Zinc Oxide Nanoparticles Improve Testicular Steroidogenesis Machinery Dysfunction In Benzo [α] Pyrene Challenged Rats

Niveen M. Daoud (dr.niveendaoud@gmail.com)
National Research Centre

Mohamed, S Aly
National Research Centre

Omaima H. Ezzo
National Research Centre

Naglaa A. Ali
National Research Centre

Research Article

Keywords: Zinc Oxide Nanoparticles, Benzo[α]Pyrene, expression of steroidogenic enzymes, Oxidative Stress Biomarkers, Histopathology, Rats

Posted Date: February 19th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-218699/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Zinc Oxide Nanoparticles improve Testicular Steroidogenesis Machinery Dysfunction In Benzo [a] Pyrene Challenged Rats

Niveen M. Daoud¹, Aly M.S.¹, Omaima H. Ezzo¹, Naglaa A. Ali ²

¹Animal Reproduction & A. I. Department, Veterinary Research Division, National Research Center, El-Buhouth Street, Dokki, Cairo, Egypt.

²Hormones Department, Medical Research Division, National Research Center, El-Buhouth Street, Dokki, Cairo, Egypt

Correspondence, Niveen M Daoud

Permanent address: Animal Reproduction & A. I. Department, veterinary Research Division, National Research Center, El-Buhouth Street, Dokki, Cairo, Egypt.

E.mail: dr.niveendaoud@gmail.com
ABSTRACT

Although Zinc oxide nanoparticles (ZnO NPs) in low doses have potentially positive effects on reproduction by their antioxidant effects, the defensive role of Zinc nanomaterials against environmental pollutants that affect male reproduction has not been adequately studied. We designed our study to assess the impact of ZnO NPs towards reproductive dysfunction induced by Benzo[a]Pyrene (B[a]P). Forty-eight mature male rats were randomly distributed into six equal groups: G1; negative control, G2&3- positive control I &II (either 10 or 30 mg ZnO NPs / kg BW); G4. (150 mg Bap / kg BW), G 5 & 6 (Co-administrated B[a]P with different concentrations of ZnO NPs). Oxidative stress biomarkers, semiquantitative real-time PCR for steroidogenic enzymes (CY11A1, StAR, and 3β- HSD), testosterone levels and histopathology in the liver, kidney, and testicles were examined for this investigation. B[a] P treated group showed significant deterioration in all reproductive parameters and induced oxidative stress. Co-administration ZnO NPs eased oxidative stress and effectively increased the expression of CY11A1, StAR, and 3β- HSD and improved histopathological changes in the examined organs. Our results using the selected doses and with Nano particle properties confirm that ZnO NPs have an obvious ameliorative effect against B[a] P.
Keywords: Zinc Oxide Nanoparticles; Benzo[a]Pyrene; expression of steroidogenic enzymes; Oxidative Stress Biomarkers; Histopathology; Rats.

1. Introduction

Nanoparticles (NPs) are materials with at least one dimension ≤ 100 nm and have a large surface-to-volume ratio. This allows them to possess unique properties that enable them to interact more effectively with biological systems. Nowadays, there are increasing interest regarding the impact of these nanoparticles on human and veterinary science, depending on the concern of these nanoparticles easily pass through the blood-brain and blood-testis barrier. ZnO NPs have become one of the most useable metal oxide nanoparticles in biological and animal science applications owing to their exceptional properties of biocompatibility, solubility, economic, and low toxicity. This allows ZnO Nps to mimic biomolecules that regulate cell cycle and cellular homeostasis. But there is a great dispute between reproductive scientists, Are Zinc nanoparticles toxic or could they play a reproductive stimulating role. This big question actually resulted in a conclusion that is the NPs effects depend on many factors like the size, the concentration used, the morphology, the synthesis process, the surface area, the cell type tested and the organism type. Small sizes, higher concentrations, and high frequency of administration doses enhance its toxic
Recently, there have been many new researches of ZnO NPs that using it as a protective effect against reproductive toxicity associated chemotherapy drugs, streptozotocin-induced diabetic and Nicotine 6–9.

Further researches are needed to reveal the defensive effects of Zinc nanomaterials against environmental pollutants that induce male reproductive dysfunction. Therefore, Our study was aimed to investigate the protective effect of ZnO NPs in different concentrations in male rats that underwent Benzo [a]pyrene.

Benzo [a] pyrene (B[a]P) is a polycyclic aromatic hydrocarbon (PAH) and most widespread environmental contaminant, originating from the incomplete burning of fossil fuels, tobacco smoke, diesel consumption, and roasted foods 10. Data conclusively clinched that, even low-to-moderate exposure to BaP has an endocrine disruptor and deleterious effects on male reproductive system and results in steroidogenic dysfunctions 11–13. B[a]P increases reactive oxygen species (ROS) production and thus oxidative stress, leads to increased lipid peroxidation and causes male infertility 14,15.

In the current study, we examined the expression of some important steroidogenic enzymes; cholesterol side-chain cleavage enzyme (CYP11A1) (Leydig cell-specific gene), steroidogenic acute regulatory protein (StAR) and 3β-hydroxysteroid dehydrogenase (3 β-HSD) using quantitative Real Time-PCR technique and support our result with scope of oxidative stress biomarkers, serum
level of testosterone, sperm count and finally validated by histopathological examination. Our result may be to add new data on the protectivity of our designed doses of ZnONPs on male fertility.

2. **Materials and methods**

2.1 **Chemicals:**

We bought Benzo[α]Pyrene and Zinc oxide nanoparticles from Sigma-Aldrich Chemicals Co., St. Louis, MO, USA. Zinc Oxide Nanoparticles Product code: 544906. They have average particle size <100nm, 10-25 m²/g specific surface area, They have a formula weight of 81.39 g mol⁻¹, and their quality level is 200. Actual surface area is 15.88 m²/g using the Brunauer-Emmett-Teller (BET) method. They have high chemical stability electrochemical coupling coefficient and high thermo-mechanical stability at ambient temperatures. We prepared our selected doses of Zinc Oxide Nanoparticles by dissolving them in distilled water.

**Animal husbandry and experimental design**

We used Forty-eight adult Wistar male rats weighing 200-250 g in this experiment. Rats were housed in stainless steel mesh cages in a naturally lit, ventilated room in the animal unit, National Research Centre, Giza, Egypt. We adjusted the lowest ambient temperature was at 30 ± 2 °C and 12h light / 12h dark cycle and fed them with a standard rat diet and water provided *ad libitum*. We put
the animals without treatment for a week to acclimate to the new conditions and then treated for 45 consecutive days.

Protocols and procedures of this experiment were approved by the Institutional Ethics Committee of National Research Centre, Egypt, and the experiments were performed as a per guideline of the National Research Centre Ethical Committee for medical research and in compliance with ARRIVE guidelines.

Rats were allocated randomly (complete randomization) into six treatments (n = 8 per treatment). **negative control (NC) group:** a normal healthy untreated animals. **ZnONPs10 and ZnONPs 30 groups:** animals were designed as positive control (PC) were treated in the amount of 1ml with 10 or 30 mg/kg BW/day Zinc nanoparticles. **BaP group:** animals were treated with Benzo [a] pyrene (98% HPLC purity) at doses 150 µg/ kg BW /day. **Bap+ZnONPs10; Bap+ ZnONP30 groups:** animals were Co- administrated B[a]P and ZnO NPs with different concentrations. All the previous chemicals were given daily to rats by oral gavage through oral cannula.

Our ZnO NPs 10 mg/kg/BW dose applied in this experiment was selected according to previously published literature, while the dose 30mg/kg/BW according to the published review that clarified the impact of Zn nanoparticles on male (in) fertility and B[a]P dose according to Kang.
At the end of the experiment, we collected blood samples via sinus orbital
puncture using un-heparinized pulled Pasteur pipettes. Subsequently, serum was
gathered after centrifugation and stored at −20°C until the assay Testosterone and
oxidative stress biomarkers.

2.3. **Determination of steroidogenesis-related genes using Quantitative Real-
Time PCR**

2.3.1. **RNA isolation and cDNA synthesis**

We dissected Epididymides from rats and froze in liquid nitrogen. Total
RNA was extracted after homogenization using the standard TRIzol® reagent
extraction method (Invitrogen, USA). We determined RNA concentrations at
260/280 nm using an ultraviolet spectrophotometer. Purified RNA was
immediately transcribed to Single-strand cDNA using a sample containing 500ng
of total RNA following the manufacturer's directions (First Strand cDNA
Synthesis Kit (MBI Fermentas, Germany). Reverse transcription (RT) was
synthesized at total volume of 25 µl using 0.5 µl poly (dT)18 primer and 13 µl
RNA. The reaction was run at 37°C for 90 minutes and ended with a step of
denaturation at 70°C for 15 min. Afterward, we preserved the cDNA containing
tubes in -20°C.
2.3.2. Quantitative Real time-polymerase chain reaction (RT-PCR) using SYBR Green I

Real-time RT-PCR analysis for StAR, 3β-HSD and cholesterol side-chain cleavage enzyme CYP450scc (CYP11A1 gene; Leydig-cell-specific biomarker) were performed on a real-time PCR detection system (iQ5-Bio-Rad Laboratories, Cepheid, USA) using Syber green PCR master mix (TaKaRa, Biotech. Co. Ltd.). The expressions level of our genes mRNAs were normalized to the β-actin housekeeping gene (Actb). We got our target gene and Actb oligonucleotides sequence from the published literatures (Table 1). PCR reactions were set up in 25 μl reaction mixtures containing 12.5 μl 1× SYBR, 0.5 μl forward primers, 0.5 μl reverse primer, 6.5 μl distilled water, and 5 μl of cDNA template. The amplification cycle started by a preliminary denaturation step at 95 °C for 3 minutes followed by 35 cycles of denaturation at 95°C for 15 sec; followed by annealing step at 55°C for 30 sec. Finally, the extension step performed at 72°C for 30 sec. Samples and controls were run in duplicate. Amplification was followed by melt curve analysis to ensure that no primer-dimer amplification occurred. The gene expressions were calculated using the formulae of Bio-Rad laboratories Inc. $Ef = 10^{-1/slope}$. Efficiency (%) = $(Ef - 1) \times 100^{20}$. We determined The relative quantification of the target to the reference by using the
ΔCₜ method if E for our target genes and the reference primer (ACTB) are the same Ratio \( \frac{\text{(reference/target)}}{\text{E}} = E^\text{CT(2)} - \text{CT(target)} \)

### 2.4. Estimation of serum testosterone and oxidative stress biomarkers:

We determined serum testosterone concentrations using an enzyme-linked immunosorbent assay kit (XEMA Co., LTD, Moscow, Russia) according to the manufacturer's instruction.

The level of serum Malondialdehyde (MDA) was estimated colorimetrically using (TBA) thiobarbituric acid method according to the standard Ohkawa method \(^{21}\).

Reduced Glutathione (GSH) activity was based on measurement of the optical density of yellow color derivative coming from GSH and DTNB (nitrobenzoic acid) reaction according to the basic method \(^{22}\). We measured The levels of serum MDA and GSH using Bio-diagnostic Co., kits.

### 2.5. Determination of sperm count

According to the previously established method \(^{23}\), we isolated Cauda epididymides and immediately immersed in normal physiological saline, gently shaken for 10 minutes, and then incubated for 2 min at 37 °C to permit spermatozoa to leave the epididymal tubules. A solution of 5 g sodium bicarbonate, 25 mg eosin and 1 ml formalin (35%) dissolved in 100 ml distilled water was prepared and mixed with 1ml supernatant fluid (1:100). An aliquot of
this diluted sperm suspension (10μl) was conveyed to each counting chamber of a hemocytometer, then counted under a light microscope at × 200 magnification.

2.6. Histopathological examination

At the termination of the experiment, we used sodium pentobarbital anesthesia for euthanasia scarification of rats. Testis, liver, and kidney specimens were collected and quickly washed with normal saline then fixed in 10% formalin for histopathological examination. Tissue samples were embedded for 24h in paraffin. We prepared tissue thickness (4-5μm) from paraffin beeswax blocks using a sledge microtome and stained them with Hematoxylin & Eosin (H&E).

We examined the sections under a light microscope (Olympus CX 41, Japan). Although liver and kidney are not a concern for our study aim, they used as a reference to standing if any cytotoxic effect of our ZnO NPs doses.

2.7. Statistical analysis of data

We analyzed the data using IBM SPSS Statistics for Windows, Version 22.0 New York, United States. Data were represented as mean ± standard deviation (SD) and statistically analyzed using one-way Analysis of Variance (ANOVA). To test the intergroup homogeneity, we used Duncan's test. Statistical significance was set at \( p < 0.05 \). We used Pearson's correlation linear regression to test whether any correlation exists between the expression of steroidogenic enzymes and serum testosterone.
3. **Results**

**3.1 mRNA gene expression of steroidogenesis-related genes using RT-PCR**

The expressions of the steroidogenesis related enzymes (StAR, CY11A1 and 3β–HSD) are illustrated in table 2 and Fig 1. Our findings recorded that there aren't significant differences of gene expressions among negative and positive control groups (p > 0.05). The BaP group showed a highly significant decrease in gene expressions reach to -81.7% for StAR, -61% for CYA11A1, and -81% for 3β–HSD when compared to the negative control group (P < 0.001). Co-administration supplementation of ZnO NPs with B[a]P recorded a significant increase in the expression of steroidogenesis related enzymes when compared to the BaP group. StAR gene expression significantly increased by 225.6%; 351%; CY11A1 increased by 167.3% and 207%, and 3β-HSD by 301%; 340 in In Bap + ZnONPs 10 and Bap + ZnONPs 30 groups respectively (P < 0.001). Pearson’s linear correlation between testosterone level and the expression of steroidogenic enzymes are highly significant (table 2-C). Although our results recorded an improvement in gene expressions after toxic exposure to BaP, they still under the negative control levels. These findings showed that ZnO NPs supplementation promoted the expression of steroidogenic enzymes, which inhibited by BaP with fairly good outcome, although it could not bring it back into control.
3.2. Antioxidant/ oxidative stress indicators (Malondialdehyde; MDA and reduced glutathione; GSH)

The results of antioxidant biomarkers including MDA and GSH are illustrated in table 3 and figure 2. Positive control I and II showed significant difference when compared with negative control group (P<0.05). The BaP group recorded a significant increase in the serum level of MDA by 35% and a significant decrease in GSH level by -37.6% when compared with the negative control (P<0.05). While Co-administration of Bap with ZnO NPs (Bap+ZnONPs10; Bap+ZnONPs 30) have an anti-Bap effect as it recorded a corresponding a significant decrease in MDA level by -26.5%, -35.5% and increase in of GSH level by 46.3% and 43.3% respectively compared to BaP group and significantly improved when compared to negative control one as it recorded non significant difference (P>0.05). These findings showed that ZnO NPs supplementation recorded antioxidant stress either at the level of negative control comparison or counteracting BaP and returning oxidative stress biomarkers to control levels.

3.3. Testosterone concentration and sperm count

The statistical analyzed data for testosterone level and sperm counts are illustrated in table 4 and figure 3. Although testosterone level recorded there is no significant difference between NC and PCI (p > 0.05), a significant increase
in its level was recorded in the PCII group. The data of BaP group recorded significant decrease (-39.2 %) than that of the negative control. Co-administrated groups (Bap+ZnO NPs10; Bap+Zn ONPs 30) reported a significant improvement in testosterone level when compared to the Bap group by 40.5%, 48.9% respectively. But its levels in co-administrated groups still recorded significant difference than NC.

Sperm count recorded that, there aren’t significant differences of gene expressions among negative and positive control groups (p > 0.05). The BaP group revealed a significant decrease in the sperm count than negative control by -50.2 % (P<0.001). Wherease, The Co-administration groups (Bap+ZnoNPs10; Bap+ZnoNPs 30) recorded a significant increase in sperm count compared to the Bap group with an increase of 59.9 % and 83.1 % respectively (P<0.001). However, the sperm counts remained significantly under the negative control group. These results indicated that, ZnO NPs supplementation stimulated testosterone synthesis accompanied by increased sperm counts inhibited by BaP with fairly good outcome, although they remained under the control.

3.4. Histopathological findings

Upon microscopic examination, animals in the negative and positive control groups revealed a normal testicular, kidney, and liver architecture and morphology. Benzo[a]Pyrene treated group alone induced various
histopathological alterations in examined tissues. In testicular tissues, B[a]P causing atrophy of the seminiferous tubules associated with severe vacuolar degeneration and desquamation of spermatogonial cells lining seminiferous tubules, lost integrity of cellular membranes, the atrophy of seminiferous tubules, lack of spermatids and spermatozoa, and altered morphology of spermatogonia and spermatocytes, with the presence of multinucleated spermatid giant cells (Symplast) (Fig. 4. B&C). While in the kidney it showed vacuolations of glomerular tufts, severe atrophy and mild degeneration of glomerular tuft associated with an increase in the Bowman’s space (Fig. 5. B), congestion of renal blood vessels and degeneration of epithelial cells lining renal tubules severe atrophy and degeneration of glomerular tuft and necrobiotic changes of epithelial lining renal tubules. Liver tissues also showed alteration of the cellular architecture pattern of the hepatic parenchyma associated with hepatic parenchyma cellular disorganization of the hepatocytes and vascular dilation and congestion, some sections showed non occluding thrombus formation , some sections showed leucocytic cells infiltration of portal area with activation of kupffer cells (Fig. 6. B&C). Improvements in the histopathological pictures were noticed in three examined organs from rats treated with Nano-Zinc Oxide and B[a]P. The aforementioned pathological alterations are depicted in the photomicrographs (Fig 4, 5& 6).
4. Discussion

Available data conclusively proved that Benzo[α] pyrene reduces male fertility. Zinc oxide can be stated as a multifunctional material due to its unique physical and chemical properties. It is known to be crucial for testosterone synthesis and spermatogenesis. While the studies of ZnO NPs effects on male fertility is still rare, either at the in vitro and in vivo levels. Therefore, this study sought to evaluate the ameliorative effect of ZnONPs supplementation on male fertility in Benzo[α] pyrene exposed rat through molecular, biochemical and histological impaction on testis and epididymis.

In our study, Benzo[a] Pyrene induced severe oxidative stress which significantly increased MDA and decrease GSH levels (P < 0.001). BaP is one member of PAHs which undergoes intracellular biotransformation by cytochrome P450 (CYP) enzymes, leading to the production of reactive oxygen species (ROS) comes from reduction of antioxidant enzymes as reduced glutathione. Free radical initiates lipid peroxidation through a chain reaction thus increasing the level of lipoperoxidation product such as MAD. This correlates logically with oxidative stress which was consistent with our results.

Regarding ZnO NPs supplementation, it recorded antioxidant stress either at the level of negative control group comparison or counteracting BaP and returning oxidative stress biomarkers to control levels. Zn is a core component of
over 200 metalloenzymes, including antioxidant enzymes and a known protector of sulfhydryl groups; it is also thought to weaken lipid peroxidation, which provides it anti-oxidative stress features. ZnO NPs dietary supplementation (10mg /kg /Bw) for nicotine-exposed rats reduces the harmful effects of exposure, through reducing oxidative stress and improvement of male fertility. Beside that, the lower doses of ZnO NPs (10 mg /kg /Bw) have a protective effect on sperm of diabetic rats owing to antioxidant properties as ZnO NPs increase the activity and mRNA expression levels of SOD, CAT, and GSH and decreased MDA levels in testicular tissue. On the contrary, Hussein et al. Recorded that ZnONPs decreased Antioxidant capacity and increased oxidative stress inducing severe reproductive toxicity in male rats. The result come from the doses used by authors as they used 100 & 400 mg/kg /BW which lead to this oxidative stress. Again, we can go back to what has been stated in Pinho’s review, The effects of ZnO NPs depend on the size, the concentration used, the morphology, the surface area. These ZnO NPs at low concentrations it act as antioxidant agents, while reactive oxygen species (ROS) can generate and induce apoptosis at high concentrations.

Testosterone level in our study was emphasized by the manifested steroidogenesis related enzymes using Pearson’s linear correlation between testosterone level and the expression of steroidogenic enzymes, which recorded highly significant correlation. Cholesterol is transferred from outside to inside the mitochondrial membrane depending mainly on the pivotal role of the
Steroidogenic Acute Regulatory (StAR) protein. Cholesterol undergoes oxidation by mitochondrial cytochrome P450 oxidase (P450scc; CYP11A1) and converts to pregnenolone. The pregnenolone is oxidized by 3β-HSD and produces androstenedione which is reduced by other enzymes and produces testosterone\(^29\).

In the BaP group, both of Testosterone and the expression of steroidogenic enzymes were significantly than negative control group (P\(<\)0.001). Our results are in harmony with previous observations recorded that StAR, CY11A1 and 3β HSD can be regulated by endogenous and exogenous agents, including environmental toxins like B[a]P which affects LH stimulated Leydig cell and serum testosterone production\(^12,30,31\). At the same context, under oxidative stress conditions, ROS activates stress leading to decrease StAR, CY11A1 and 3β-HSD gene expressions\(^13\). This implies a potent strength opposing correlation between oxidative stress and testicular steroidogenesis\(^32,33\).

Regarding Co-administrated groups showed an increasing the testosterone level in parallel with an improvement in gene expression of steroidogenic enzymes in comparing with BaP group. This agrees with that recorded previously by Le et al. who stated that, ZnO NPs induces up-regulation of the genes and increasing gene expression dependent on the exposure time and concentration\(^34\). Recently, Bara and his co-authors\(^35\) examined the direct effect of ZnO NPs in vitro using different concentrations on mouse testicular Leydig cells (TM3) and recorded significant amplification of the expression of steroidogenic enzymes.
(STAR and CYP11A1). Recently, Mohamed et al. have reported that ZnO NPs supplementation with a dosage of 10mg/kg/BW caused an increase in testicular gene expression of StAR and cytochrome P450scc as in parallel with the level of testosterone in nicotine exposed rats. In contrast, Tang et al. reported that ZnO NPs decrease testosterone production through the downregulation of StAR. This difference come from the dose which the author used (50, 150, and 450 mg/kg). Although several literature discusses the protective effect of ZnO NPs against drugs or toxic substances, to date, the molecular mechanism of ZnO NPs is still absence, especially in vivo.

Sperm count showed an significant decrease in B[a]P group (P<0.001) as compared to negative control one. This result is predictable as testosterone levels highly decreased. Testosterone is the androgen in the testis that supports spermatogenesis. BaP as an oxidative stress inducer, it damages DNA in the sperm nucleus and increases apoptosis at a specific stage of the germinal cycle. Our results were further supported by histopathological changes in testis as showed the deleterious effects of B[a]P on the testis. The alteration in seminiferous tubules architecture, altered morphology of spermatogonia and spermatocytes, and atrophy of seminiferous tubules showed B[a]P interferes with the process of spermatogenesis.

ZnO NPs Co-administration improves sperm count and histopathological findings. These results agree with other investigations recorded that the
administration of ZnO NPs prevented testicular toxicity and sperm damage via an antioxidant mechanism, against doxorubicin \(^7,43\) and Nicotine in adult rats \(^8\).

Our histopathological findings, which including nephrotoxic adverse effects of B[a]P, including degeneration, atrophy of glomerular tuft and necrotic lining epithelium and disorganization of the hepatic parenchyma, necrosis, and leucocytic cells infiltration agree with previously published studies \(^{44-46}\). And can be interpreted by a previously published study who recorded that increasing free radical and ROS production by BaP reinforce tissue damages and considered the central causative factor responsible for pathological finding through membrane lipid peroxidation and DNA mutations \(^{47}\).

Nano-Zinc Oxide is known for its antioxidant and anti-inflammatory properties. This antioxidant activity mainly occurs through neutralizing and scavenging free radicals \(^{48-51}\). This concept is supported by its ability to protect cell membrane integrity by increasing the antioxidant enzyme levels and decreasing MDA and free radical levels \(^{52}\). Zinc has anti-apoptotic properties that guard cells against different pro-apoptotic molecules \(^{53}\). Our histopathological findings are in line with these concepts as all examined tissues recorded normal histology like the control group. This is a sign that ZnO NPs are helpful for tissue regeneration to reverse damage caused by Benzo [a]Pyrene.
Conclusion

Our findings at the designed doses and with the properties of nanoparticles in this study concluded that ZnO NPs have an obvious ameliorative effect against B[a] P through decreasing oxidative stress and increasing expression of steroidogenic enzymes, repair tissue abnormalities which may progress a new hope in both reproductive toxicology and nanomedicine fields. Further researches are needed to discover its different mechanisms in improving male fertility.

Table (1): Primers sequences for RT-PCR

| Gene      | Oligonucleotides sequence (5′–3′)                              | Accession Number   |
|-----------|----------------------------------------------------------------|-------------------|
| StAR      | F: TCT CTA GTG TCT CCC ACT GCA TAG C                           | NM_011485.5       |
|           | R: TTA GCA TCC CCT GTT CG TAG CT                               |                   |
| CYP11A1   | F: ACAT GGC CAA GAT GGT ACA GTT G                             | NM_019779         |
|           | R: ACG AAG CAC CAG GTC ATT CAC                                 |                   |
| 3β-HSD    | F: ACAT GGC TCT GGG AGT TAT AAG GT                             | NM_008293         |
|           | R: TTA GTG ACT GGC AAG GCT TCT G                              |                   |
| B-actin   | AGA AGA TCT GGC ACC ACA CC TAC GAC CAG AGG CAT ACA GG          | NM_007393.5       |

† Abbreviations: F: forward primer; R: reverse primer. StAR; steroidogenic acute regulatory protein; CYP11A1- P450sc-c-cholesterol side-chain cleavage enzyme; 3 β-HSD -3β-hydroxysteroid dehydrogenase-1.
Tables (2) Effect of Zinc Oxide Nanoparticles on the relative expression of Steroidogenic enzymes in Bap-challenged male rats.

A- Statistical comparison among groups using ANOVA test

| Groups                      | StAR      | CY11A1     | 3β-HSD    |
|-----------------------------|-----------|------------|-----------|
| Negative Control (NC)       | 1.006± .0115 | 1.010± .0173 | 1.000± .000 |
| ZnO NPs 10 (PC)             | 1.016± .015 | 1.006± .011  | 1.010± .010  |
| ZnO NPs 30 (PC)             | 1.020± .010 | 1.003± .005  | 1.010± .010  |
| Bap                         | 0.183± .045 * | 0.621± .052 * | 0.190± .113 * |
| Bap + ZnO NPs 10            | 0.596± .070 * | 1.660± .150 * | 0.763± .028 * |
| Bap+ ZnO NPs 30             | 0.826± .109 * | 1.910± .120 * | 0.836.075 * |

† Values are represented as mean ± standard deviation (SD).
‡ Values with superscript * within the same column means a significant difference from NC group at P < 0.05.

B. Statistical comparison among B[a]P and co-administrated groups using ANOVA test

| Groups                      | StAR      | CY11A1     | 3β-HSD    |
|-----------------------------|-----------|------------|-----------|
| Bap                         | 0.183± .045 * | 0.621± .052 * | 0.190± .113 * |
| Bap + ZnO NPs 10            | 0.596± .070 * | 1.660± .150 * | 0.763± .028 * |
| Bap+ ZnO NPs 30             | 0.826± .109 * | 1.910± .120 * | 0.836.075 * |

† Values are represented as mean ± standard deviation (SD).
‡ Values with superscript * within the same column means a significant difference from Bap group at P < 0.05.

§ Abbreviations: StAR ; Steroidogenic Acute Regulatory protein ; CYP11A1; P450Scc cholesterol side-chain cleavage enzyme ; 3 β -HSD - 3β-Hydroxysteroid Dehydrogenase 1; Bap- Benzo [a] Pyrene ; ZnO NPs- zinc Oxide Nanoparticles.
### C- Pearson's correlation analysis between the expression of Steroidogenic enzymes and Testosterone level

|                      | StAR   | CY11A1 | 3β-HSD |
|----------------------|--------|--------|--------|
| Pearson correlation coefficient with testosterone | $r = 0.975$ | $r = 0.392$ | $r = 0.985$ |
| P value              | .000   | .022   | .000   |

¶ StAR: Steroidogenic acute regulatory protein; CYP11A1: cholesterol side-chain cleavage enzyme; 3β-HSD: 3β-hydroxysteroid dehydrogenase. P significant at < 0.05.
Table (3) Effect of ZnO NPs on oxidative stress biomarkers in serum of B[a]p-challenged male rats.

**A. Statistical comparison among groups using ANOVA test**

| Groups                        | Parameters | MDA nmol/ml | GSH mg/dl |
|-------------------------------|------------|-------------|-----------|
| Negative Control (NC)         |            | 7.52 ± 0.05 | 2.18 ± 0.10 |
| ZnO NPs 10 (PC I)            |            | 6.10 ± 0.30 * | 2.77 ± 0.12 * |
| ZnO NPs 30 (PC II)           |            | 6.24 ± 0.14 * | 2.80 ± 0.14 * |
| Bap                          |            | 10.17 ± 0.21 * | 1.36 ± 0.17 * |
| Bap + ZnO NPs 10             |            | 7.47 ± 0.02 | 1.99 ± 0.03 |
| Bap + ZnO NPs 30             |            | 6.55 ± 0.03 * | 1.95 ± 0.02 |

† Values are represented as mean ± standard deviation (SD).
‡ Values with superscript * within the same column means a significant difference from NC group at P < 0.05.

**B. Statistical comparison between B[a]P and supplemented groups using ANOVA test**

| Groups                   | Parameters | MDA nmol/ml | GSH mg/dl |
|--------------------------|------------|-------------|-----------|
| Bap                      |            | 10.17 ± 0.21 | 1.36 ± 0.17 |
| Bap + ZnO NPs 10         |            | 7.47 ± 0.02 * | 1.99 ± 0.03 * |
| Bap + ZnO NPs 30         |            | 6.55 ± 0.03 * | 1.95 ± 0.02 * |

† Values are represented as mean ± standard deviation (SD).
‡ Values with superscript * within the same column means a significant difference from Bap group at P < 0.05.

§ Abbreviations: MDA, Malondialdehyde; GSH; reduced glutathione, Bap- Benzo [a] Pyrene; ZnONPs- zinc Oxide Nanoparticles.
Table (4): Effect of zinc Oxide nanoparticles on serum testosterone and sperm counts in Bap-challenged male rats.

A. Statistical comparison among groups using ANOVA test

| Groups             | Sperm count (X10^6/ml) | Testosterone ng /dl |
|--------------------|------------------------|---------------------|
| Negative Control (NC) | 73.66 ± 6.51           | 5.32± 0.18          |
| ZnO NPs 10 (PCI)    | 68.83 ± 3.76           | 5.49 ± 0.12         |
| ZnO NPs 30 (PCII)   | 73.83 ± 6.30           | 5.88 ± 0.11*        |
| Bap                | 36.67 ± 2.65*          | 3.23 ± 0.05*        |
| Bap + ZnO NPs 10    | 58.67 ± 7.44*          | 4.54 ± 0.04*        |
| Bap+ ZnO NPs 30     | 67.17 ± 3.25*          | 4.81± 0.14*         |

† Values are represented as mean ± standard deviation (SD).
‡ Values with superscript * within the same column means a significant difference from NC group at P < 0.05.

B. Statistical comparison among B[a]P and Co-administrated groups using ANOVA test

| Groups             | Sperm count (X10^6/ml) | Testosterone ng /dl |
|--------------------|------------------------|---------------------|
| Bap                | 36.67 ± 2.65*          | 3.23 ± 0.05*        |
| Bap + ZnO NPs 10   | 58.67 ± 7.44*          | 4.54 ± 0.04*        |
| Bap+ ZnO NPs 30    | 67.17 ± 3.25*          | 4.81± 0.14*         |

† Values are represented as mean ± standard deviation (SD).
‡ Values with superscript * within the same column means a significant difference from Bap group at P < 0.05.

§ Abbreviations: Bap- Benzo [α] Pyrene; ZnO NPs- zinc Oxide Nanoparticles.
FIGURE 1: The Effect of Zinc Oxide Nanoparticles against Benzo [a] pyrene challenged rats.

Results are represented as expression controlled by B actin. Bap group showed highly significant decrease the expression of all steroidogenic enzymes when compared to the negative control group. Co-administration of ZnO NPs with B[a] P resulted in a significant increase in the expression of them when compared with the Bap group although it could not bring it back into control.

# Means with different superscripts (a, b, c and d) among different groups are significant at P < 0.05.

Abbreviations: StAR; Steroidogenic Acute Regulatory protein; CYP11A1; P450 Sc - cholesterol side-chain cleavage enzyme; 3 ß -HSD - 3ß-Hydroxysteroid Dehydrogenase 1; Bap- Benzo [a] Pyrene; ZnO NPs- Zinc Oxide Nanoparticles.
FIGURE 2: The antioxidant Effect of Zinc Oxide Nanoparticles (ZnO NPs) against Benzo[a]pyrene (Bap) challenged rats. The Bap group significantly increased the serum level of Malondialdehyde (MDA) accompanied by significant decrease level of reduced glutathione (GSH) when compared with negative control. While Co-administration of Bap with ZnO NPS recorded correspondingly significant decrease in MDA and increase in serum level of GSH which counteract the effect of Bap and back their levels to normal levels especially in GSH.

Means with different superscripts (a, b and c) in different groups are significant at P < 0.05.
FIGURE 3: Effect of zinc Oxide nanoparticles (ZnO NPs) on serum testosterone and sperm count in Benzo[a]pyrene (Bap)-challenged male rats. Testosterone and sperm count are significantly decreased in B[a]P group compared with the negative control. Co-administrated groups recorded a significant increase in testosterone concentration by nearly one third and sperm count by nearly half and two forth respectively when compared with the Bap group proving that ZnO NPs can guard sperm against toxic effect of B[a]P.

# Means with different superscripts (a, b, c and d) between different groups are significant at P < 0.05.
FIGURE 4. Photomicrograph of testis of control rat showing no histopathological change (A). In B[a]P treated group shows severe desquamation of spermatogonial cells in testis of male rat (B). and degeneration and pyknosis of spermatogonial cells associated with giant cells formation (Symplast) (Arrows) (C) (H & E X 200). D and E Co-administration of Nano-Zinc Oxide and B[a]P improved these histopathological alterations. F and G show normal testicular section in male rats treated with Nano-Zinc Oxide (H & E X100).
FIGURE 5. Photomicrograph of Kidney in negative control rat showing no histopathological change (A). Benzo [a] pyrene group shows severe atrophy and degeneration of glomerular tuft (Arrow) with vacuolization and necrobiotic changes of epithelial lining renal tubules (B) and (C) shows necrobiotic changes of epithelial lining renal tubules associated with severe congestion (Arrows). D and E show normal histologic features in groups exposed to nano-Zinc Oxide and B[a]P (H & E X200). F and G show normal histology in male rats treated with nano-Zinc Oxide. (H & E X200).
**FIGURE 6.** Photomicrograph of Liver of negative control rat showing no histopathological change (A).

In Benzo (a) pyrene (B) shows disorganization of the hepatocytes and vascular dilation, congestion and non-occluding thrombus associated with thickening of blood vessel wall (H & E X100). And also shows degenerative changes of hepatocytes associated with leucocytic cells infiltration (C). D and E show normal hepatic tissue in male rat exposed to nano-Zinc Oxide and B[a] P. F and G show normal hepatic histology in male rats treated with nano-Zinc Oxide (positive control groups) (H & E X200).
The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

References

1. Roduner, E. Size matters: why nanomaterials are different. *Chem Soc Rev* **35**, 583–592 (2006).

2. Jha, R. K., Jha, P. K., Chaudhury, K., Rana, S. V. S. & Guha, S. K. An emerging interface between life science and nanotechnology: present status and prospects of reproductive healthcare aided by nano-biotechnology. *Nano Rev.* **5**, 22762 (2014).

3. McAuliffe, M. E. & Perry, M. J. Are nanoparticles potential male reproductive toxicants? A literature review. *Nanotoxicology* **1**, 204–210 (2007).

4. Vizirianakis, I. S. Nanomedicine and personalized medicine toward the application of pharmacotyping in clinical practice to improve drug-delivery outcomes. *Nanomedicine* **7**, 11–17 (2011).

5. Pinho, A. R., Rebelo, S. & de Lourdes Pereira, M. The impact of zinc oxide nanoparticles on male (In)fertility. *Materials (Basel).* **13**, 1–18 (2020).

6. Torabi, F., Malekzadeh Shafaroudi, M. & Rezaei, N. Combined protective effect of zinc oxide nanoparticles and melatonin on cyclophosphamide-induced toxicity in testicular histology and sperm parameters in adult Wistar rats. *Int J Reprod Biomed* **15**, 403–412 (2017).

7. Badkoobeh, P., Parivar, K., Kalantar, S. M., Hosseini, S. D. & Salabat, A. Effect of nano-zinc oxide on doxorubicin- induced oxidative stress and sperm disorders in adult male Wistar rats. *Iran J Reprod Med* **11**, 355–364 (2013).

8. Mahmoud, A. R. H. & Shalaby, N. M. M. Ameliorative Effect of Zinc Oxide Nanoparticles on Nicotine Induced Testicular Dysfunction; Biochemical and Histological Study. *Toxicol. Environ. Health Sci.* **11**, 104–113 (2019).

9. Afifi, M., Almaghrabi, O. A. & Kadasa, N. M. Ameliorative effect of zinc oxide nanoparticles on antioxidants and sperm characteristics in streptozotocin-induced diabetic rat testes. *Biomed Res. Int.* **2015**, (2015).
10. Guerreiro, C. B. B., Horalek, J., de Leeuw, F. & Couvidat, F. Benzo(a)pyrene in Europe: Ambient air concentrations, population exposure and health effects. *Env. Pollut* **214**, 657–667 (2016).

11. Banerjee, B. *et al.* Benzo (a) pyrene induced p53 mediated male germ cell apoptosis: Synergistic protective effects of curcumin and resveratrol. *Front. Pharmacol.* **7**, 245 (2016).

12. Chung, J.-Y. *et al.* Benzo [a] pyrene reduces testosterone production in rat Leydig cells via a direct disturbance of testicular steroidogenic machinery. *Environ. Health Perspect.* **119**, 1569–1574 (2011).

13. Banerjee, B., Chakraborty, S., Chakraborty, P., Ghosh, D. & Jana, K. Protective Effect of Resveratrol on Benzo(a)Pyrene Induced Dysfunctions of Steroidogenesis and Steroidogenic Acute Regulatory Gene Expression in Leydig Cells. *Front Endocrinol* **10**, 272 (2019).

14. Archibong, A. *et al.* Effects of benzo (a) pyrene on intra-testicular function in F-344 rats. *Int. J. Environ. Res. Public Health* **5**, 32–40 (2008).

15. Inyang, F. *et al.* Disruption of testicular steroidogenesis and epididymal function by inhaled benzo (a) pyrene. *Reprod. Toxicol.* **17**, 527–537 (2003).

16. Mboyi, A., Kamika, I. & Momba, M. B. Detrimental effects of commercial zinc oxide and silver nanomaterials on bacterial populations and performance of wastewater systems. *Phys. Chem. Earth, Parts A/B/C* **100**, 158–169 (2017).

17. Kang, H. G., Jeong, S. H., Cho, M. H. & Cho, J. H. Changes of biomarkers with oral exposure to benzo (a) pyrene, phenanthrene and pyrene in rats. *J. Vet. Sci.* **8**, 361–368 (2007).

18. Bouma, G. J., Hart, G. T., Washburn, L. L., Recknagel, A. K. & Eicher, E. M. Using real time RT-PCR analysis to determine multiple gene expression patterns during XX and XY mouse fetal gonad development. *Gene Expr Patterns* **5**, 141–149 (2004).

19. Akanda, M. R. *et al.* Neuroprotective Effects of Sagesbeckia pubescens Extract on Glutamate-Induced Oxidative Stress in HT22 Cells via Downregulation of MAPK/caspase-3 Pathways. *Cell. Mol. Neurobiol.* **38**, 497–505 (2018).

20. Daoud, N. M., Mahrous, K. F. & Ezzo, O. H. Feed restriction as a biostimulant of the production of oocyte, their quality and GDF-9 gene expression in rabbit oocytes. *Anim. Reprod. Sci.* **136**, 121–127 (2012).

21. Ohkawa, H., Ohishi, N. & Yagi, K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.* **95**, 351–358 (1979).

22. Beutler, E. Improved method for the determination of blood glutathione. *J. lab. clin. Med.* **61**, 32
23. Yokoi, K., Uthus, E. O. & Nielsen, F. H. Nickel deficiency diminishes sperm quantity and movement in rats. *Biol. Trace Elem. Res.* **93**, 141–153 (2003).

24. Bancroft, J. D. & Gamble, M. *Theory and practice of histological techniques*. (Elsevier health sciences, 2008).

25. Briede, J. J. *et al.* In vitro and in vivo studies on oxygen free radical and DNA adduct formation in rat lung and liver during benzo[a]pyrene metabolism. *Free Radic Res* **38**, 995–1002 (2004).

26. Dawei, A. I., Zhisheng, W. & Anguo, Z. Protective Effects of Nano-ZnO on the Primary Culture Mice Intestinal Epithelial Cells in in vitro Against Oxidative Injury. *J. Anim. Vet. Adv.* **8**, (2010).

27. Aitken, R. J. & Roman, S. D. Antioxidant systems and oxidative stress in the testes. *Oxid. Med. Cell. Longev.* **1**, 15–24 (2008).

28. Hussein, M. M. A., Ali, H. A., Saadeldin, I. M. & Ahmed, M. M. Querectin Alleviates Zinc Oxide Nanoreprotoxicity in Male Albino Rats. *J. Biochem. Mol. Toxicol.* **30**, 489–496 (2016).

29. Miller, W. L. & Auchus, R. J. The Molecular Biology, Biochemistry, and Physiology of Human Steroidogenesis and Its Disorders. *Endocr. Rev.* **32**, 81–151 (2011).

30. Chung, J. Y. *et al.* Cellular defense mechanisms against benzo[a]pyrene in testicular Leydig cells: implications of p53, aryl-hydrocarbon receptor, and cytochrome P450 1A1 status. *Endocrinology* **148**, 6134–6144 (2007).

31. Hu, J., Zhang, Z., Shen, W. J. & Azhar, S. Cellular cholesterol delivery, intracellular processing and utilization for biosynthesis of steroid hormones. *Nutr Metab* **7**, 47 (2010).

32. Turner, T. T. & Lysiak, J. J. Oxidative stress: a common factor in testicular dysfunction. *J Androl* **29**, 488–498 (2008).

33. Abidi, P. *et al.* Oxidative stress-induced inhibition of adrenal steroidogenesis requires participation of p38 mitogen-activated protein kinase signaling pathway. *J Endocrinol* **198**, 193–207 (2008).

34. Lee, S. H. *et al.* Effects of zinc oxide nanoparticles on gene expression profile in human keratinocytes. *Mol. Cell. Toxicol.* **8**, 113–118 (2012).

35. Bara, N. & Kaul, G. Enhanced steroidogenic and altered antioxidant response by ZnO nanoparticles in mouse testis Leydig cells. *Toxicol Ind Heal.* **34**, 571–588 (2018).

36. Zhao, C. Y. *et al.* Effects of dietary zinc oxide nanoparticles on growth performance and antioxidative status in broilers. *Biol Trace Elem Res* **160**, 361–367 (2014).
37. Ramesh, A. *et al.* Metabolism, bioavailability, and toxicokinetics of Benzo(α)pyrene in F-344 rats following oral administration. *Exp. Toxicol. Pathol.* **53**, 275–290 (2001).

38. Aitken, R. J. Reactive oxygen species as mediators of sperm capacitation and pathological damage. *Mol Reprod Dev* **84**, 1039–1052 (2017).

39. Revel, A. *et al.* Resveratrol, a natural aryl hydrocarbon receptor antagonist, protects sperm from DNA damage and apoptosis caused by benzo(a)pyrene. *Reprod Toxicol* **15**, 479–486 (2001).

40. Raychoudhury, S. S. & Kubinski, D. Polycyclic aromatic hydrocarbon-induced cytotoxicity in cultured rat Sertoli cells involves differential apoptotic response. *Environ. Health Perspect.* **111**, 33–38 (2003).

41. Hassan, A. M., Alam, S. S., Abdel-Aziem, S. H. & Ahmed, K. A. Benzo-a-pyrene induced genotoxicity and cytotoxicity in germ cells of mice: Intervention of radish and cress. *J. Genet. Eng. Biotechnol.* **9**, 65–72 (2011).

42. Mohamed el, S. A. *et al.* The transgenerational impact of benzo(a)pyrene on murine male fertility. *Hum Reprod* **25**, 2427–2433 (2010).

43. El-Maddawy, Z. K. & Abd El Naby, W. S. H. Protective effects of zinc oxide nanoparticles against doxorubicin induced testicular toxicity and DNA damage in male rats. *Toxicol Res* **8**, 654–662 (2019).

44. Chen, X. *et al.* The combined toxicity of dibutyl phthalate and benzo(a)pyrene on the reproductive system of male Sprague Dawley rats in vivo. *J Hazard Mater* **186**, 835–841 (2011).

45. Deng, C. *et al.* Acute benzo[a]pyrene treatment causes different antioxidant response and DNA damage in liver, lung, brain, stomach and kidney. *Heliyon* **4**, e00898–e00898 (2018).

46. Kolade, O. Y. & Oladiji, T. A. Protective Effects Of Curcumin Against Benzopyrene Induced Liver Toxicity In Albino Rats. *IOP Conf. Ser. Earth Environ. Sci.* **210**, 12013 (2018).

47. El-Agamy, D. S. Comparative effects of curcumin and resveratrol on aflatoxin B(1)-induced liver injury in rats. *Arch Toxicol* **84**, 389–396 (2010).

48. Nagajyothi, P. C. *et al.* Antioxidant and anti-inflammatory activities of zinc oxide nanoparticles synthesized using Polygala tenuifolia root extract. *J Photochem Photobiol B* **146**, 10–17 (2015).

49. Li, J. *et al.* ZnO nanoparticles act as supportive therapy in DSS-induced ulcerative colitis in mice by maintaining gut homeostasis and activating Nrf2 signaling. *Sci Rep* **7**, 43126 (2017).
50. Falchi, L., Khalil, W. A., Hassan, M. & Marei, W. F. A. Perspectives of nanotechnology in male fertility and sperm function. *Int. J. Vet. Sci. Med.* **6**, 265–269 (2018).

51. Kim, M. H., Seo, J. H., Kim, H. M. & Jeong, H. J. Zinc oxide nanoparticles, a novel candidate for the treatment of allergic inflammatory diseases. *Eur J Pharmacol* **738**, 31–39 (2014).

52. Siddiqi, K. S., Ur Rahman, A., Tajuddin & Husen, A. Properties of Zinc Oxide Nanoparticles and Their Activity Against Microbes. *Nanoscale Res. Lett.* **13**, 141 (2018).

53. Swain, P. S., Rao, S. B. N., Rajendran, D., Dominic, G. & Selvaraju, S. Nano zinc, an alternative to conventional zinc as animal feed supplement: A review. *Anim. Nutr. (Zhongguo xu mu shou yi xue hui)* **2**, 134–141 (2016).

---

**Additional Information**

**Author contributions statements**

Niveen M Daoud: designed and wrote edited the final manuscript text, analyzed the data statistically, prepared figures and tables. Mohamed S Aly: Prepared and examined tissues for histopathology; determined sperm counts; wrote this part in the manuscript. Naglaa A Ali: treated and observed The experiment animals, determined hormonal and biochemical parameters and wrote draft preparation. Omaima H Ezzo; Niveen M Daoud and Naglaa A Ali: shared in the experiment’s funding and gene expression detection. All Authors reviewed the manuscript.

**competing interests statement**

We declare that the authors have no competing interests as defined by Nature Research, or other interests that might be perceived to influence the results and/or discussion reported in this paper.
The Effect of Zinc Oxide Nanoparticles against Benzo [a] pyrene challenged rats. Results are represented as expression controlled by B actin. Bap group showed highly significant decrease the expression of all steroidogenic enzymes when compared to the negative control group. Co-administration of ZnO NPs with
B[a] P resulted in a significant increase in the expression of them when compared with the Bap group although it could not bring it back into control. # Means with different superscripts (a, b, c and d) among different groups are significant at P < 0.05. Abbreviations: StAR; Steroidogenic Acute Regulatory protein; CYP11A1; P450 Scc - cholesterol side-chain cleavage enzyme; 3β-HSD - 3β-Hydroxysteroid Dehydrogenase 1; Bap- Benzo [a] Pyrene; ZnO NPs- Zinc Oxide Nanoparticles.

Figure 2
The antioxidant Effect of Zinc Oxide Nanoparticles (ZnO NPs) against Benzo [a] pyrene (Bap) challenged rats. The Bap group significantly increased the serum level of Malondialdehyde (MDA) accompanied by
significant decrease level of reduced glutathione (GSH) when compared with negative control. While Co-administration of Bap with ZnO NPS recorded correspondingly significant decrease in MDA and increase in serum level of GSH which counteract the effect of Bap and back their levels to normal levels especially in GSH. Means with different superscripts (a, b and c) in different groups are significant at \( P < 0.05 \).
Effect of zinc Oxide nanoparticles (ZnO NPs) on serum testosterone and sperm count in Benzo[a] pyrene (Bap)-challenged male rats. Testosterone and sperm count are significantly decreased in B[a]P group compared with the negative control. Co-administrated groups recorded a significant increase in testosterone concentration by nearly one third and sperm count by nearly half and two forth respectively when compared with the Bap group proving that ZnO NPs can guard sperm against toxic effect of B[a]P.

# Means with different superscripts (a, b, c and d) between different groups are significant at P < 0.05.
Photomicrograph of testis of control rat showing no histopathological change (A). In B[a]P treated group shows severe desquamation of spermatogonial cells in testis of male rat (B). and degeneration and pyknosis of spermatogonial cells associated with giant cells formation (Symplast) (Arrows) (C) (H & E X 200). D and E Co-administration of Nano-Zinc Oxide and B[a]P improved these histopathological alterations. F and G show normal testicular section in male rats treated with Nano- Zinc Oxide (H & E X100).

Figure 5
Photomicrograph of Kidney in negative control rat showing no histopathological change (A). Benzo[a]pyrene group shows severe atrophy and degeneration of glomerular tuft (Arrow) with vacuolization and necrobiotic changes of epithelial lining renal tubules (B) and (C) shows necrobiotic changes of epithelial lining renal tubules associated with severe congestion (Arrows). D and E show normal histologic features in groups exposed to nano-Zinc Oxide and B[a]P (H & E X200). F and G show normal histology in male rats treated with nano-Zinc Oxide. (H & E X200).
Photomicrograph of Liver of negative control rat showing no histopathological change (A). In Benzo (a) pyrene (B) shows disorganization of the hepatocytes and vascular dilation, congestion and non-occluding thrombus associated with thickening of blood vessel wall (H & E X100). And also shows degenerative changes of hepatocytes associated with leucocytic cells infiltration (C). D and E show normal hepatic tissue in male rat exposed to nano-Zinc Oxide and B[a] P. F and G show normal hepatic histology in male rats treated with nano-Zinc Oxide (positive control groups) (H & E X200).