Histopathological Evaluation of Toxic Effect of Zinc Oxide Nanoparticles on Reproductive System of Male Rats

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***Abstract*—**Despite the beneficial effects of zinc oxide nanoparticles (ZnO-NPs) on different biomedical applications, including their antioxidant and anti-inflammatory ones, it might have cytotoxic and genotoxic impacts on the male reproductive system. Although some researchers were using zinc oxide nanoparticles are beneficial to body organs due to its antioxidant and anti-inflammatory effects, it has also been found to have a harmful effect on the male reproductive system. *Objective:* The current study aimed to revealed the effect of zinc oxide nanoparticles on male rats' reproductive performance and histopathology changes for sexual organs for example testes. *Materials and Methods*: a number of twenty healthy white male rats, aged approximately 7-9 weeks, were randomly divided into two groups and subjected to the following treatments. Group 1 (n=10), were treated with 0.5 ml of aseptic normal saline save as the control negative group. Group 2 (n=10): were Injected with (0.5ml) of ZnO-NPs 350 mg/kg b.w orally for 2 weeks. At day7 and day 21 post injection, rats were anesthetized and sacrificed for histopathological examination. *Results:* It was shown that ZnO-NPs have a harmful effect on the male reproductive system by distorted seminiferous tubules, severe desquamation and necrosis and absence of spermatocytes and most Sertoli cells *Conclusion:* It can be concluded that zinc oxide nanoparticles, administered at 350 mg/kg, had the most harmful and toxic effect on male reproductive system.

***Keywords* —** Zinc Oxide Nanoparticle; Testes; Pathological changes.

INTRODUCTION

Nanoparticles (NPs) are materials with at least one dimension ≤ 100 nm and have a large surface-to-volume ratio. This characteristic endows NPs with unique properties that enable them to interact more effectively with biological systems [1,2]. There is an increasing interest on the impact of NPs in the fields of human and veterinary sciences, particulary on whether could easily pass through the blood–brain and blood–testis barrier [3]. NPs have several applications such as their ability to inhibit the growth of bacteria [4], fungus [5] detect SARS-CoV-2 virus [6], and treat cancer [7].

Zinc is the second most abundant trace metal in animals after iron and is found in bone, muscle, liver, kidneys, brain, and skin, ZnO reduces inflammation and oxidative stress in the intestinal mucosa and supports better feed conversion, zinc deficiency may induce impairment in growth, fertility and immune competence. ZnO and its nanoparticles show antimicrobial effects against pathogens, NPs that have attracted much attention in the biological and animal fields, due to their excellent properties of biocompatibility, solubility, nontoxicity rather than being cheap [8]. Their structural features enable ZnO-NPs to act like a biomolecule and they can modulate cell cycle regulation and cellular homeostasis. However, whether ZnO-NPs are toxic [9], or stimulate the reproductive system is a major argument in the field of reproduction, the effects of NPs depend on various factors including size, concentration, morphology, synthesis method and surface area of NSs but also both tested cell type organism. Small size, high concentration and frequent administration is conducive to the toxic effect of NPs [10]. Increases the production of reactive oxygen species (ROS) and thus the oxidative stress, leading to increased lipid peroxidation and causing male infertility [11,12].

Zinc oxide nanoparticles ZnO-NPs have received considerable attention. This is due to their low production cost, ability to form diverse structures and various biological applications including drug delivery, bio-imaging probes, cancer treatment, antibacterial and immunomodulatory agent [13]. ZnO-NPs have highly photo-catalytic and is expected to be relatively biocompatible compared to Titanium Dioxide (TiO2). They are currently employed in the food industry to retain color among others, preventing spoilage by antimicrobial activity. The antibacterial activity of ZnO-NPs is mainly due to the damage of bacterial cell wall and interference with DNA replication. The particles generate ROS and release metal ions inside the cells. It has been reported that ZnO-NPs nanoparticles have the high ROS producing ability. This may be the cause for this high ZnO activity in cell wall damage, membrane permeability increase, and NPs internalization via destruction of the PMF [14].

Spermatogenesis is an intricate process leading to formation of the most specialized of male germ cells. It involves a series of tightly controlled stages i.e. proliferation/differentiation of spermatogonia, meiosis and/or spermiogenesis [15]. Spermatogonia cells undergo many rounds of mitotic divisions to maintain their own population (self-renew), however, also allow the production of undifferentiated diploid spermatogonia. Following a number of cycles differentiation 63, meiotic divisions see to it that cells which initially arose from the first differentiation wave might be just like younger ones.57 During the second spermatogenic phase round haploid spermatids are formed. These spermatids undergo dramatic morphological changes during the course of spermiogenesis, resulting in elongated spermatids and ultimately mature spermatozoa [16,17]. Recently, novel insights about the biological relevance of nuclear envelope proteins for mammalian spermatogenesis and male fertility were reviewed [18]. Green biosynthesis, compared to physical or chemical synthesis processes, may be the best and most efficient method used in manufacturing nanomaterials. It is environmentally friendly, cost-effective, less toxic, easier to adapt, and safer [19].

MATERIALS AND MTHODS

Preparation of Zinc Oxide Nanoparticles

The synthesis of zinc oxide nanoparticles was conducted using biological methods at the Department of Biotechnology, College of Science, University of Baghdad, Iraq. The Allium porrum leaves were collected from the cultivation site in Kerbela, Iraq, rinsed with tap water, and thoroughly desiccated. The desiccated foliage was pulverized into a fine powder. Next, 250 grams of the powder were combined with 500 ml of deionized water. The combination was then stirred for one hour and then stored at 4°C for 24 hours. Next, the mixture was centrifuged at 8,000 r.p.m. for 10 minutes. The supernatant was promptly collected and placed in the refrigerator for the subsequent procedure. 200 cc of the prior preparation was combined with 20 g of zinc acetate and agitated overnight in a shaker. Next, the mixture was briefly centrifuged to gather the nanoparticles. After being rinsed twice with 5 ml of deionized water, the precipitated nanoparticles were centrifuged at 8,000 r.p.m. for 5 minutes, recovered, and transferred to a sterile Petri plate and maintained at 37°C for 24 hours to dry. Finally, the end product was characterized to verify the incorporation of nanoparticles [20].

Preparations for in vivo administration

Amounts of ZnO-NPs (size of nanoparticle was 65.49 nm), dose are given orally at 350 mg/kg ZnO NPs according to [21].

Histopathological examination

At the end of the experiment, testis, samples were collected, immediately washed with normal saline, and then fixed in 10% formalin. Tissue samples were washed and then routinely processed, dehydrated in graded series of alcohol followed by clearance in xylol, and finally embedded in paraffin wax. Using a sledge microtome, we prepared 5 µm-thick specimens from the paraffin beeswax blocks and then stained them with hematoxylin and eosin[22].

In vivo experimental Design:

Twenty healthy white male rats, ages ranging between 7-9 weeks, were randomly allocated into two groups and administered the treatments as follows:

Group 1 n=10: Rats oral gavage with 0.5 ml of sterile normal saline serving as a negative control group.

Group 2 n=10: Rats oral gavage with 0.5ml (350mg/kg) of ZnO nanoparticles for two weeks.

Five rats were sacrificed from each group at day 7 and 21 post treatment and samples from internal organs (testis) were taken for histopathology examination

RESULT AND DISCUSSION

Histopathological examination

Histopathological examination showing the normal testicular cellular structure, normal seminiferous tubules figures (1,2), distorted seminiferous tubules with elongation and significant germinal epithelial lining of tubule separation figures (3), distorted seminiferous tubules and the wide interstitial spaces in between some tubules, figure(4), dilation of interstitial spaces due to presence deep staining proteintious substance figure(5),necrosis, and severe desquamation, cellular epithelial necrosis and congested blood vessels, figures(6), increase of the (collagen fibers) thickness of the tunica albuginea and around basement membrane of severely damaged seminiferous tubule as well as congested blood vessels (figure7), degeneration and necrosis of tubular epithelia and around basement membrane of seminiferous tubule with inflammatory reaction and blood vessels congestion with fibrosis (figure8). Degeneration and necrosis of tubular epithelia and around basement membrane of seminiferous tubule with inflammatory reaction and blood vessels congestion with fibrosis figure (9).

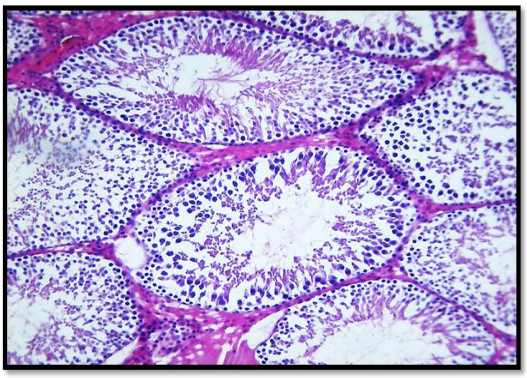

Figure 1. Histological section in testis of rat showing the normal testicular cellular structure, normal seminiferous tubules of the control group (H and E stain 100x).

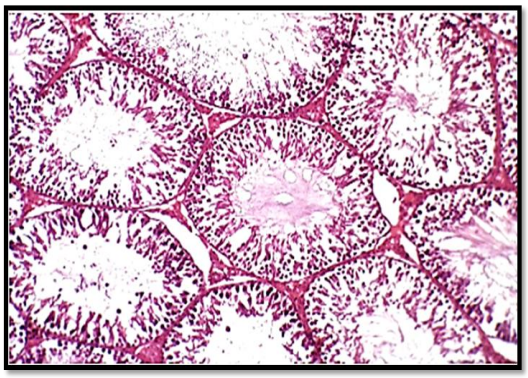

Figure 2. Histological section in testis of rat showing the normal testicular cellular structure, normal seminiferous tubules of the control group (H and E stain 100x).

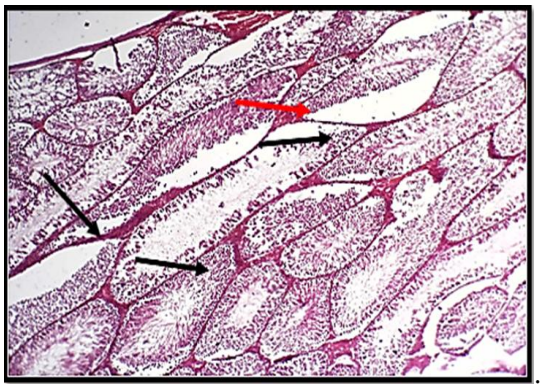

Figure 3. Histopathological section in testis of rat showing distorted seminiferous tubules with elongation (black arrow) and significant germinal epithelial lining of tubule separation (red arrow). (H and E stain 40x).

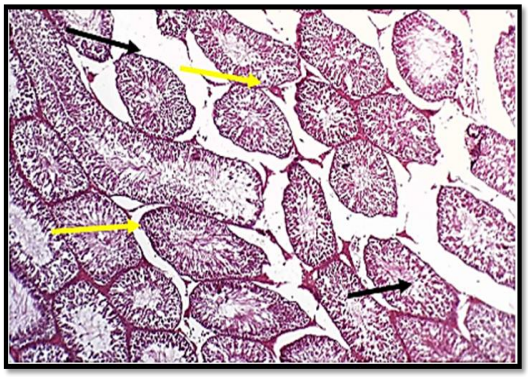

Figure 4. Histopathological section in testis of rat showing distorted seminiferous tubules (yellow arrow) and the wide interstitial spaces in between some tubules (black arrow) (H and E stain 40x).

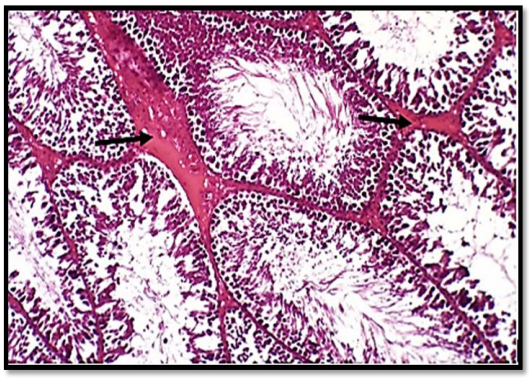

Figure 5. Histopathological section in testis of rat showing dilation of interstitial spaces due to presence deep staining proteintious substance (black arrow) (H&E stain 100X).

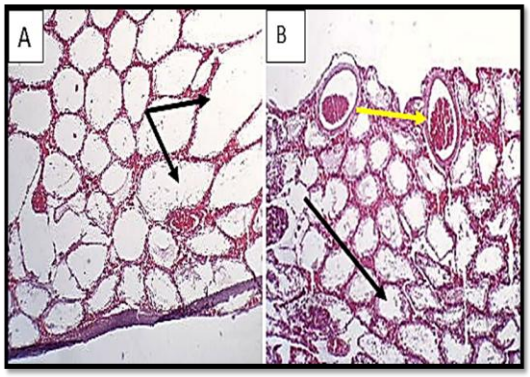

Figure 6 Histopathological section in testis of rat **(A)** showing necrosis and severe desquamation (black arrow) **(B)** showing cellular epithelial necrosis (black arrow), and congested blood vessels (yellow arrow) (H and E stain 40x).

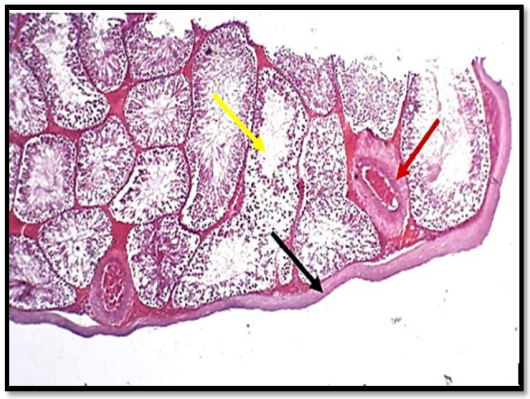

Figure 7. Histopathological section in testis of rat showing increase thickness of the tunica albuginea (black arrow) and around basement membrane of severely damaged seminiferous tubule (yellow arrow) as well as congested blood vessels (red arrow) (H and E stain 100x).

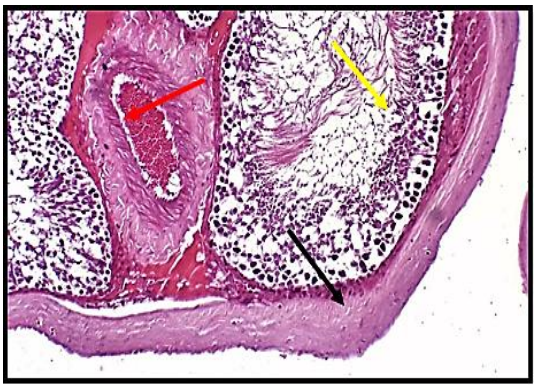

Figure 8. Histopathological section in testis of rat showing increase of the collagen fibers of the tunica albuginea (black arrow) and around basement membrane of seminiferous tubule with inflammatory reaction (yellow arrow) and blood vessels congestion with fibrosis (red arrow) (H and E stain 400x).

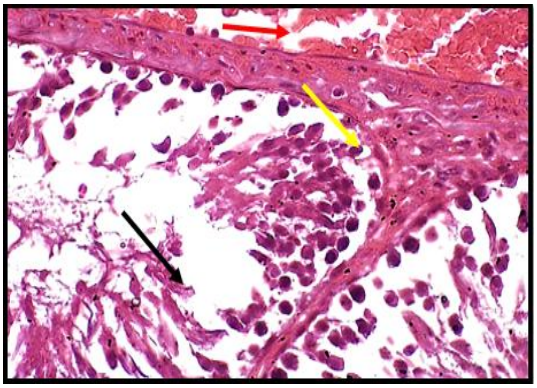

Figure 9. Histopathological section in testis of rat showing degeneration and necrosiss of tubular epithelia (black arrow) and around basement membrane of seminiferous tubule with inflammatory reaction (yellow arrow) and blood vessels congestion with fibrosis (red arrow) (H and E stain 400x).

Based on the results of this work, zinc oxide nanoparticles (ZnO-NPs) may have cytotoxic effects on the male reproductive system. Even though it has been showing beneficial impact on certain medicinal uses since it can reduce inflammatory and free radicals [23]. More, with the widespread application of ZnO-NPs in daily human life, their potential impacts on the human body, especially the male reproductive system, considering their smaller size and ability to cross the blood-testicular barrier [24], have attracted increasing attention. Thus, the current project shed the light on the side effect of ZnO-NPs mainly on reproductive system, in addition, testes are negatively impacted by (ZnO-NPs) in a dose-dependent manner. Interestingly, in animal models, ZnO-NPs mainly cause lower testosterone levels, testicular injury, and decreased sperm quality and fertility [25]. Compared to bigger, non-nano zinc particles, these harmful consequences are more noticeable. in addition to increased morphological defects، induced oxidative stress and DNA damage, exposure to ZnO-NPs causes a dose-dependent reduction in sperm count, motility, and viability [26,27]. Decreased testosterone levels by interfering with Leydig cell activity and down-regulating important enzymes (such as StAR and CYP17A1) necessary for testosterone synthesis [28]. Zinc oxide nanoparticles negatively affect pregnancy rates and the number of viable offspring, leading to reduced male fertility, if taken in high doses and for extended periods [29].

Although some research indicates that larger nanoparticles may also be extremely harmful, perhaps as a result of differential accumulation or other mechanisms, higher dosages and smaller particles typically show more toxicity. While toxicity is the most common finding at larger levels, several investigations indicate possible protective effects at extremely low concentrations [30].

Given these concerns regarding toxicity, several studies have specifically investigated the adverse effects of ZnO NPs on testicular function. For instance, exposure to ZnO NPs has been shown to induce oxidative stress and inflammation in testicular cells, leading to histopathological changes and reduced sperm quality in animal models [31,32].

Histopathological examination show that distorted seminiferous tubules with loosely arranged detached spermatogenic cells and the wide interstitial spaces in between some tubules, thickening of basement membrane due to presence protientious substance, severe desquamation and necrosis and absence of spermatocytes and most Sertoli cells, as well as congested blood vessels, increase thickness of the tunica albuginea(collagen fibers) and around basement membrane of severely damaged seminiferous tubule. Additionally, the morphology of seminiferous tubules, a decrease in the diameter and height of the epithelium resulting from germ cell loss is notable, owing to the apoptotic effect of ZnO-NPs on spermatogenic cells [33]. In addition to the testis and epididymis, other studies analysed histological changes in seminal vesicles [34]. In seminal vesicles, the changes were also significative, with the detection of mononuclear cells infiltrating the stroma and the appearance of mild to moderate proliferation of epithelial cells [34]. According to these data, exposure to ZnO-NPs has repercussions on all male reproductive systems in a dose-dependent manner [29].

CONCLUSION

The current study demonstrated that oral administration of zinc oxide nanoparticles at a dose of 350 mg/kg b.w induced significant adverse effects on the male reproductive system in rats. Histopathological examination of the testis revealed histopathological change, indicating reproductive toxicity, it is possible that ZnO-NPs there by destroying the microenvironment necessary for spermatogenesis, which may lead to poor reproduction in rats.

REFERENCES

1) Roduner E. Size matters: why nanomaterials are different. Chemical society reviews. 2006;35(7):583-92.
2) Jha RK, Jha PK, Chaudhury K, Rana SV, Guha SK. An emerging interface between life science and nanotechnology: present status and prospects of reproductive healthcare aided by nano-biotechnology. Nano reviews. 2014 Jan 1;5(1):22762.

3) McAuliffe ME, Perry MJ. Are nanoparticles potential male reproductive toxicants? A literature review. Nanotoxicology. 2007 Jan 1;1(3):204-10.
4) Hassanen EI, Ragab E. In vivo and in vitro assessments of the antibacterial potential of chitosan-silver nanocomposite against methicillin-resistant Staphylococcus aureus–induced infection in rats. Biological Trace Element Research. 2021 Jan;199(1):244-57.
5) He L, Liu Y, Mustapha A, Lin M. Antifungal activity of zinc oxide nanoparticles against Botrytis cinerea and Penicillium expansum. Microbiological research. 2011 Mar 20;166(3):207-15.
6) Hassanzadeh P. The significance of bioengineered nanoplatforms against SARS-CoV-2: From detection to genome editing. Life sciences. 2021 Jun 1;274:119289.
7) Sztandera K, Gorzkiewicz M, Klajnert-Maculewicz B. Gold nanoparticles in cancer treatment. Molecular pharmaceutics. 2018 Nov 19;16(1):1-23.
8) Rahman HS, Othman HH, Abdullah R, Edin HY, AL-Haj NA. Beneficial and toxicological aspects of zinc oxide nanoparticles in animals. Veterinary Medicine and Science. 2022 Jul;8(4):1769-79.
9) Greco F, Courbière B, Rose J, Orsière T, Sari-Minodier I, Bottero JY, Auffan M, Perrin J. Toxicity of nanoparticles on reproduction. Gynecologie, Obstetrique & Fertilite. 2014 Dec 19;43(1):49-55.
10) Pinho AR, Rebelo S, Pereira MD. The impact of zinc oxide nanoparticles on male (in) fertility. Materials. 2020 Feb 13;13(4):849.
11) Archibong AE, Ramesh A, Niaz MS, Brooks CM, Roberson SI, Lunstra DD. Effects of benzo (a) pyrene on intra-testicular function in F-344 rats. International journal of environmental research and public health. 2008 Mar 30;5(1):32-40.
12) Inyang F, Ramesh A, Kopsombut P, Niaz MS, Hood DB, Nyanda AM, Archibong AE. Disruption of testicular steroidogenesis and epididymal function by inhaled benzo (a) pyrene. Reproductive Toxicology. 2003 Sep 1;17(5):527-37.
13) De Angelis I, Barone F, Zijno A, Bizzarri L, Russo MT, Pozzi R, Franchini F, Giudetti G, Uboldi C, Ponti J, Rossi F. Comparative study of ZnO and TiO2 nanoparticles: physicochemical characterisation and toxicological effects on human colon carcinoma cells. Nanotoxicology. 2013 Dec 1;7(8):1361-72.
14) Sirelkhatim A, Mahmud S, Seeni A, Kaus NH, Ann LC, Bakhori SK, Hasan H, Mohamad D. Review on zinc oxide nanoparticles: antibacterial activity and toxicity mechanism. Nano-micro letters. 2015 Jul;7(3):219-42.
15) Dutta S, Sengupta P, Slama P, Roychoudhury S. Oxidative stress, testicular inflammatory pathways, and male reproduction. International journal of molecular sciences. 2021 Sep 17;22(18):10043.
16) Guraya SS. Biology of spermatogenesis and spermatozoa in mammals. Springer Science & Business Media; 2012 Dec 6.
17) Hess RA, De Franca LR. Spermatogenesis and cycle of the seminiferous epithelium. Molecular mechanisms in spermatogenesis. 2009:1-5.
18) Pereira CD, Serrano JB, Martins F, da Cruz e Silva OA, Rebelo S. Nuclear envelope dynamics during mammalian spermatogenesis: new insights on male fertility. Biological Reviews. 2019 Aug;94(4):1195-219.
19) Raja A, Ashokkumar S, Marthandam RP, Jayachandiran J, Khatiwada CP, Kaviyarasu K, Raman RG, Swaminathan M. Eco-friendly preparation of zinc oxide nanoparticles using Tabernaemontana divaricata and its photocatalytic and antimicrobial activity. Journal of Photochemistry and Photobiology B: Biology. 2018 Apr 1;181:53-8.
20) Nallal VU, Prabha K, VethaPotheher I, Ravindran B, Baazeem A, Chang SW, Otunola GA, Razia M. Sunlight-driven rapid and facile synthesis of Silver nanoparticles using Allium ampeloprasum extract with enhanced antioxidant and antifungal activity. Saudi journal of biological sciences. 2021 Jul 1;28(7):3660-8.
21) Hong X, Shao N, Yin L, Li C, Tao G, Sun Y, Qian K, Yang J, Xiao P, Yu X, Zhou Z. Exposure to zinc oxide nanoparticles affects testicular structure, reproductive development and spermatogenesis in parental and offspring male rats. Annals of translational medicine. 2022 Jul;10(13):751.
22) Bancroft JD, Gamble M. Fixation of tissues. In: Layton C, Bancroft JD, Suvarna SK, editors. *Theory and practice of histological techniques*. 8th ed. London: Churchill Livingstone Elsevier; 2019. p. 40–63. doi:10.1016/B978-0-7020-6864-5.00004-9.
23) Rehman N, Jabeen F, Asad M, Nijabat A, Ali A, Khan SU, Luna-Arias JP, Mashwani ZU, Siddiqa A, Karthikeyan A, Ahmad A. Exposure to zinc oxide nanoparticles induced reproductive toxicities in male Sprague Dawley rats. Journal of Trace Elements in Medicine and Biology. 2024 May 1;83:127411.
24) Pinho AR, Rebelo S, Pereira MD. The impact of zinc oxide nanoparticles on male (in) fertility. Materials. 2020 Feb 13;13(4):849.
25) Tang Y, Chen B, Hong W, Chen L, Yao L, Zhao Y, Aguilar ZP, Xu H. ZnO nanoparticles induced male reproductive toxicity based on the effects on the endoplasmic reticulum stress signaling pathway. International Journal of Nanomedicine. 2019 Dec 4:9563-76.
26) Sengul AB, Asmatulu E. Toxicity of metal and metal oxide nanoparticles: a review. Environmental Chemistry Letters. 2020 Sep;18(5):1659-83.d o i . o r g / 1 0 . 1 0 0 7 / s 1 0 3 1 1 - 0 2 0 - 0 1 0 3 3 – 6
27) Iqbal S, Jabeen F, Chaudhry AS, Shah MA, Batiha GE. Toxicity assessment of metallic nickel nanoparticles in various biological models: An

interplay of reactive oxygen species, oxidative stress, and apoptosis. Toxicology and Industrial Health. 2021 Oct;37(10):635-51.

28) Pinho AR, Rebelo S, Pereira MD. The impact of zinc oxide nanoparticles on male (in) fertility. Materials. 2020 Feb 13;13(4):849.

29) Gonzalez E, Guengerich FP. Kinetic processivity of the two-step oxidations of progesterone and pregnenolone to androgens by human cytochrome P450 17A1. Journal of Biological Chemistry. 2017 Aug 11;292(32):13168-85.

30) Crisponi G, Nurchi VM, Lachowicz JI, Peana M, Medici S, Zoroddu MA. Toxicity of nanoparticles: etiology and mechanisms. InAntimicrobial nanoarchitectonics 2017 Jan 1 (pp. 511-546). Elsevier.

31) Ahmed DH, El-Sayed WM. Large diameter zinc oxide nanoparticles reduced testicular CYP17A1 and viability and elevated testicular inflammation in adult male rats. The Journal of Basic and Applied Zoology. 2025 Dec;86(1):102.

32) Han Z, Yan Q, Ge W, Liu ZG, Gurunathan S, De Felici M, Shen W, Zhang XF. Cytotoxic effects of ZnO nanoparticles on mouse testicular cells. International journal of nanomedicine. 2016 Oct 11:5187-203.

33) Erfani Majd N, Hajirahimi A, Tabandeh MR, Molaei R. Protective effects of green and chemical zinc oxide nanoparticles on testis histology, sperm parameters, oxidative stress markers and androgen production in rats treated with cisplatin. Cell and Tissue Research. 2021 May;384(2):561-75.

34) Salman RA. The influence of ZnO NPs on reproductive system tissues of albino male mice. Histopathological study. Int J Sci Res. 2017;6(7):2021-5.