-NPs		204.66 ± 3.44	167.66 ± 4.36 *c
Control	9 days	199.83 ± 2.66	222.00 ± 1.94 *a●
20 mg/kg Cu-NPs		198.17 ± 1.68	167.17 ± 1.85 *b●▲
40 mg/kg Cu-NPs		197.66 ± 0.66	146.17 ± 2.21 *c ●▲

*Data are mean ±SEM (n = 6 rats/treatment). * Significantly different between initial body weights and final body weights (ANOVA, P < 0.05).Values with different letters within column were significantly different (ANOVA, p ≤ 0.05). ● Significantly different between day 3 and 9 at the same treatment (ANOVA, p ≤ 0.05). ▲ Significantly different between day 6 and 9 at the same treatment (ANOVA, p ≤ 0.05) ■ Significantly different between day 3 and 6 at the same treatment (ANOVA, p ≤ 0.05)*

The coefficient reproductive organs weights (Testis, epididymis head, epididymis tail, seminal vesicle and prostate glands) were statistically significant increased ($p \leq 0.05$) with both 20 and 40 mg/kg of Cu-NPs at different time points compared to the control groups (table 2). More increasing in the coefficient reproductive organs weights were observed over a long time of exposure. The coefficient weight of tunica albuginea was statistically significant increased ($p \leq 0.05$) with high concentration of Cu-NPs at day 9 compared to the control group (table 2). A concentration effect was observed in the coefficient weight of prostate gland and a time effect detected in the coefficient weights of tunica albuginea, epididymis tail and prostate gland (table 2).

Table (2): The coefficient –relative organs weight in albino rats injected with 20 and 40 mg/kg of Cu-NPs for 3, 6 and 9 days

Parameters			Time/ Days	Control	20 mg/kg Cu-NPs	40 mg/kg Cu-NPs
A coefficient-relative organs weights	Testes	Tunica albuginea	3	0.024 ± 0.002 a	0.024 ± 0.001 a	0.024 ± 0.000 a
			6	0.025 ± 0.001 a	0.025 ± 0.000 a	0.027 ± 0.001 a
			9	0.024 ± 0.002 a	0.029 ± 0.002 a●▲	0.035 ± 0.001 b▲
		Testis tissues	3	0.555 ± 0.025 a	0.576 ± 0.021 a	0.578 ± 0.021 a
			6	0.517 ± 0.009 a	0.578 ± 0.022 a	0.662 ± 0.032 b
			9	0.486 ± 0.057 a	0.677 ± 0.036 b	0.675 ± 0.022 b
	Epididymes	Head	3	0.109 ± 0.005 a	0.113 ± 0.005 a	0.106 ± 0.005 a
			6	0.098 ± 0.004 a	0.114 ± 0.006 b	0.127 ± 0.003 b
			9	0.093 ± 0.008 a	0.128 ± 0.008 b	0.135 ± 0.003 b
		Tail	3	0.078 ± 0.002 a	0.086 ± 0.005 ab	0.105 ± 0.009 b
			6	0.081 ± 0.005 a	0.108 ± 0.003 b■	0.125 ± 0.009 b■
			9	0.069 ± 0.004 a	0.124 ± 0.007 b●	0.118 ± 0.009 b●
	Seminal vesicle gland		3	0.271 ± 0.021 a	0.565 ± 0.010 b	0.567 ± 0.033 b
			6	0.224 ± 0.008 a	0.417 ± 0.041 b	0.626 ± 0.108 b
			9	0.276 ± 0.012 a	0.592 ± 0.056 b	0.638 ± 0.124 b
	Prostate gland		3	0.172 ± 0.020 a	0.337 ± 0.057 b	0.313 ± 0.043 b
			6	0.226 ± 0.006 a	0.410 ± 0.048 b	0.356 ± 0.045 b
			9	0.142 ± 0.010 a	0.386 ± 0.027 b▲	0.263 ± 0.030 c▲

Data are mean ±SEM (n = 6 rats/treatment). Values with different letters within row were significantly different (ANOVA, p ≤ 0.05). ● Significantly different between day 3 and 9 at the same treatment (ANOVA, p ≤ 0.05). ▲Significantly different between day 6 and 9 at the same treatment (ANOVA, p ≤ 0.05) ■ Significantly different between day 3 and 6 at the same treatment (ANOVA, p ≤ 0.05)

Effect of Cu-NPs on sperm parameters in testes and epididymis

The effect of daily injected to 20 and 40 mg/kg Cu-NPs for 3, 6 and 9 days on sperm cells life, sperm concentration and sperm abnormalities in testis and epididymes (head and tail) of albino rats were shown in tables (3-5). The percentage of sperm life in the testis and epididymes (head and tail) showed significant decrease ($p \leq 0.05$) compared to control groups of each treatment and time point with more decreasing with high concentration of Cu-NPs and longtime of exposure (table 3).

The sperm concentrations in the testis and epididymes (head and tail) were exhibited significant increased ($p \leq 0.05$) with both Cu-NPs concentration compared to the control (table 4). A concentration effects of Cu-NPs were observed in the testis and epididymis tail with less increase with 40 mg/kg Cu-NPs when compared to 20 mg/kg Cu-NPs at the same time point (table 4).The sperm concentration in the testis and epididymis (head, tail) showed a time effect (table 4). Table 5 reveals significant increase ($p \leq 0.05$) in the percentage of sperm abnormalities in the testis and epididymis head and tail with both Cu-NPs concentrations over longtime of exposure .The elevation of sperm abnormalities in the testis and epididymis (head, tail) showed concentration and time effects with more increasing with high Cu concentration at day 9 when compared to low Cu concentration at day 3 and 6 (table 5).

The sperm abnormalities that observed with both Cu-NPs concentration at day 3,6 and 9 involved deformed head, detached head, coiled head, coiled tail, curved tail and loss of head spine when compared to normal sperm structure that consist of head with spine, middle piece, and tail (figure 1).

Table (3): The percentage of sperms viability (sperms life) in rats injected with 20 and 40 mg/kg/BW of Cu-NPs for 3, 6 and 9 days

Parameters			Time/Days	Control	20 mg/kg Cu-NPs	40 mg/kg Cu-NPs
Percentage of sperm viability	Testes		3	96.67 ± 0.21 a	66.17 ± 0.47 b	64.33 ± 0.88 c
			6	96.50 ± 0.43 a	63.83 ± 0.31 b■	61.67 ± 0.33 c■
			9	97.33 ± 0.33 a	60.83 ± 0.48 b●	56.17 ± 0.48 c●
	Epididymis	Head	3	97.83 ± 0.31 a	64.17 ± 0.48 b	60.33 ± 0.66 c
			6	97.67 ± 0.21 a	61.33 ± 0.33 b■	58.17 ± 0.31 c■
			9	97.83 ± 0.31 a	59.17 ± 0.31 b●	53.67 ± 0.49 c●
		Tail	3	98.17 ± 0.31 a	63.67 ± 0.49 b	59.17 ± 0.47 c
			6	98.83 ± 0.17 a	60.17 ± 0.47 b■	57.33 ± 0.42 c■
			9	98.83 ± 0.17 a	58.50 ± 0.43 b●▲	51.50 ± 0.43 c●▲

Data are mean ± SEM (n = 6 rats/treatment). Values with different letters within row were significantly different (ANOVA, p ≤ 0.05). ● Significantly different between day 3 and 9 at the same treatment (ANOVA, p ≤ 0.05). ▲ Significantly different between day 6 and 9 at the same treatment (ANOVA, p ≤ 0.05) ■ Significantly different between day 3 and 6 at the same treatment (ANOVA, p ≤ 0.05)

Table (4): The sperms concentration in rats injected with 20 and 40 mg/kg/BW of Cu-NPs for 3, 6 and 9 days

Parameters			Time/Days	Control	20 mg/kg Cu-NPs	40 mg/kg Cu-NPs
Sperms concentrations *10^3	Testes		3	51979.2 ± 408.8 a	71666.7 ± 2000.4 b	68125.0 ± 2060.3 b
			6	54270.8 ± 987.1 a	54270.8 ± 1254.3 a■	57500.0 ± 2212.7 a■
			9	51354.2 ± 298.3 a	66562.5 ± 576.2 b▲	54270.8 ± 746.8 c▲
	Epididymis	Head	3	26541.7 ± 150.2 a	45958.3 ± 318.9 b	42666.7 ± 333.3 c
			6	31208.3 ± 1309.3 a	32291.7 ± 1918.6 a■	31958.3 ± 3159.9 a■
			9	26708.3 ± 135.7 a	32041.7 ± 1109.5 b●	29916.7 ± 1394.4 b●
		Tail	3	30750.0 ± 129.1 a	68291.7 ± 881.3 b	65833.3 ± 1247.8 b
			6	36000.0 ± 766.5 a	36125.0 ± 1938.4 a■	50375.0 ± 1311.4 b■
			9	34125.0 ± 201.6 a	40541.7 ± 439.8 b●	30416.7 ± 1457.3 c●

Data are mean ±SEM (n = 6 rats/treatment). Values with different letters within row were significantly different (ANOVA, $p \leq 0.05$). ● Significantly different between day 3 and 9 at the same treatment (ANOVA, $p \leq 0.05$). ▲ Significantly different between day 6 and 9 at the same treatment (ANOVA, $p \leq 0.05$) ■ Significantly different between day 3 and 6 at the same treatment (ANOVA, $p \leq 0.05$).

Table (5): The percentage of sperms abnormalities in rats injected with 20 and 40 mg/kg/BW of Cu-NPs for 3, 6 and 9 days

Parameters			Time/Days	Control	20 mg/kg Cu-NPs	40 mg/kg Cu-NPs
Percentage of sperms abnormalities	Testes		3	7.83 ± 0.31 a	46.83 ± 0.60 b	57.33 ± 0.49 c
			6	8.17 ± 0.40 a	67.83 ± 0.40 b■	76.33 ± 0.67 c■
			9	8.33 ± 0.33 a	88.33 ± 0.33 b●▲	95.33 ± 0.33 c●▲
	Epididymis	Head	3	6.50 ± 0.22 a	42.17 ± 0.60 b	54.50 ± 0.42 c
			6	6.83 ± 0.31 a	63.67 ± 0.67 b■	73.17 ± 0.79 c■
			9	5.67 ± 0.33 a	61.17 ± 0.48 b●▲	70.83 ± 0.47 c●▲
		Tail	3	5.50 ± 0.22 a	40.50 ± 0.43 b	51.17 ± 0.60 c
			6	5.67 ± 0.33 a	61.17 ± 0.48 b■	70.83 ± 0.47 c■
			9	5.83 ± 0.17 a	82.50 ± 0.50 b●▲	87.17 ± 0.31 c●▲

Data are mean ±SEM (n = 6 rats/treatment). Values with different letters within row were significantly different (ANOVA, $p \leq 0.05$). ● Significantly different between day 3 and 9 at the same treatment (ANOVA, $p \leq 0.05$). ▲ Significantly different between day 6 and 9 at the same treatment (ANOVA, $p \leq 0.05$) ■ Significantly different between day 3 and 6 at the same treatment (ANOVA, $p \leq 0.05$)

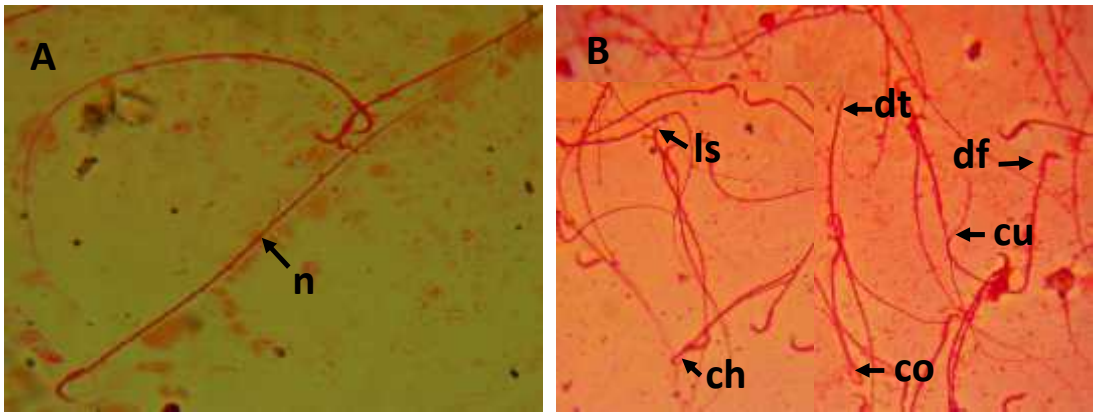

Figure (1): (A) (cu) normal sperm shape and (B) types of sperm abnormalities in rats intraperitoneal injection with 20 and 40 mg/kg Cu-NPs for 9 days. Normal sperm (n) consist of head with spine, middle piece, and tail. The sperm abnormalities involved deformed head (df); detached head (dt); loss of head spine (ls); coiled head (ch); coiled tail (co) and curved tail (cu).

DISCUSSION

Body and reproductive organs weights

Decline in the average body weight reported in this study are consistent with Zhang *et al.* (17), who found that the intraperitoneal injection of gold nanoparticles after 14 days in mice induced a decline in the body weight. The authors suggested that this way of exposure may cause some toxicity. Decreasing the average body weight observed in this study may attributed to the disturbance in different metabolic activity that resulted from intraperitoneal injection. Other types of nanoparticles (25 mg/kg TiO_2 NP) also caused decrease in the rat body weight when intraperitoneal injection (18). Another ways of exposure to nanoparticles also caused decrease in the body weight (13, 17). For example, Kim *et al.* (13) found that oral exposure to 30, 125, and 500 mg/kg of Ag-NPs for 13 weeks in male rats produced a significant decrease ($P < 0.05$) in the bodyweight after 4 weeks of exposure. A study by Zhang *et al.* (17) found that oral administration of 1100 µg/kg gold nanoparticles over 28 days caused a decrease in the body weight and the authors suggested that oral exposure to NPs could produce some effects on the digestive system. The body weight of male albino rats fed 1% and 2% TiO2 NP showed decline after 65 days of exposure (19).

The results of this work revealed that intraperitoneal injection of 20 and 40 mg/kg Cu-NPs resulted in significant increase in testis, epididymis head and tail, seminal vesicle and prostate glands relative weights compared to control group at all-time point (3, 6 and 9 days). The elevation of relative sex organs weights observed in this study may be related to histopathological changes in sex organs (20) or to the level of serum testosterone that play a major role in the maintenance of structural integrity and functional efficacy of the sex accessary glands (21). Kong *et al.* (20) illustrated that administration of different doses Ni NPs caused increase in the ratio of epididymis weight over body weight and testes pathologies. A study by Bakare *et al.* (22) also found that intraperitoneal administration of 0.5 ml TiO_2 NPs in mice caused pathological changes in the testicular tissue such as congestion of the inter-stitiumoedema, vacuolation and necrosis. Garcia *et. al.* (23) found that intravenously injected of 1 mg/kg Ag NPs in mice caused elevation serum and intratesticular testosterone concentration after 15 days of injection.

Sperm parameters

The present study revealed that intraperitoneal injection of 20 and 40 mg/kg Cu-NPs at different time points caused a decrease in the percentage of sperm life in the testis and epididymes (head and tail) with concentration and time effect. This reduction is occur due to the effects of nanoparticles on the germ cells, which lead to disturbance of spermatogenic process. Gromadzka-Ostrowska *et al.* (24) exhibited that nanoparticles have the ability to penetrate the blood-testis barrier and cause toxic effects on male germ cells and reduced sperm quality. A study by Xu *et al.* (25) illustrated mitochondrial damaging and decline the levels of ATP resulting oxidative stress in the testis, DNA damaging and decrease in the quantity and quality of epididymal sperm in mice injected with 20 mg/kg of silica nanoparticles for 15 and 35 days.

An increase in the percentage of sperm abnormalities and sperm concentration that observed in the current study may be caused due to the effect of Cu-NPs on the sertoli cells function or oxidative stress. The results of this study is in consistence with the suggestion of Kruszewski *et al.* (26) who reported that Ag-NPs could react with cellular DNA and stimulated inflammation, oxidative damage and cellular dysfunction that created genetic mutation and sperm cells with abnormal morphology. In testes, the sperm cells are almost immature, and they are getting mature through passing the epididymides tail (27). Talebi *et al.* (28) found that oral administration of 50 and 300 mg/kg zing nanoparticles for 35 consecutive days caused the presence of vacuoles in the cytoplasm of sertoli cells and affect the function of these cells which led to increase the percentage of sperm abnormalities. Exposure to silver nanoparticles can cause the inflammation and oxidative damage as well as increase the rates of sperm abnormalities and genetic mutations (29). Smith *et al.* (30) found that at 4–8 days post-injection of anatase titanium dioxide nanoparticles in mice induce structural and functional sperm deficiency associated with infertility, and DNA damage via oxidative stress. The oral administration with 200 mg/kg of Ag NPs in mice for 5, 10, and 15 days caused decrease in the percentage of sperm viability and sperm concentration as well as an increase in the percentage of sperm abnormalities that observed in the testis and epididymis (14). In rats, the oral exposure to 100 mg/kg of TiO_2 NPs for 8 weeks also caused decline in the percentage of sperm motility, viability and sperm concentration as well as an increase in the percentage of sperm abnormalities (31).

Generally, the intraperitoneal injection of Cu-NPs could affect immature sperm cells in the testes and mature one that stocked in the epididymis. The reduction in the number of sperm viability, and the elevation of sperm concentration and abnormalities in the testis and epididymis in this study, depending on concentration and time of exposure. However, Gromadzka-Ostrowskaa *et al.* (24) found that a dose-dependent (5 and 10 mg/kg body mass) and time-dependent (24 hrs, 7 and 28 days) decline the sperm count in the rat epididymis, and elevation in number of dead sperms after intravenously administrated with Ag-NPs.

CONCLUSION

The present obtained results demonstrated that copper nanoparticles can be considered as a reproductive toxicant by diminish the number of sperm parameter indices in addition to decline the body weight, which led to a negative effects on the activity of male reproductive system. The effects of nanocopper on reproductive indices are concentration and time-dependent. Further research is needed to clarify exposure by measuring the hormone levels and pathological changes.

RECOMMENDATION

The researchers recommend to study the effect of Cu-NPs on female reproductive system and embryonic development in addition to investigate the effect of these materials on immuno-histo system.

REFERENCES

1. Roduner E. (2006). Size matters: why nanomaterials are different. Chem. Soc. Rev. 35: 583 – 592.
2. Shaw BJ. and Handy RD. (2011). Physiological effects of nanoparticles on fish: A comparison of nanometals versus metal ions. Envir. Int. 37: 1083-1097.
3. Chen Z.; Meng H.; Xing G.; Chen C.; Zhao Y.; Jia G.; Wang T.; Yuan H.; Ye C.; Zhao F.; Chai Z.; Zhuc C.; Fang X.; Mac B. and Wan L. (2006). Acute toxicological effects of copper nanoparticles in vivo. Toxicol. Lett. 163: 109–120.
4. Brownheim SV. (2011). Characterization and in vitro toxicity of copper nanoparticles in murine neuroblastoma (N2A) cells. Department of Systems and Engineering Management, Graduate School of Engineering and Management, Air Force Institute of Technology. Air University USA.
5. McAuliffe ME. and Perry MG. (2007). Are nano-particles potential male reproductive toxicant? A literature review. Nano-Toxicol. 1: 204-210.
6. Suker DK. and Albadran RM. (2013). Cytotoxic effects of titanium dioxide nanoparticles on rat embryo fibroblast REF-3 cell line *in vitro*. Europ. J. Exper. Biol. 3(1): 354-363.
7. Yousefi BV.: Najafi L.: Najafi A.: Gholami H.: BeigiZarji ME.: Golzadeh J.: Amraie E. and Shirband A. (2012). Evaluation of iron oxide nanoparticles effects on tissue and enzymes of liver in rats. J. Pharmaceut. Biomed. Sci. 23:1-5.
8. Ahamed M.; Siddiqui MA.; Akhtar MJ.; Ahmad I.; Pant AB. And Alhadlaq HA. (2010). Genotoxic potential of copper oxide nanoparticles in human lung epithelial cells. Biochem. Biophys. Res. Commun. 396 (2): 578–583.
9. Fu X. (2015). Oxidative stress induced by CuO nanoparticles (CuO NPs) to human hepatocarcinoma (HepG2) cells. J. Cancer Therap. 6: 889-895.
10. Ostaszewska T.; Chojnacki M.; Kamaszewski M. and Sawosz-Chwalibóg E. (2016). Histopathological effects of silver and copper nanoparticles on the epidermis, gills, and liver of *Siberian sturgeon*. Environ. Sci. Pollut. Res. 23:1621–1633.
11. Al-Bairuty GA.; Shaw BJ.; Handy RD. and Henry TB. (2013). Histopathological effects of waterborne copper nanoparticles and copper sulphate on the organs of rainbow trout (*Oncorhynchus mykiss*). Aqua. Toxicol. 126: 104–115.
12. Thakur M.; Gupta H.; Singh D.; Mohanty IR.; Maheswari U.; Vanage G. and Joshi DS. (2014). Histopathological and ultra-structural effects of nanoparticles on rat testis following 90 days (Chronic study) of repeated oral administration. J. Nanobiotechnol. 12(42):1-13.
13. Kim YS.; Song MY.; Park JD.; Song KS.; Ryu HR.; Chung YH.; Chang HK.; Lee JH.; Oh KH.; Kelman BJ.; Hwang IK. and Yu IJ. (2010). Sub chronic oral toxicity of silver nanoparticles. Part Fibre Toxicol. 7:20–23.
14. Taha MN. and Ali AMJ. (2016). Changes in the weights of the testes and epididymes as well as sperm characteristic of male albino mice treated with silver nanoparticles. Ibn Al-Haitham J. Pure. Appl. Sci. 28(3):309-316.
15. Ema M.; Kobayashi N.; Naya M.; Hanai S. and Nakanishi J. (2010). Reproductive and developmental toxicity studies of manufactured nanomaterials. Reprod. Toxicol. 30: 343–352.
16. Bearden HJ and Fuquay JW. (1997). Applied animal reproduction. 4th ed., Prentice Hall, Upper Saddle River, New Jersey: p:351.
17. Zhang X-D.; Wu H-Y.; Wu D.; Wang Y-Y.; Chang J-H.; Zhai Z-B.; Meng A-M.; Liu P-X.; Zhang L-A. and Fan F-Y. (2010). Toxicologic effects of gold nanoparticles in vivo by different administration routes. Int. J. Nanomed. 5:771-781.
18. Amara S.; Khemissi W.; Mrad I.; Rihane N.; Ben Slama I.; Mir LE.; Jeljeli M.; Ben Rhouma K.; Abdelmelek H. and Sakly M. (2013). Effect of TiO_2 nanoparticles on emotional behavior and biochemical parameters in adult Wistar rats. General Physiol. Biophys. 32(2):229-234.
19. El- Sharkawy NI.; Hamza SM. and Abou-Zeid EH. (2010). Toxic impact of titanium dioxide (TiO_2) in male albino rats with special reference to its effect on reproductive system. J. Amer. Sci. 6(11):865-872.
20. Kong L.; Tang M.; Zhang T.; Wang D.; Hu K.; Lu W.; Wei C.; Liang G. and Pu Y. (2014). Nickel nanoparticles exposure and reproductive toxicity in healthy adult rats. Int. J. Molec. Sci. 15: 21253-21269.
21. Moor CR.; Price D. and Gallagher TF. (1930). Rat prostate cytology as testis-hormone indicator and prevention of castration changes by testis extract injection. Amer. J. Anatom. 45: 71-75.
22. Bakare AA.; Udoakang AJ.; Anifowoshe AT.; Fadoju OM.; Ogunsuyi OI.; Alabi OA.; Alimba CG. and Oyeyemi IT. (2016). Genotoxicity of titanium dioxide nanoparticles using the mouse bone marrow micronucleus and sperm morphology assays. J. Pollut. Effects. Control. 4(2): 1-7.
23. Garcia TX.; Costa GMJ.; Franca LR. and Hofmann M. (2014). Sub-acute intravenous administration of silver nanoparticles in male mice alters Leydig cell function and testosterone levels. Reprod. Toxicol. 45:59-70.
24. Gromadzka-Ostrowska J.; Dziendzikowska K.; Lankoff A.; Dobrzyoska M.; Instanes C.; Brunborg G.; Gajowik A.; Radzikowska J.; Wojewódzka M. and Kruszewski M. (2012). Silver nanoparticles effects on epididymal sperm in rats. Toxicol. Lett. 214(3):251-258.
25. Xu Y.; Wang N.; Yu Y.; Li Y.; Li Y-B.; Yu Y-B.; Zhou X-Q and Sun Z-W. (2014). Exposure to silica nanoparticles causes reversible damage of the spermatogenic process in mice. PLOS ONE. 9(7): e101572.
26. Kruszewski M.; Brzoska K.; Brunbor G.; Asare N.; Dobrzynska M.; Duzinska M.; Fjellsbo LM.; Georgantzopoulou A.; Gromadzka-Ostrowska J. and Gutleb AC. (2011). Toxicity of silver nanomaterials in higher eukaryotes. Advan. Molec. Toxicol. 5: 179-259.

27. Seligman J.; Kosower NS. and Shalgi R. (1992). Effects of caput ligation on rat sperm and epididymis: protein thiols and fertilizing ability. Biol. Reprod. 46: 301-308.
28. Talebi AR.; Khorsandi L. and Moridian M. (2013). The effect of zinc oxide nanoparticles on mouse spermatogenesis. J. Assist. Reprod. Genet. 30:1203–1209.
29. Nel A.; Xia T.; Mädler L. and Li N. (2006). Toxic potential of materials at the nano-level. Science. 311(5761): 622-627.
30. Smith MA.; Michael R.; Aravindan RG.; Dash S.; Shah SI.; Galileo DS. and Martin-De Leon PA. (2015). Anatase titanium dioxide nanoparticles in mice: evidence for induced structural and functional sperm defects after short, but not long-term exposure. Asian J. Androl. 17: 261–268.
31. Morgan AM.; Abd El-Hamid MI. and Noshy PA. (2015). Reproductive toxicity investigation of titanium dioxide nanoparticles in male albino rats. World J. Pharm. Pharmaceut. Sci. 4 (10): 34-49.