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The Exposure Impact of Copper Oxide Nanoparticles on Testicular Toxicity in Male Rats

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Abstract

Background: Copper oxide nanoparticles (CuO NPs) are metallic nanoparticles recognized for their diverse applications, including high conductivity, catalytic activity, and antimicrobial properties. However, their potential cytotoxicity and the specific mechanisms by which exposure affects male testicular function remain largely unknown. This study aims to evaluate the impact of CuO NPs on reproductive health, specifically focusing on hormonal levels and sperm quality.

Patients and Methods: In this investigation, 12 adult male white mice were administered a single dose of 20 mg/kg of copper oxide nanoparticles both orally and dermally. After a 24-week period, biological samples were collected, including blood via heart puncture, semen from the cauda epididymis, and testicular tissue. The assessment included serum testosterone levels, sperm density, viability, motility, and a detailed histological evaluation of the testicular structure.

Results: The results demonstrated that copper oxide nanoparticles significantly reduced serum testosterone levels and inhibited sperm density, viability, and motility. Furthermore, histological analysis of the testicular tissues revealed clear degeneration in germ cells, Sertoli cells, Leydig cells, and spermatogonia. These findings indicate a profound disruption of the physiological and structural integrity of the male reproductive system following exposure

Conclusion: In conclusion, the aforementioned results offer evidence that copper oxide nanoparticles (CuO NPs) exert a negative and irreversible effect on testicular function and the physiological characteristics of sperm. These harmful effects were notably prominent following the administration of high doses, highlighting the need for further investigation into the environmental and occupational risks posed by these nanoparticles to male fertility

تأثير التعرض لجسيمات أكسيد النحاس النانوية على سمية الخصية لدى ذكور الجرذان

زينب عبد الامير حسين، احمد هادي اليساري، رحاب جاسم محمد

الخلاصة:

المقدمة: تُعد جزيئات أكسيد النحاس النانوية (CuO NPs) مواد ذات أهمية تكنولوجية وطبية بالغة نظراً لخصائصها الفيزيائية والمضادة للميكروبات. ومع ذلك، فإن المخاطر المحتملة لهذه الجزيئات على الجهاز التناسلي الذكري وآليات تأثيرها على وظيفة الخصية لا تزال تفتقر إلى الفهم الكامل. لذا، فإن دراسة السمية الخلوية لهذه الجزيئات وتأثيرها على المعايير الهرمونية والنسيجية أمر ضروري لتقييم السلامة البيئية والمهنية.

المرضى وطرق العمل: أجريت هذه الدراسة التجريبية باستخدام 12 فأراً أبيضاً بالغاً من الذكور، حيث تم تعريضهم لجرعة واحدة قدرها 20 ملغ/كغ من جزيئات أكسيد النحاس النانوية عبر المسارين الفموي والجلدي. بعد فترة متابعة استمرت 24 أسبوعاً، تم جمع عينات الدم عن طريق ثقب القلب لقياس مستويات التستوستيرون، كما استُخلص السائل المنوي من ذيل البربخ لتقييم الكثافة والحيوية والحركة، بالإضافة إلى إجراء فحص نسيجي دقيق لأنسجة الخصية.

النتائج: أظهرت النتائج أن التعرض لجزيئات أكسيد النحاس النانوية أدى إلى انخفاض معنوي في مستويات هرمون التستوستيرون في المصل، وصاحب ذلك تثبيط واضح في كثافة الحيوانات المنوية وحيويتها وقدرتها على الحركة. نسيجياً، كشفت الفحوصات عن تدهور ملحوظ وتغيرات تنكسية في الخلايا الجرثومية، وخلايا سيرتولي، وخلايا لايدك، بالإضافة إلى تضرر الخلايا الأم (Spermatogonia)، مما يعكس خللاً بنيوياً في النسيج الخصوي.

الاستنتاج: تقدم هذه النتائج رؤية واضحة حول التأثيرات السلبية وغير العكسية لجسيمات أكسيد النحاس النانوية على الوظائف الفسيولوجية والنسيجية للخصية. تؤكد الدراسة أن التعرض بجرعات عالية يؤدي إلى سمية تناسلية حادة، مما يستدعي ضرورة وضع ضوابط وقائية للحد من مخاطر هذه الجزيئات، وفتح آفاق جديدة للبحث في السبل الممكنة لتقليل هذه الأضرار.

1. Introduction

Nanotechnology is a revolutionary field experiencing rapid growth, leading to the emergence of a new generation of nanomaterials and nanoparticles with unique physical and chemical properties. Among these, copper oxide nanoparticles (CuO NPs) are widely used in numerous industries and applications, including electronics, antimicrobial coatings, textiles, catalysts, cosmetics, and even in some medical applications (Garipov et al., 2019). This widespread use is due to its unique properties such as high surface area, catalytic activity, and antimicrobial properties (Gupta S, 2021) As the production and use of CuO NPs increases, as a result there are concerns about their environmental fate and potential impacts on human and animal health (Ghareeb, 2023). CuO NPs can enter the body through various exposure routes such as inhalation, ingestion, and dermal contact (Jiang W, 2017). Once they enter the body, these particles can travel through the blood and accumulate in various organs, including the reproductive organs (Sharma S, 2019). Recent epidemiological studies indicate a concerning decline in sperm quality and fertility rates among men worldwide (Lewis et al., 2025) , which is partially attributed to exposure to toxic environmental factors. Given that CuO NPs are becoming a common component in the urban and industrial environment, it is crucial to assess their potential effects on the male reproductive system The toxicity of CuO NPs is primarily attributed to their ability to produce reactive oxygen species (ROS) such as hydroxyl radicals (OH.), and hydrogen peroxide ($H2O_2$) leads to excessive accumulation of ROS, causing oxidative stress and damage to key cellular components such as lipids (lipid peroxidation), proteins (protein oxidation), and DNA (DNA damage) (Gupta et al., 2021) The male reproductive system, particularly sperm, is especially sensitive to oxidative stress due to the high content of polyunsaturated fatty acids in their plasma membranes and their limited DNA repair capacity (Qiu et al., 2020) as well as the release of copper ions in biological environments, where CuO NPs can partially dissolve. In biological fluids, leading to the release of free copper ions. Although copper is an essential element, high levels can be toxic, as copper ions can interfere with cellular enzymes, participate in Fenton reactions to produce ROS, and disrupt the ionic balance within cells (Vo et al., 2024) Exposure to CuO NPs can lead to pathological changes in testicular structure, such as atrophy of the seminiferous tubules, damage to Sertoli cells and Leydig cells, and the presence of gaps in the tissue. A significant decrease in sperm count, motility, and viability after exposure to CuO NPs were reported (Hassan et al., 2024a) It also influences reproductive hormone levels such as testosterone, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) (Vassal et al., 2021a; Wu et al., 2023) However, most of these studies have been conducted on the in vivo oral route of administration. Owing to the increasing prevalence of CuO NPs in products and the environment, understanding the associated health risks is crucial. In line with this context, this research aims to first explorer and investigate the effects of CuO NPs administrated

transdermal on male fertility, focusing on the involved molecular and cellular mechanisms, and the biological indicators of toxicity CuO.

2. Materials and Methods

2.1.Synthesis of CuO Nanoparticles

CuO NPs were synthesized using the sol-gel method (Jabbar, 2017a) Acquired 0.9 g of $CuCl_2$ was dissolved in 25 mL of ethanol, whereas 1.5 g of NaOH was dissolved in 80 mL of ethanol. The produced NaOH solution was added dropwise to the $CuCl_2$ solution, with steady stirring at room temperature for 30 minutes. Reaction happens, and the color changes from dark blue to black. Filter paper is used to filter the gel and then rinsed with H2O. The sample was allowed to dry at ambient temperature before being annealed in a furnace at 1200 degrees Celsius Show in Fig.1.

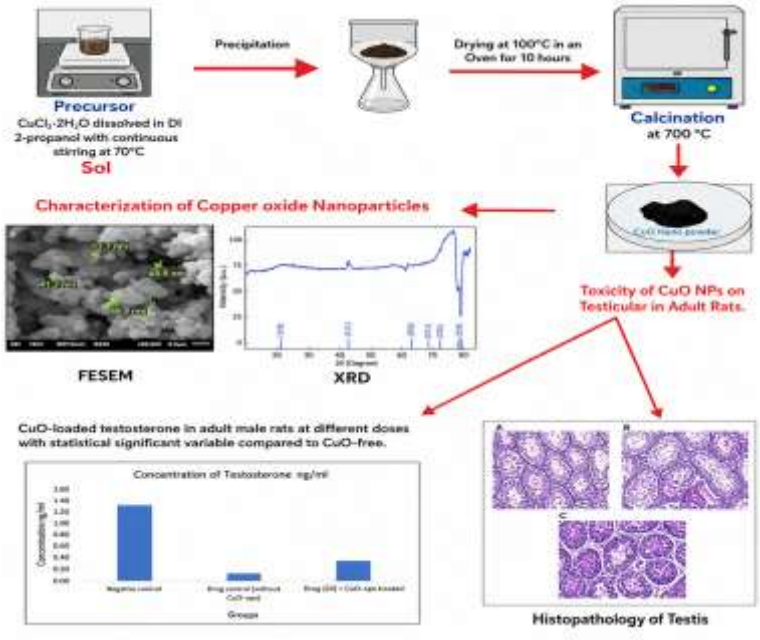

Figure 1: Copper Oxide Nanoparticles Were Synthesized Using the Sol-Gel Method

2.2.Characterization of Copper Oxide Nanoparticles

2.2.1. FE-SEM of Copper Oxide NPs

Using a focused electron beam, a scanning electron microscope (Fe-SEM) creates pictures of copper oxide nanoparticles (CuO NPs) samples by scanning their surface. Information on the size distribution sample composition and surface morphology is revealed by the electrons' overlap with the atoms. To investigate the material, Tescan Mira3 SEM/French was used.

2.2.2. FTIR of Copper Oxide NPs

Fourier transform infrared spectroscopy (FT-IR) was used to identify the functional groups of the synthesized CuO NPs. The CuO NPs were measured using a Shimadzu (8400S)/Japan FT-IR spectrometer with a KBr disk as the absorption medium in the wavenumber range of 400 to 400 cm-1.

2.2.3. XRD of Copper Oxide NPs

X-ray Diffraction (XRD) analysis was conducted to identify and describe the composition of copper oxide nanoparticles (CuO NPs). The radiation used was k=1.50456, which is associated with the copper atoms.

2.2.4. EDX Energy Dispersive X-ray Spectroscopy

(EDX) analysis was performed to detect and quantify nanoscale copper oxide particles (CuO NPs. The samples were then mounted on a sample holder and placed inside the chamber of the EDX-equipped Scanning Electron Microscope (SEM) system The EDX measurements were carried out at an accelerating voltage of 20 kV with a working distance of ~10 mm. The elemental composition of the samples was obtained from the spectra, focusing on copper (Cu) and oxygen (O) peaks to confirm the presence of CuO nanoparticles. The data were analyzed using the system's dedicated software and expressed as weight % and atomic % for each element detected.

2.3. Experimental Animals and Exposure Design

The laboratory of the College of Pharmacy, University of Karbala, conducted this investigation. Between 1/3/2024 and 1/8/2024, 18 male albino rats were collected, weighting between 200 and 225 grams. And at 10-12 weeks old.The rats were randomly divided into 3 groups, each containing six animals. Each group was given (20 mg/kg) of aluminum oxide nanoparticles once daily, orally and dermally, for 24 weeks.

2.3.1 Samples' Collection and Process

Under light anesthesia, the rats were sacrificed after 48 hours of the last dose. Blood samples were collected through the retro-orbital route, and testosterone levels were estimated from the isolated serum. To investigate the various sperm parameters, a pair of epididymites were collected in 5 ml of phosphate-buffered saline (PBS) to release sperm. To monitor histological changes, the right testy of each animal was selected and preserved in formalin (10%).

2.4. Biochemical and Hormonal Assay Methods

2.4.1. Superoxide Dismutase SOD1

The Rat SOD1 (Superoxide Dismutase 1, Soluble) ELISA Kit from Reed Biotech Ltd (Catalog Number: RE1626R) was used to measure the concentration of the SOD1 enzyme in rat serum samples, according to the manufacturer's protocol.

2.4.2. Catalase CAT

The Rat CAT(Catalase) ELISA Kit from Reed Biotech Ltd (Catalog Number: RE9196R) was used to measure the concentration of the CAT enzyme in rat serum samples, according to the manufacturer's protocol

2.4.3. Malondialdehyde MDA

The Rat MDA(Malondialdehyde) ELISA Kit from Reed Biotech Ltd (Catalog Number: RE10165) was used to measure the concentration of the CAT enzyme in rat serum samples, according to the manufacturer's protocol.

2.4.4. Testosterone Hormone levels

The Rat Testosterone ELISA Kit from Reed Biotech Ltd (Catalog Number: RE10175) was used to measure the concentration of the Testosterone enzyme in rat serum samples, according to the manufacturer's protocol.

2.5. Sperm Analysis

The left epididymis was removed, the tail was isolated, and placed in a Petri dish containing 1 ml of physiological saline. And he mashed it well into very small pieces using a sharp scalpel to release the sperm contained within. The mixture was left for 3-5 minutes in an incubator at 37 degrees Celsius. After that, a drop of the mixture was placed on a clean, dry glass slide, covered with a cover slip, and examined under an electron microscope specifically designed for semen analysis at 40X magnification to assess sperm parameters including (sperm motility, sperm viability, sperm concentration)

2.6.Metal Concentration Analysis

Inductively coupled plasma optical emission spectrometry (ICP-OES) was employed to determine the quantity of copper. resulting from copper oxide nanoparticles (CuO) in rat serum samples. The samples were subjected to wet digestion using nitric acid (HNO_3) and hydrogen peroxide (H_2O_2) within a closed microwave system After digestion was complete, the solution was diluted with deionized water to the final volume and then filtered to remove particulate impurities. Measurements were taken using an ICP-OES (Shimadzu, Japan).

2.7.Histopathology of Testes

Microscopic alterations in testis tissues were examined by Hematoxylin and Eosin (H&E) staining. The tissues were then fixed with formalin, embedded in paraffin wax, and tissue Sections were 5 μm thick. The slides were suitably stained and inspected under an optical microscope.

2.8.Statistical Analysis

The mean ± standard error (SE) was determined from the statistical information using SPSS for instances where a one-way analysis of variance (ANOVA) was followed by Tukey's test. The degree of statistical significance was determined at $P<0.05$.

3. Results and Discussion

3.1.FT-IR Spectrum of CuO NPs

The FTIR spectrum is a helpful tool for the identification of functional groups in a molecule; each specific chemical bond typically has a unique absorption spectrum, and can be used to acquire structural information and bond strengths of a complex in order to study the nature and strength of its bond. Fig.2. shows the FT-IR spectrum of the prepared copper oxide sample. In this picture, only a single prominent peak is observed at 547 cm-1, which is the absorption of the stretching vibration of the Cu-O bond. Other peaks that correspond to organic impurities are impossible to appear because the samples are heated to a high temperature (Mgheer et al., 2023; Raul et al., 2014)

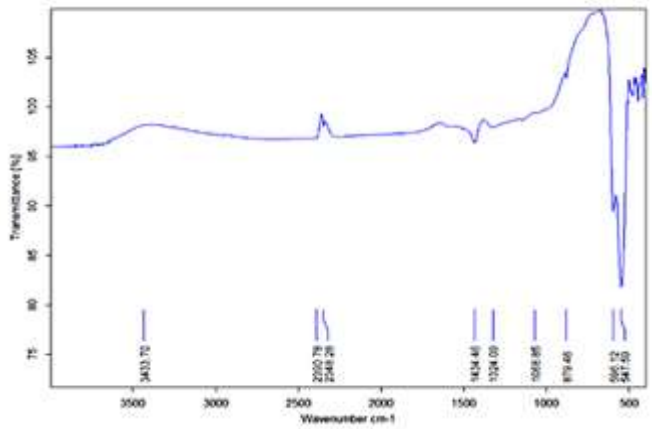

Figure2: FT-IR Spectrum of the CuO NPs

3.2.X-ray Diffraction Pattern of CuO NPs

The patterns of crystalline structure of the nanoparticles that were being studied and analyzed using an X-ray equipment. Fig.3. exhibits the XRD pattern's (XRD) intensity peaks that are located at 35.4173°, 38.6523° and 48.6751°, which are associated with the atomic planes (111), (111) and (-202) (Jabbar, 2017b).

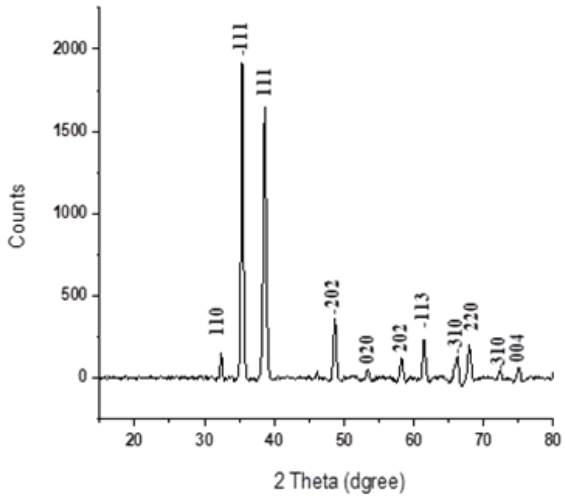

Figure3: XRD of the CuO NPs

3.3.FE-SEM of CuO NPs

The morphologies of the samples were studied using a field emission scanning electron microscope (FESEM). The typical micrograph of CuO prepared by SEM is demonstrated in Fig.4. The SEM micrograph demonstrated the presence of irregular-shaped particles of CuO. The SEM observation revealed the presence of aggregated nanoparticles and the average size of the grains was determined using SEM and was different at 53.29, 56.06, 74.97 and 75.90. The surface of the grains was evaluated and exhibited a variety of average diameters: D1 (53.29), D2 (56.06), D3 (74.97) and D4 (75.90) (Javed et al., 2017).

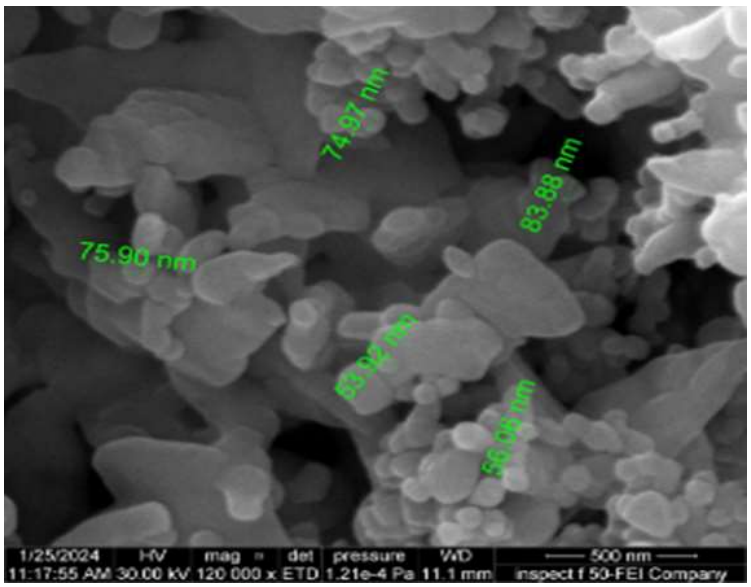

Figure4: FE-SEM Analysis of CuONPs

3.4.Energy-Dispersive X-ray Spectroscopy (EDX/EDS) of CuO NPs

The energy-dispersive X-ray spectrometer is utilized to examine the structure and analytical composition of materials. The EDX technique can qualitatively and quantitatively identify elements. The EDX spectrum shows the presence of copper and oxygen in the copper oxide nanoparticles synthesized by the sol-gel process, as illustrated in Fig.5. And Table1 (Thamer et al., 2025)

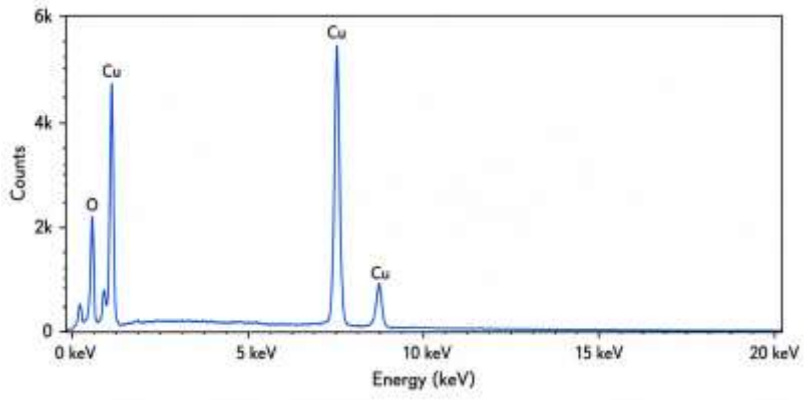

Figure5: EDS of the CuO NPs

Table1: Elemental Ratio of Cuo Nano Particles

Element	Atomic %	Atomic % Error	Weight %	Weight % Error
O	50.7	0.5	20.5	0.2
Cu	49.3	0.3	79.5	0.4

3.5.Testosterone Hormone Level

A significant decrease ($p < 0.05$) in testosterone hormone concentration was observed in rats exposed to CuO NPs compared to the control group Table2 and Fig 6. The study showed that exposure of rats to CuO NPs at a dose of 20 mg/kg orally and dermally led to a significant decrease in testosterone hormone concentration compared to the control group and a sharp decline in testosterone levels, as CuO nanoparticles can cross the blood-testis barrier (Hassan et al., 2024b) The results confirm that CuO NPs can biologically accumulate in target organs, including the testes, once they enter the bloodstream (Lewis et al., 2025) Moreover, exposure to CuO NPs may cause damage to Leydig cells with problems in steroidogenesis in the testes of mice, which can lower testosterone levels (Xiangqin Zheng a b 1, 2023) Additionally, the increase in blood copper concentration reduces the ability of testosterone to bind to receptors, thereby lowering its level in the blood, which affects biological processes that depend on hormone concentration (Vassal et al., 2021b) According to a study conducted by Klaudia Jomova (Jomova et al., 2023) on male C57BL/6 mice, the current results

are consistent with their findings, which confirm that exposure to copper oxide nanoparticles during puberty is the main cause of decreased testosterone levels. Another possible scenario, CuO NPs may affect the gene expression of the protein that plays a key role in transport Cholesterol to the mitochondrial membrane and increased steroid synthesis, which leads to the prevention of conversion Cholesterol to pregnenolone and reduces testosterone levels (Rone et al., 2009) Notably, the level of testosterone hormone in animals transdermally exposed to CuO NPs decreases to up to 3 times lower than control animals.

Table2: The Level of Testosterone Hormone in Control Animals and Animals Injected With 20 Mg/Kg of Cuo Nps.

Groups	Testosterone ng/ ml	P value
Group1 control	1.557 ± 0.434^{A}	-
Group2 CuO NPs Oral	0.138 ± 0.011^{C}	0.01**
Group3 CuO NPs Transdermal	0.427 ± 0.050^{B}	0.05*
*All the values were stated as Mean ± SE (n=6). By using one way ANOVA followed by Tukey comparison test, Statistical analysis was achieved and the statistical significance was considered at P< 0.05. * Different letters between any two means signify the existence of significant differences.		

3.6. Sperm Analysis

A significant drop ($p<0.05$) was observed in the concentration of sperm, their viability, and their movement, all of which were decreased in the rate exposed to CuO NPs in comparison to the control group (see Table3).

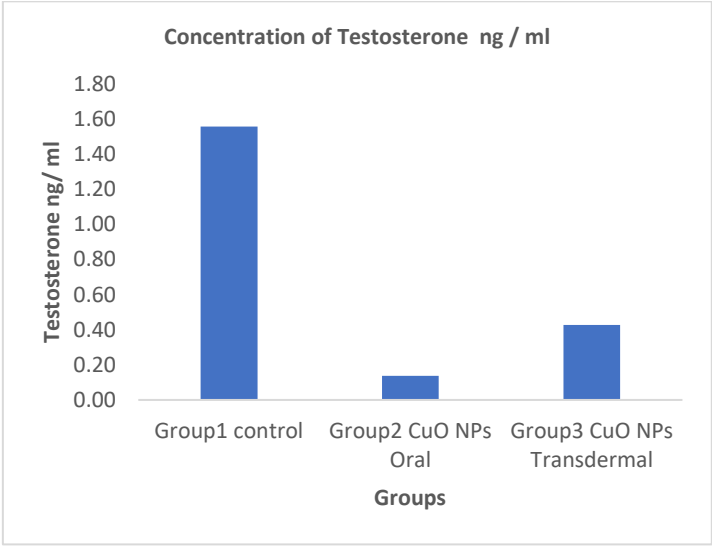

Figure6: The Testosterone Level in The Serum of Control Animals and Animals Injected With 20 Mg/Kg of Cuo Nps.

The traits of sperm are an indication of testicular toxicity that is manifested. As chemicals are present, the sperm must follow a proper and orderly progression of events to produce mature sperm during the process of spermatogenesis. The alterations observed in sperm's concentration, vitality, and motion could be attributed to multiple causes that either individually or together lead to morphological and functional changes. Literature research (Xuan et al., 2023) demonstrated that the stress associated with nanoparticles causes an increase in the production of radical molecules. Reactive oxygen species in sperm and the oxidization of unsaturated fatty acids to acids in the plasma membrane that causes the death of cells with a programmed fate and affects the quality of sperm and the increase in the percentage of abnormal sperm and the suppression of sperm production. The outcomes of the current investigation demonstrate that as the length of time spent with NPs increased, as well as increasing concentrations, various alterations in the properties of sperm were observed. From the sperm's semen, the concentration, vitality, and motion of the sperm has been reduced, this is in agreement with the results of Xuan L (Xuan et al., 2023)

Table3: The Semen Characteristics in Control Animals and Animals Injected With 20 Mg/Kg of Cuo Nps

Experimental groups	Parameter		
	Sperm concentration (1×10^6 ml^{-1})	Sperm motility (%)	Sperm viability (%)
Group1 control	83.03± 2.46 A	77.47 ± 3.10 A	62.67 ±3.50 A
Group2 CuO NPs Oral	41.08± 1.72 B	33.88 ±0.93 B	26.50 ±1.78 B
Group3 CuO NPs Transdermal	14.75± 1.18 C	23.35 ±1.39 C	17.83 ±1.33 C

* All the values were stated as Mean ± SE (n=6). By using one way ANOVA followed by Tukey comparison test, Statistical analysis was achieved and the statistical significance was considered at P< 0.05.
* Different letters between any two arithmetic means signify the existence of significant differences.

3.7.Oxidative stress (MDA) and Antioxidants (CAT and SOD)

Data presented in Table4 demonstrated that administration of copper oxide nanoparticles (CuO NPs) at a dose of 20 mg/kg body weight to rats resulted in a significant ($p \leq 0.05$) increase in malondialdehyde (MDA) levels, accompanied by a significant reduction in superoxide dismutase (SOD) activity and catalase (CAT) activity when compared with the control group (G1). Furthermore, both higher nanoparticle concentrations and prolonged exposure periods were associated with further increases in MDA levels and more pronounced decreases in SOD and CAT activities. This phenomenon may be attributed to excessive production of MDA radicals and reactive oxygen species (ROS), leading to an imbalance between antioxidant defenses and pro-oxidant agents, which consequently induces oxidative stress and structural as well as functional alterations in testicular tissue. Due to their nanoscale size, CuO NPs can readily cross cell membranes and biological barriers, allowing them to interact with intracellular organelles such as mitochondria, thereby enhancing ROS production. Elevated MDA levels observed in the testes and sperm of CuO NP-exposed rats indicate

oxidative damage, likely caused by the inability of antioxidant enzymes to neutralize accumulated oxidants in the testicular microenvironment. As a lipid peroxidation byproduct, MDA serves as a reliable biomarker for predicting tissue injury. The large surface area-to-volume ratio of nanoparticles further increases their reactivity, exacerbating oxidative stress in both human and animal tissues. These findings are consistent with previous reports demonstrating that exposure to metal oxide nanoparticles is associated with enhanced lipid peroxidation in biological tissues. In experiments with Wistar rats administered different oral doses of CuO NPs, oxidative stress was shown to be dose-dependent, with more substantial declines in antioxidant enzyme activity observed with longer exposure durations. This reduction in SOD activity is particularly relevant, as SOD constitutes the first line of defense against oxygen toxicity by preventing excessive free radical formation. Suppression of SOD activity under higher nanoparticle doses is likely due to increased generation of free radicals from intracellular hydrogen peroxide (H_2O_2), which accelerates oxidative injury. Numerous previous studies have confirmed that increased tissue MDA levels following exposure to metal oxide nanoparticles contribute to the induction of apoptosis and autophagy in testicular cells. The current results are in line with prior research showing that CuO NP exposure elevates lipid peroxidation in sperm and reduces overall antioxidant capacity. Supporting evidence comes from an experimental study in which mice were orally administered varying doses of copper (25,30 mg/kg) for 14 days, leading to a significant increase in MDA levels due to intensified lipid peroxidation in the testes. These observations suggest that CuO NPs are a major factor in disrupting peroxidase activity, thereby promoting oxidative stress, free radical-mediated damage, and oxidation of cellular proteins and lipids (Al-Musawi et al., 2022).

Table4: The Level of Oxidative Stress (MDA) And Antioxidants (SOD And CAT) In Control Animals and Animals Injected With 20 Mg/Kg of Cuo Nps.

Experimental groups	Parameter		
	MDA Concentration ng/ml	SOD1 Concentration ng/ml	CAT Concentration ng/ml
Group1 control	123.06 ± 0.46 C	8.40 ± 0.07 ^{A}	19.84 ± 2.87 ^{A}
Group2 CuO NPs Oral	759.37 ±41.56 A	3.37 ± 0.02 ^{C}	11.22 ± 0.43 ^{C}
Group3 CuO NPs Transdermal	615.89 ± 23.22 B	3.85 ± 0.017 ^{B}	12.39 ± 0.71 ^{B}

∗ All the values were stated as Mean ± SE (n=6). By using one way ANOVA followed by Tukey comparison test, Statistical analysis was achieved and the statistical significance was considered at P< 0.05.
∗ Different letters between any two arithmetic means signify the existence of significant differences.

3.8.Histopathological Examination

Fig.7. showed the microscopic images of the right testis from the different groups. The seminiferous tubules with normal structure showed regularly arranged rows and a complete set of germinal epitheliums in the control group. Otherwise, the seminiferous tubules in the rat treated with copper oxide (CuO NPs) in Figures 6a, 6b and 6c showed atrophy and degenerative and necrotic changes, particularly in the spermatogenic layer and in some other areas (Larijani et al., n.d.). Histological examination of the sections taken from the rat testes in the group showed control G1 in Fig.7.a histologically normal Seminiferous tubules with normal Basement membrane Containing Spermatogenesis in different Stages of maturation. Multiple mature sperms also seen. As for the groups treated with nano-copper oxide orally and through the skin, groups (G2, G3) at a concentration of (Larijani et al., n.d.) 20 mg/kg body weight, in fig 6b is observed histologically normal looking seminiferous tubules. Contains Spermatogenesis in different stages of maturation - Some tubules show spermatogenesis arrest In Fig 7c is histologically normal seminiferous tubules with normal basement membrane different stages of maturation mature sperms. as for the changes observed in the treated groups, they are due to the accumulation of copper oxide nanoparticles and their penetration of biological barriers where most tubules show spermatogenesis maturation arrest. The production of free radicals due to oxidative stress from copper oxide nanoparticles causes toxic effects on cells, manifested by their interaction with DNA, leading to disruptions in its replication and structure, and subsequently resulting in cellular dysfunction and eventual cell death (Thakur et al., 2014) The generated free radicals cause lipid peroxidation in the cell membrane and mitochondria, leading to changes in membrane permeability and significant dysfunction, resulting in. Fig 6: Photomicrographs of testicular sections of different groups, hematoxylin, and eosin. 6a Testicular sections from control respectively, showing normal histological features of seminiferous tubules. 6b Testicular section from CuO NPs oral rat Section shows seminiferous tubules with severe atrophy Severe Spermatogenesis arrest, tubules show only few Spermatogenesis without presence of only maturation. 6c CuO NPs in skin Section shows histologically normal Seminiferous tubules. Most tubules show spermatogenesis maturation rest.

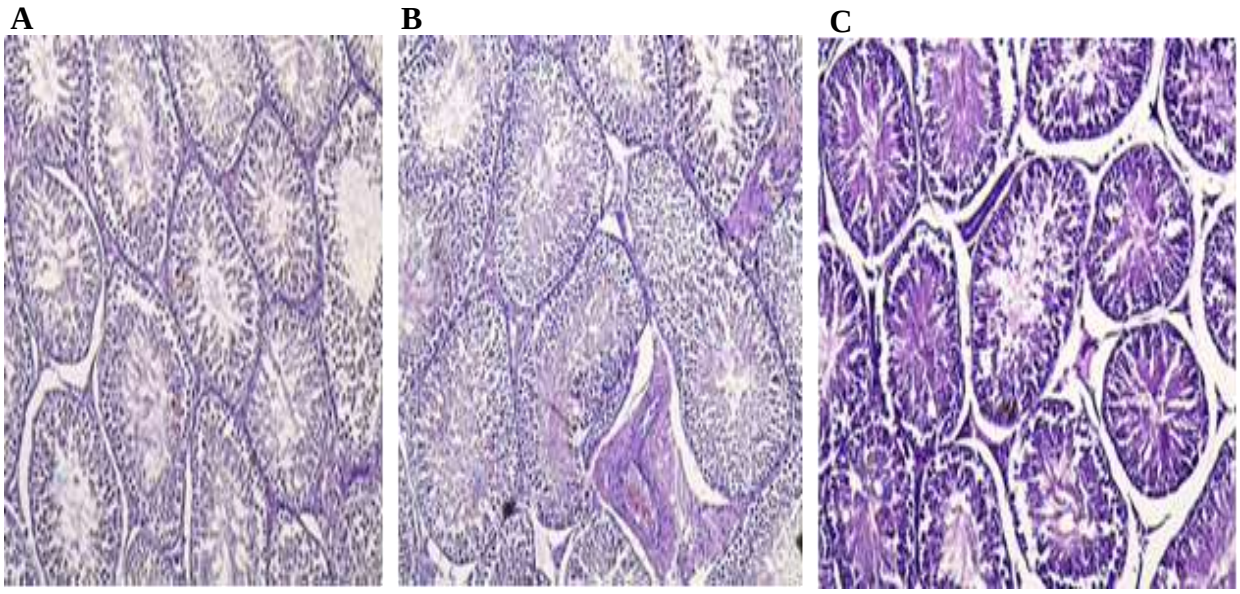

Figure7: Representative Photomicrographs of Hematoxylin and Eosin (H&E)-Stained Sections of The Right Testis from The Experimental Groups. (A) Control group (G1) demonstrating normal seminiferous tubules with intact basement membranes, complete spermatogenic cell layers, and abundant mature spermatozoa. **(B)** Oral CuO-NP-treated group (G2; 20 mg/kg body weight) showing severe seminiferous tubular atrophy, degeneration and necrosis of the germinal epithelium, and marked spermatogenic arrest. **(C)** Dermal CuO-NP-treated group (G3; 20 mg/kg body weight) showing largely preserved seminiferous tubular morphology with mild-to-moderate spermatogenic arrest in some tubules.

Conclusion

The main conclusion is symmetrized. In a study on the male reproductive system of mice inside the bodies of rats. The results revealed that the effects caused by copper nanoparticles are significant, necessitating a reduction in the safe dosage level for living organisms to an insignificant level. However, further investigation is needed to distinguish between the underlying mechanisms associated with the direct effect through particle accumulation or the indirect effect through secondary oxidative stress during exposure to copper and its nanoparticles.

Ethical Approval

The experimental protocol was implemented in accordance with the regulations of the Research Ethics Committee of the College of Science, University of Karbala, which approved the current study methodology (Approval Number: 003CSE in 10/6/2024). Competing interests, the authors declare no competing interests.

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