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RESEARCH PAPER

Nanosized TiO_2 Induced Ovarian Alterations in NMRI Mice Treated with Isoniazid

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ABSTRACT

Titanium dioxide (TiO_2) is a widely used material in various products, including cosmetics, food additives, and medical devices. However, its small size (nanoscale) has raised concerns about its potential impacts on human health and the environment. One area of concern is the effect of nanosized TiO_2 on the female reproductive system, particularly the ovaries. The purpose of this study is to look at how isoniazid-treated NMRI mice's ovarian tissue alters in response to nanosized titanium dioxide (nano-TiO_2) treatment. The 50 adult female mice used in this research were split into five groups of 10 at random. The experimental groups included the control group (no medication), sham group (0.5 mg/kg of normal saline), first experimental group (45 mg/kg isoniazid), second experimental group (45 mg/kg isoniazid and 0.5 mg/kg nano-TiO_2), and the third experimental group (45 mg/kg isoniazid and 0.45 mg/kg nano-TiO_2). All injections were given for 20 days. After that, the ovarian tissue from each animal was isolated and put in an 10% formalin solution before tissue analyses were carried out using hematoxylin and eosin (H&E) stain. In comparison to the control group, there was a reduction in the quantity of corpus luteum and secondary follicles in all experimental groups. In comparison to the first experimental group, there is a significant increase in the number of unilaminar primary follicles in the rest of the experimental groups. The damaging effects of isoniazid on ovarian tissue can be lessened by using nanoparticles in small amounts.

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INTRODUCTION

Tuberculosis (TB) is a serious and highly contagious bacterial infection that primarily affects the lungs, but can also impact other parts of the body such as the lymph nodes, bones, joints, kidneys, and brain [1–3]. It is caused by the bacterium Mycobacterium tuberculosis and is spread through the air when an infected person coughs, sneezes, or talks [4]. Isoniazid (INH) is a first-line antibiotic that is commonly used to treat

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TB [5]. It is a type of drug known as a bactericidal, which means it works by killing the bacteria that cause TB, rather than simply suppressing their growth [6]. Androgen lipid peroxidation is a process by which the fatty acids in cell membranes are damaged by the formation of highly reactive molecules known as free radicals [7]. This process can lead to cellular damage and death, and has been identified as a potential contributing factor in the cytotoxicity (cell-killing effects) of the TB drug INH [8]. The cytotoxicity of INH has implications for the treatment of TB, as it can increase the risk of liver toxicity and other adverse effects associated with the drug [9].

Nanoparticles (NPs) have been a common tool in biomedical research in recent years. According to studies, magnetic NPs are being utilized more frequently in clinical settings to treat and diagnose diseases, as well as for drug withdrawal, MRI, and other medical procedures [10–13]. With numerous properties and prospective uses in the treatment of diseases, nanotechnology has emerged as a promising method for developing novel materials. Titanium dioxide (TiO_2) is a widely used material in the medical field due to its unique properties such as high biocompatibility, low toxicity, and photocatalytic activity [14,15]. Nanosized TiO_2, or titanium dioxide NPs, have garnered significant attention in recent years for their potential in various medical applications [16]. TiO_2 NPs have a high surface area-to-volume ratio, making them ideal carriers for drugs that need to be delivered to specific target cells in the body [17]. Additionally, their photocatalytic activity can be used to trigger the release of drugs in response to light [18]. In the field of wound healing, nanosized TiO_2 has been shown to have antibacterial properties, making it useful for preventing infections in wounds [19,20]. Furthermore, TiO_2 NPs can also stimulate angiogenesis (the growth of new blood vessels) and promote tissue regeneration, making them a promising material for wound healing [21].

According to previous studies, using a small amount of TiO_2 NPs instead of a large one is more beneficial for curing and treating illnesses [22–24]. Animals exposed to high concentrations of TiO_2 (4.0 mg/kg) exhibited lung inflammation and cytotoxicity, while those exposed to medium concentrations (1 mg/kg) manifested milder symptoms [25,26]. Studies have shown that exposure to TiO_2 NPs can cause oxidative stress and inflammation in testicular somatic cells, leading to cellular damage and decreased cell viability [9,27,28]. NPs penetration into cells and its therapeutic and molecular impact on cellular mechanisms increase with decreasing NP diameter [29]. Given the aforementioned details, the significance of the reproductive system for the survival of the generation, and the contradictory findings regarding the toxic and non-toxic effects of TiO_2 NPs in treating diseases and their antioxidant properties, the purpose of the current study is to examine the impact of TiO_2 NPs on histopathological changes of the ovary in NMRI mice treated with INH in order to investigate potential side effects.

MATERIALS AND METHODS

In this experimental research, 50 female NMRI mice aged 8-12 weeks and weighing 25-30 g were obtained from the ACE - Animal Care in Egypt. The animals were housed under settings that included 12 hrs. of light and 12 hrs. of darkness, a temperature of 22-26°C, and free access to water and commercial pelleted food throughout the course of the study [30]. The mice were then distributed at random into five groups of 10. Control group: received enough food and water and did not receive any drug treatment; Sham group: physiological serum solution of 0.5 mg/kg INH administered through gavage; First experimental group: treated with INH by gavage at 45 mg/kg of body weight; Second experimental group: 0.5 mg/kg of body weight intraperitoneal (IP) injections of TiO_2 NPs and the INH medication through gavage at 45 mg/kg of body weight; Third experimental group: 1 mg/kg of body weight IP injections of TiO_2 NPs and the INH medication through gavage at 45 mg/kg of body weight. Scanning electron microscope (SEM) image, X-ray diffraction (XRD) pattern, and Fourier-transform infrared spectroscopy (FTIR) of the TiO_2 NPs employed in this investigation are all displayed in Fig. 1. The creation of a high purity product was demonstrated by FTIR analyses of the TiO_2 NPs. The Ti-O-O bond's vibration is what causes the peak at 590 cm^{-1} to be visible. The FTIR spectrum strongly indicates that the end product has Ti-O bonds and does not contain peroxo or -OH groups.

For two weeks, the animals received treatment. The mice were then anesthetized with diethyl ether in a desiccator 24 hours after the last treatment. Ovarian tissue was separated for microscopic studies and placed in 10% formalin

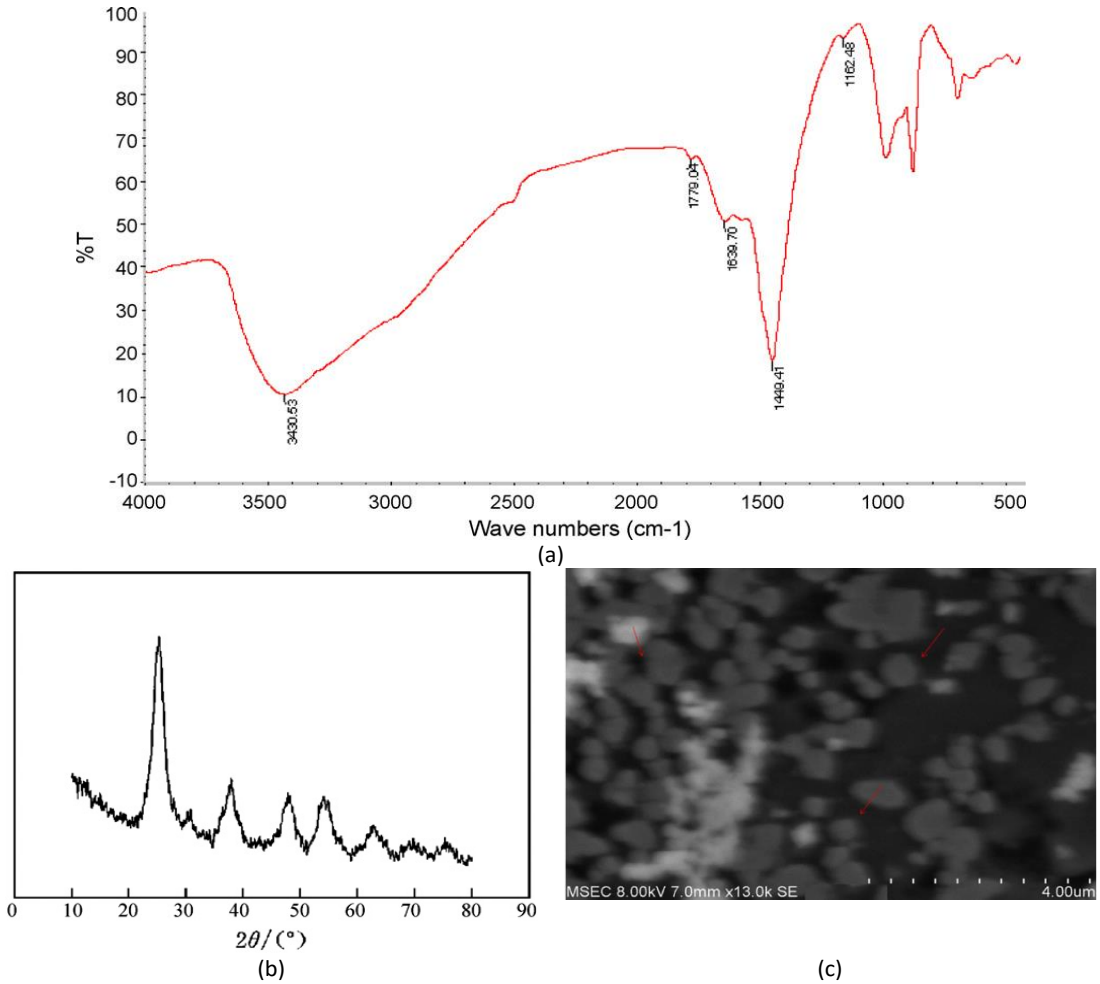

Fig. 1. Analysis results of TiO_2 NPs: (a) FTIR spectroscopy, (b) XRD measurement, and (c) SEM image.

fixative. Following the customary steps of tissue passage and pathological section preparation, 10 consecutive sections, each with a thickness of 5 microns, were obtained. A total of 10 sections from each sample were stained using the hematoxylin and eosin (H&E) stain [31]. By using a light microscope, pathological studies were conducted, including changes in the number of primordial, primary (unilaminar and multilaminar), secondary (or antral), and mature (or Graafian) follicles, and corpus luteum. A statistical analysis was performed on the results from counting the number of follicles in various groups. The one-way ANOVA was used to examine the data using the SPSS version 23.0, and the Duncan's new multiple range test (MRT) were employed to compare the groups. A significant difference of $p<0.05$ was considered.

RESULTS AND DISCUSSION

As seen in Table 1, it was discovered that the first (INH by gavage at 45 mg/kg of body weight) and second (0.5 mg/kg of body weight IP injections of TiO_2 NPs) experimental groups had significantly fewer primordial follicles than the control group. There are no significant differences between the first and second experimental groups. A significant difference was observed between the first and third (1 mg/kg of body weight IP injections of TiO_2 NPs) experimental groups, whereas the difference between the control group and the third experimental group is not significant. Additionally, the first experimental group revealed a significantly lower number of primordial follicles than all other groups under study. In addition, a significant increase was seen in the second and third experimental groups

Table 1. Comparing several experimental group parameters to those in the control and sham groups.

	Control, %	Sham, %	INH, %	INH+0.5 mg/kg TiO_2, %	INH+1 mg/kg TiO_2, %
Primary follicles	8.67^{c}	7.65^{bc}	3.74^{a}	5.95^{ab}	6.80^{bc}
Unilaminar primary follicles	6.12^{b}	5.78^{b}	3.06^{a}	4.76^{b}	4.76^{b}
Multilaminar primary follicles	5.27^{b}	5.10^{ab}	3.40^{a}	4.25^{ab}	4.42^{ab}
Secondary follicles	7.31^{c}	6.29^{bc}	3.40^{a}	4.59^{ab}	4.93^{ab}
Graafian follicles	4.93^{c}	4.42^{bc}	1.70^{a}	3.06^{ab}	3.40^{bc}
Corpus luteum	5.44^{b}	4.42^{b}	2.72^{a}	2.38^{a}	2.55^{a}

Note: Common letters have no statistical significance ($p<0.05$)

compared to the first experimental group, but there was no significant difference compared to the control group. When compared to the control group, the first experimental group exhibits a significantly lower number of primary multilaminar follicles. In comparison to the first experimental group, the increase seen in second and third experimental groups is not statistically significant. In comparison to the control group, the first, second and third experimental groups

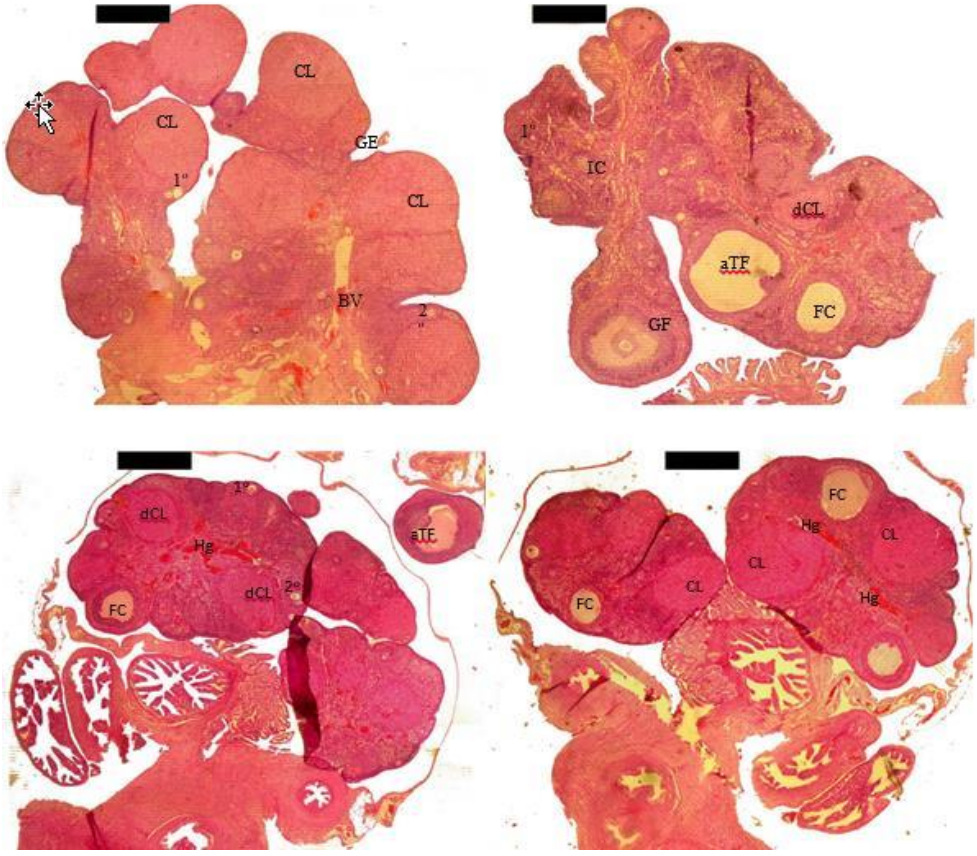

Fig. 2. Photomicrograph of ovarian tissue in the control group (upper left), NMRI mice treated with INH (upper right), NMRI mice treated with INH and 0.5 mg/kg TiO_2 NPs (lower left), NMRI mice treated with INH and 1 mg/kg TiO_2 NPs (lower right). [Note: germinal epithelium (GE), hemorrhage (Hg), blood vessels (BV), interstitial stromal cell (IC), follicular cysts (FC), degenerating corpus luteum (dCL), corpus luteum (CL), atretic tertiary follicle (aTF), graafian follicle (GF), secondary follicle (2^{o}), primary follicle (1^{o})].

all had significantly fewer secondary follicles. When compared to the first experimental group, the second and third experimental groups did not significantly increase. In comparison to the control group, the first, second and third experimental groups all had significantly fewer Graafian follicles. In comparison to the first experimental group, the third experimental group exhibits a significant increase. As compared to the control group, there are significantly fewer corpus luteum in the first, second and third experimental groups.

It was discovered that the ovarian tissue in the control group (no medication) is in good health and free of necrosis and cell damage. In contrast to the control tissue, it was discovered that the ovarian tissue in the sham group is healthy and free of any cell damage (see Fig. 2). Additionally, in contrast to the control group, the ovarian follicles displayed in the Fig. 2 are normal. When ovarian follicles and corpus luteum were counted, it was shown that the groups receiving INH exhibited tissue damage and had less ovarian follicles than the control group. INH also produced cell necrosis, cell disintegration, and atretic follicles in the ovarian tissue. In the groups receiving INH, there was an increase in ovarian tissue hyperemia and a decrease in the number of follicles, as shown in Fig. 2. The findings demonstrated that, in comparison to the control group, there was a reduction in ovarian follicles and tissue degradation in the group receiving INH and the least amount of TiO_2 NPs. Also, there was a reduction in ovarian follicles in the group receiving the highest dose of TiO_2 NPs and INH compared to the control group.

According to several studies [31–33], TiO_2 NPs demonstrate their toxicity through promoting cell divisions. It's possible that the current study's finding that ovarian follicles increased in comparison to the INH group did not necessarily point to TiO_2 NPs' beneficial effects. As stated in Ref. [34], the use of a smaller amount of TiO_2 NPs has a more positive effect, and produces favorable results. In the present study, the value of 45 mg/kg of TiO_2 NPs has been able to reduce the destructive effect of INH on primary and Graafian follicles to some extent, which is consistent with the research results of Ref. [32].

CONCLUSION

TiO_2 NPs have been studied for their potential therapeutic effects, including their ability to reduce the harmful effects of certain drugs. INH is an antibiotic medication used to treat tuberculosis, but it can have adverse effects on the reproductive system, including the ovaries. According to the results, TiO_2 NPs in smaller doses may be able to lessen the destructive effects of INH on ovarian tissue. Additional tests are advised to examine the potential impact of TiO_2 NPs. Additionally, it's likely that a variety of variables, including time, the way NPs were administered, the length of the experiment, the age of the lab animal, have an impact on the outcomes in terms of the ability of NPs to lessen the negative effects of INH on ovarian tissue. However, it's important to note that animal studies may not necessarily translate directly to humans, and more research is needed to fully understand the potential risks of TiO_2 NPs on reproductive health in humans.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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