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Ameliorative effect of zinc oxide nanoparticles on cyclophosphamide induced testicular injury in adult rat

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ABSTRACT

Despite its wide range of application, cyclophosphamide (CP) exhibits a wide range of adverse effects including reproductive toxicity. The emerging field of zinc oxide nanoparticles (ZnO NPs) therapy may provide a new hope for prevention of CP induced gonadal toxicity. Herein, we aim to investigate the possible role of ZnO NPs as a new strategy to protect against CP induced testicular injury. Sixty adult male albino rats were divided into 3 groups; control, CP treated and CP + ZnO NPs treated groups. CP group was injected with CP (5 mg/kg/day), whereas CP + ZnO NPs group was concomitantly injected with CP and ZnO NPs (5 mg/kg/day). Testicular specimens were processed for histological, ultrastructural and c-kit immunohistochemical study. Biochemical analysis for tissue malondialdehyde and serum testosterone was done in addition to sperm morphology assay and cytogenetic study. Our results revealed that CP induced deleterious testicular histopathological, biochemical and genetic alterations that were effectively prevented by ZnO NPs.

1. Introduction

Cyclophosphamide (CP), a cyclic phosphoramide ester, is an orally active alkylating agent belongs to the class of nitrogen mustard. It introduces alkyl radicals into DNA strands of cells and stops cancer cells from growing, so, it had been classified as one of the top cancer drugs. It has also an immunosuppressive effect as it suppresses the body's natural immune response (De Jonge et al., 2005; Perini et al., 2007).

CP is commonly used in combination with other chemotherapeutic agents for treatment of various malignant and non-malignant tumors (Shanafelt et al., 2007). It has been used in treatment of severe manifestations of systemic autoimmune diseases as rheumatoid arthritis, systemic lupus erythematosus, and multiple sclerosis and for prevention of graft rejection (Vernet et al., 2004). In dermatology, it has been used as asteroid-sparing adjunct for severe or refractory autoimmune disease like autoimmune vasculitides, autoimmune bullous disease and autoimmune connective tissue diseases (Kim and Chan, 2017).

CP is an inactive drug. In the liver, it is converted by cytochrome P-450 enzymes to the active metabolite 4-hydroxycyclophosphamide, which forms and exists in equilibrium with aldophosphamide. Aldophosphamide is cleaved intracellularly to its active metabolites phosphoramide, mustard and acrolein (Zhang et al., 2005). CP and its metabolites are renally excreted in urine (Haubitz et al., 2002).

According to Monach et al. (2010), phosphoramide and mustard have the ability to introduce alkyl radicals into DNA strands which interferes DNA replication by forming DNA cross-linkage. Cross-linked cancer cell DNA is unable to complete normal cell division. Thus, it stops cancer cells from growing, causing them to die. Kern and Kehrer (2002) suggested that phosphoramide and mustard have the antineoplastic and immunosuppressive effects, while the acrolein may be responsible for toxic side effects of CP, such as cell death, necrosis, apoptosis and oxidative damage.

The capability of CP to interfere with rapidly proliferating tissue is the basis of its therapeutic and many of its toxic properties. Testis is highly sensitive to this drug, due to the presence of rapidly dividing cells, resulting in testicular injury (Mulvihill and Garlow, 2007). The high susceptibility of the testis to oxidative damage is due to the abundance of reactive oxygen species (ROS) generating systems and high concentrations of poly-unsaturated fatty acids thus, the antioxidant system plays a crucial role in protecting this tissue from oxidative damage (Aitken and Roman, 2008).

Zinc (Zn) is one of the important trace elements in the body (Prasad, 2009). It is a cofactor for more than 80 enzymes involved in DNA transcription and protein synthesis (Cortese et al., 2008). High Zn concentration is required in testis and prostate to maintain their normal physiology. It maintains the redox balance of the two glands by modulating several Zn-dependent enzymes like metallothionein, matrix metalloproteinases, nuclear factor- erythroid 2–related factor 2 (Nrf2)

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and many others. So, Zn is important for germ cell development and for reproduction (Coyle et al., 2002).

Nanoparticles (NPs) are small particles (< 100 nm) with special characters regarding nano surface, nano size, nano structure, self- assembly, dissolution, aggregation and concentration (Yan et al., 2011). Nanotechnology helps the controlled synthesis of materials at nanoscale level (1–100 nm); this enables precision engineering to control nanoparticles' physicochemical properties, and their interactions with biological systems (Wang, 2008).

Over the last 20 years, the medical application of nanotechnology referred to as 'nanomedicine'. Nanomedicine has improved both therapeutics and diagnostics in several medical fields, including cardiology and oncology. It helps delivering a new set of tools, devices and therapies for treatment of disease (Singh and Singh, 2013). The medicinal preparations based on metals NPs (gold, silver, iron and others) with size of 5–16 nm are considered to be the most promising for practical applications. It has been found that high-disperse powders of nano-metals (zinc, copper, magnesium, etc.) as well as their oxides show a high biological activity (Andrusishina, 2011).

Zinc oxide nanoparticles (ZnO NPs) are one of the better-known materials used for medical applications. ZnO NPs can be considered magic material because of its wide area of applications and flexibility of preparation in different morphologies and different properties (Padmavathy and Vijayaraghavan, 2008).

Interest is growing regarding the use of ZnO and other metal oxide nanomaterials as biomarkers for cancer imaging, screening and diagnosis (Shen et al., 2008). Much attention has been paid for their applications in cancer therapy. Cancer cells are the most susceptible to ZnO NPs induced cytotoxicity, whereas quiescent cells are the least sensitive (Zhang et al., 2011; Premanathan et al., 2011).

So, the present study was designed to investigate the possible protective role of ZnO NPs as a new strategy against CP induced testicular and chromosomal injury in adult rats.

2. Materials and methods

2.1. Chemicals

CP: white to light yellow crystalline odorless powder; CAS No.50-18-0 with > 95% purity was purchased from (Sigma-Aldrich, Steinheim, Germany). It was dissolved in distilled water and stored in refrigerator at 4 °C all the time of the experiment.

ZnO NPs: white liquid dispersion; CAS No.1314-13-2 with the following properties: concentration 50 wt. % in H2O, the average NP size < 35 nm, the particle size distribution (hydrodynamic diameter) < 100 nm using dynamic light scattering (DLS) technique, pH 7 ± 0.1 (for aqueous systems) and density 1.7 g/mL ± 0.1 g/mL at 25 °C (Fig. 1). It was purchased from (Sigma-Aldrich, Steinheim, Germany).

2.2. Experimental animals

The study was carried out on 60 adult male Sprague Dawley albino rats with average weight of 180–200 g m. They were housed at the Breading Animal House of the Faculty of Medicine, Zagazig University, in a controlled room (temperature 25–27 °C, artificially illuminated12-h light/12-h dark cycle and relative humidity of 40–70%) with food and water ad-libitum. They were acclimatized for one week before starting the experiment. All experimental procedures were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee and accepted by the Faculty of Medicine; Zagazig University.

2.3. Experimental design

After one week acclimatization period, the rats were divided into 3 groups as follow:

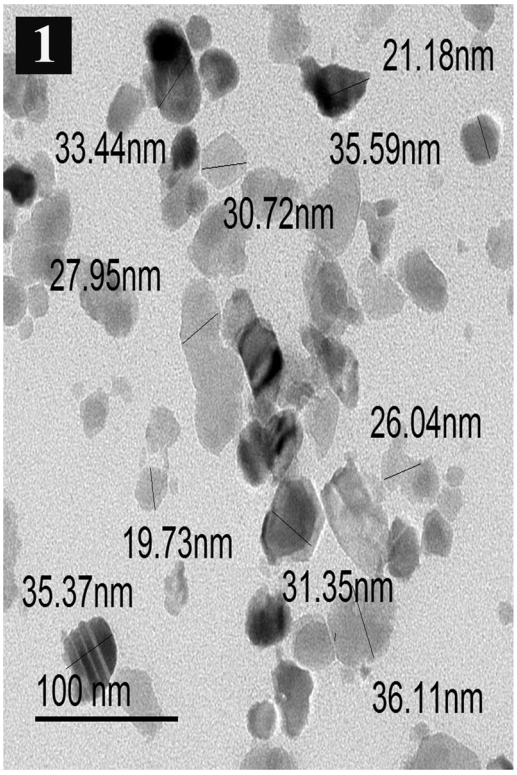

Fig. 1. An electron micrograph showing the morphology of ZnONPs used in the experiment and indicating variable dimensions.

(1) Group I (control group): 36 rats were equally subdivided into 3 subgroups:

- **Subgroup (Ia):** received no treatment till the end of the experiment.
- **Subgroup (Ib):** received an intraperitoneal injection of 0.25 ml distilled water once daily for 4 weeks. Water is the solvent of both CP and ZnO NPs.
- **Subgroup (Ic):** received ZnO NPs intraperitoneally at a dose of (5 mg/kg/day) dissolved in 0.25 ml of distilled water once daily for 4 weeks (Alkaladi et al., 2014; Reza et al., 2014 and Mozaffari et al., 2015)

(2) Group II (CP treated group): (12 rats) which received CP intraperitoneally at a dose of (5 mg/kg/day) (Dalouchi et al., 2014) dissolved in 0.25 ml distilled water once daily for 4 weeks (Das et al., 2002).

(3) Group III (CP + ZnO NPs-treated group): (12 rats) which received an intraperitoneal injection of CP as group II concomitant with an intraperitoneal injection of ZnO NPs as Subgroup Ic

At the end of the experiment, all rats were injected intraperitoneally with colchicine (4 mg/kg of body weight) 2 h before scarification to arrest mitosis in the metaphase (Tripathi and Jena, 2009). Rats from each group were anaesthetized with 50 mg/kg body weight of sodium phenobarbital through intraperitoneal injection (Wen et al., 2016). Then, the following specimens were extracted:

1) Blood samples were collected from retro orbital venous plexus of all animals in heparinized tubes for measuring serum testosterone hormone level.
2) Testes: an incision was made in the lower abdominal region. Then, the testes were dissected out carefully. The right one processed for histopathological study (half for light microscopic preparation and the other half for electron microscopic preparation). The left testes