

## The Synergic Effect of Zinc Oxide Nanoparticles and Coconut Oil as Antioxidant, and Their Effect on the Reproductive Systems of Female Rats

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### Abstract

The purpose of this study was to detect the synergic effect of zinc oxide nanoparticles (ZnO-NPs) and coconut oil effect as antioxidant, and their effect on the reproductive systems of female rats. The experiments included experimentally infected the animals with candida albicans. Rats were infected with a 1 ml inoculum ( $3 \times 10^9$  yeast/ml) on day 0 and stressed immediately after the infection and during the following 2 days as single dose (3 ml) after dilution the main culture. After that the ZNO and coconut oil were used to know the effect of both on the reduction of the oxidative effect of fungus infection. The doses of 5 mg/kg of ZnO, and 0.5 ml of coconut oil were administered to infected Wistar rats. Hormonal and oxidative parameters were accessed after (30) days of experiment. The histopathological evaluations of tissues were also performed for uterus and ovary. The level of (LH, FSH, and progesterone) was observed to detect the effect of both treatments on the fertility level and oxidative markers. All the treated groups were comparable to control group at the end of the experiment. The results showed a significant decrease of hormones in infected female with *Candida albicans*, while the level of these hormones began to return to its normal value after ZNO and coconut oil treatment. Significant differences of oxidation markers were obtained in this study.

**Keywords:** Nanoparticle, Antifungal, Coconut Oil, *Candida Albicans*, Antioxidant

### Introduction

Nowadays fungal infections resulted from opportunistic fungi have been common especially in people talented in being affected by special conditions like immune weakness, pregnancy and diseases like HIV and the candidates of affection to these infections (Lee & Lau, 2017).

The fungus *C. albicans* and to a lesser extent other *Candida* species are present in the oral cavity of up to 75% of the population (Wasan et al., 2021). In healthy individuals this colonization generally remains benign. However, mildly immune compromised individuals can frequently suffer from recalcitrant infections of the oral cavity. These oral infections with *Candida* species are termed "oral candidiasis" (OC) (Vandeputte et al., 2012).

It is estimated that approximately 75% of all women suffer at least once in their lifetime from vulvovaginal candidiasis (VVC), with 40–50% experiencing at least one additional episode of infection, a small percentage of women (5–8%) suffer from at least four recurrent VVC per year (da Silva Dantas et al., 2015). Predisposing factors for VVC are less well defined than for OC and include diabetes mellitus, use of antibiotics, oral contraception, pregnancy and

hormone therapy, Despite their frequency and associated morbidity, superficial *C. albicans* infections are non-lethal (Enjalbert et al., 2003).

Coconut oil has been touted in a recent years as a ‘super food’ with claims of numerous benefits ranging from ability to promote increased weight loss, improved (healthier) lipid profile during dieting to its antimicrobial, antiviral and antifungal properties. Although the health benefits of coconut oil are regularly suggested to be plentiful in the media (much like several other ‘miracle foods’), there seems to be some scientific support for the claims for coconut oils (Ogbolu et al., 2007).

The use of nanotechnology in medicine offers some exciting possibilities. Some techniques are only imagined, while others are at various stages of testing, or actually being used today. The use of nanotechnology in the field of medicine could revolutionize the way we detect and treat damage to the human body and disease in the future, and many techniques only imagined a few years ago are making remarkable progress towards becoming realities (Kumar et al., 2013).

Zinc oxide nanoparticles are nanoparticles of zinc oxide (ZnO) that have diameters less than 100 nanometers. They have a large surface area relative to their size and high catalytic activity. The exact physical and chemical properties of zinc oxide nanoparticles depend on the different ways they are synthesized. Iosub et al. (2017) *recent stududy* showed that (ZnO) nanoparticles (NPs) at the tested concentrations significantly inhibited the germination of many fungal species such as candida and caused structural damage to the fungal cells (Kasemets et al., 2009).

The close association between oxidative stress and lifestyle-related diseases has become well known. Oxidative stress is defined as a “state in which oxidation exceeds the antioxidant systems in the body secondary to a loss of the balance between them.” It not only causes hazardous events such as lipid peroxidation and oxidative DNA damage, but also physiologic adaptation phenomena and regulation of intracellular signal transduction. From a clinical standpoint, if biomarkers that reflect the extent of oxidative stress were available, such markers would be useful for physicians to gain an insight into the pathological features of various diseases and assess the efficacy of drugs.

The biological antioxidant refers to any compound that is capable of delaying or preventing the oxidation of a substrate. Thus, antioxidants decrease oxidants such as RONS, avoiding oxidative stress. In this way, antioxidants attenuate cell biomolecule damage, such as lipids, proteins, and DNA (Beauchamp & Fridovich, 1971). Since the damaging effects on these biomolecules contribute to cellular toxicity and disease, antioxidants prevent these effects (Aranda-Rivera et al., 2022). The antioxidants group includes enzymes such as SOD, CAT, and GPx, which reduce oxidants or free radicals, with the ability to induce the production of other free radicals. After entering the host system, *C. albicans* cells are exposed to a wide variety of toxic chemicals, most important of which being the reactive oxygen species (ROS) produced by host phagocytes, such as neutrophils and macrophages. The pathogenic fungi, on its part, elicit a strong response against the ROS by activating/regulating multiple signaling pathways involving the stress-activated protein kinase Hog1, the DNA damage kinase Rad53, and the transcription factor Cap1 in order to survive under the oxidative stress condition (Bispo e al., 2017).

## **Methods**

### **Animals and experimental design**

A total (18) albino female rate average weight (135-160 gm) were used. Animals were divided in to 4 experimental groups. The first group contain (5) animals was given the water and food as a normal condition and conceder as control group (A).; the second group contain 15 animals

was infected with the fungi *Candida albicans*, since it given by drinking water after the dilution of the main cultural as single dose; this group then distributed to group (B) contain 5 infected animals without any treatment, group (C) treated with Nystatin drug (1mg/kg) daily all the period of experiment (and group (D) which was treated with Zinc oxide nanoparticle solution and coconut oil(0.5ml) .

At the end of the experimental period, which was one month, the animals were sacrificed. Blood samples were collected in sterile plastic test tube and, serum samples were separated and used for analysis of biochemical parameters.

### **Preparation of samples for histological study**

The organs tissue (ovary and uterus) were prepared for histological examination according to Brown et al. (2009).

### **Preparation of zinc oxide nanoparticle**

White powder of zinc oxide nanoparticles (Pars Lima Company, the Particle size of 20 nm and purity of (90%) addition, distilled water was used as the solvent.

### **Investigation of Oxidative marker**

#### ***Malondialdehyde Assessment***

Measurement of MDA level is based on thiobarbituric acid reaction (TBARS). Tissue MDA level was measured by the method described by Kaya (18). The tissue MDA levels were expressed as nmol/ml tissue.

#### ***Superoxide Dismutase (SOD) activity***

Superoxide Dismutase activity was determined by modifications of methods described by Cruz et al. (2013) using its ability to inhibit the auto-oxidation of epinephrine determined by the increase in absorbance at 480 nm.

#### ***Hormones estimation***

Hormones were measured according to the manufacturer's instructions and using an ELISA method.

### **Statistical analysis**

The variables were analyzed as means  $\pm$  Standard Deviation, and estimated by the one way ANOVA, followed by t-test. Differences were considered to be statistically significant if  $p \leq 0.05$ .

### **Results and Discussion**

Table 1. The level of Reproductive hormones, oxidative marker (MAD and SOD) after 1 month.

<b>Parameters measured</b>	<b>A (Control)</b>	<b>B</b>	<b>C</b>	<b>D</b>
LH (mIU/ml)	0.96 $\pm$ 0.29	0.69 $\pm$ 0.24*	0.78 $\pm$ 0.26*	0.85 $\pm$ 0.32
FSH (mIU/ml)	0.58 $\pm$ 0.08	0.29 $\pm$ 0.05*	0.38 $\pm$ 0.09*	0.45 $\pm$ 0.1
Estrogen (pg/ml)	19.57 $\pm$ 0.63	18.44 $\pm$ 0.56	18.22 $\pm$ 0.48	18.27 $\pm$ 0.39
MDA (nmol/ml)	11.54 $\pm$ 0.75	17.63 $\pm$ 0.41*	14.66 $\pm$ 0.32*	12.29 $\pm$ 0.58
SOD (min/mg/protein)	40.37 $\pm$ 0.71	58.28 $\pm$ 0.33*	49.39 $\pm$ 0.29*	42.54 $\pm$ 0.37

Values are expressed as mean  $\pm$  SD.

\*  $p < 0.05$  compared with A.

## **Effect of fungus *Candida Albicans* on vagina (vaginitis)**

Clinical signs and infection diagnosis: When a yeast infection develops, inflammation occurs along with symptoms of pruritus (itching), a thick white or yellow discharge, and odor.: small sample of vaginal discharge was taken and examined under a microscope to sure of the infection.

### **The levels of reproductive hormones**

At the end of the study, there were significant differences ( $p < 0.05$ ) in LH, FSH hormones in B and C group compared to control group A (Table 1) since the levels of these hormones decreased significantly in treated groups, while there was no-significant differences between group A and D for these hormones which return to its normal value after the treatment with zinc oxide Nano particle and coconut oil.

For Estrogen levels the results showed non-significant differences between the control group and B, C ,D groups, after end of the period of the study, there was a significant increase( $p < 0.05$ ) in MDA and SOD levels of group B and C compared to group A. There was no significant difference between the group A and D which treated with zinc oxide Nano particle and coconut oil (Table 1).

*C. albicans* However, they typically only cause disease in the female reproductive tract under conditions that compromise the host's defenses. While there are at least 20 *Candida* species of clinical importance, *C. albicans* is the species most commonly responsible for fungal vaginitis (Enjalbert et al., 2007).

As discussed earlier, lactobacilli in the vagina inhibit the growth of other organisms, including bacteria and *Candida*, but disruptions can allow *Candida* to increase in numbers. Gunsalus et al. (2016) Typical disruptions include antibiotic therapy, illness (especially diabetes), pregnancy, and the presence of transient microbes, Immunosuppression can also play a role, and the severe immunosuppression associated with HIV infection often allows *Candida* to thrive. Ighodaro & Akinloye (2018) This can cause genital or vaginal candidiasis, a condition characterized by vaginitis and commonly known as a yeast infection. When a yeast infection develops, inflammation occurs along with symptoms of pruritus (itching), a thick white or yellow discharge, and odor (Ighodaro & Akinloye, 2018).

Following phagocytosis, *C. albicans* can evade oxidative-killing by macrophages and neutrophils by switching from budding to filamentous cells, which can pierce the phagosomal membrane (Jamieson et al., 1996). Not only does this allow the pathogen to escape, but this also results in the *C. albicans*-mediated killing of the phagocyte. It has recently been demonstrated that the ability of *C. albicans* to mount robust oxidative stress responses is vital for this polymorphic pathogen to filament in the phagosome (Lorenz et al., 2004).

The ROS generated within the phagosome creates a toxic environment that induces oxidative stress in *C. albicans*. Indeed, exogenous ROS can induce programmed cell death in this fungal pathogen, ROS interact with proteins, lipids and nucleic acids causing irreversible damage to the pathogen. DNA damage caused by ROS can result Lorenz et al. (2004).

Nanoparticles have been incorporated into many consumer industries, including industrial, health, food, feed, space, chemical, and cosmetics, necessitating a green and environmentally friendly approach to their synthesis (Luna, 1968). Recent studies shodd that ZnO-NPs could be good anti-fungal and anti-bacterial properties (Mashrai et al., 2017) The antimicrobial mechanism of metallic NPs is still being discussed in many aspects. However, based on the scientific reports, the principal mechanisms proposed are the formation of reactive oxidative species (ROS) and the release of metal ions from the NPs due to the interaction of NPs with

the cell membrane causing inhibition of cell wall synthesis, enzyme activities, and cell signalling, DNA damage, ribosome disassembly, inactivation of protein synthesis, and structure modification of essential proteins. In addition to the membrane dysfunction caused by the accumulation of positively charged Zn<sup>2+</sup> from the dissolution of ZnO NPs on the surface of the cell membrane, the internalisation of ZnO NPs disrupts microbial metabolic activity, eventually causing microbial cell death (Odel Nitbani & Jumina, 2020).

The beneficial antioxidative effects of coconut oil have been studied and include effects on several antioxidant pathways in the body. The antioxidant activity of specific compounds and extracts of natural products (such as coconut oil) is typically measured through both chemical and automated assays and technologies; this includes analyzing the scavenging properties of various free radicals/reactive oxygen species as well as looking at chelation and reducing properties (Phillips et al., 2003). Numerous studies have shown that polyphenolic compounds have antioxidant properties; the characterization of polyphenols and their properties has gained significant interest in recent years (Singh & Nanda, 2013; Shahidi & Zhong, 2015). Polyphenols can scavenge free radicals and chelate metals, resulting in LDL-cholesterol oxidation inhibition, which can help prevent atherosclerosis and resultant heart disease (Phillips et al., 2003).

CO is rich in medium-chain saturated fatty acids, which are amphiphilic just like the macrolide antifungal drugs (Sirelkhatim et al., 2015), is the most promising of natural antifungal treatments. While VCO fatty acids too would get sequestered inside the biofilm matrix-like their synthetic counterparts and must (probably) be aided by beta-1,3 glucanase treatment, our interest is in the amazing ability of coconut oil in the diet to reduce colonization by *C. albicans* (Valadez-Carmona et al., 2016).

## **Histological study**

### ***Ovary***

#### **1-controle group.**

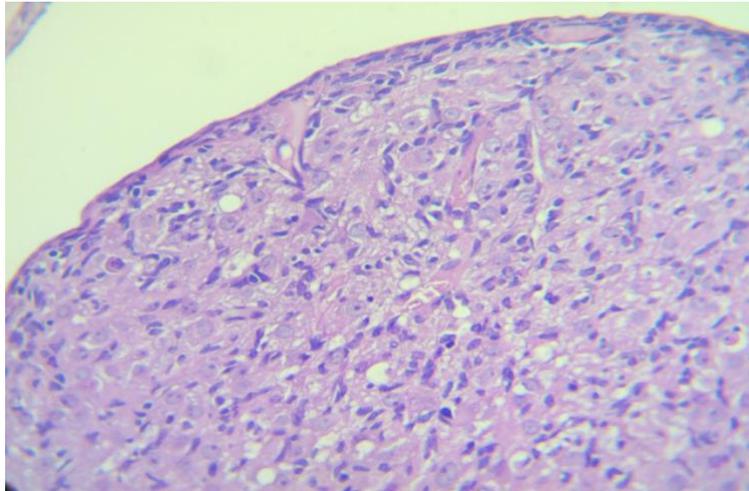


Figure 1. Cortex of ovary, degenerated germinal epithelium (A), collagen bundles (B), WBCs (C) (H&E X40).

The cortex of ovary was containing a great number of collagen bundles associated with multiple fibroblasts and scattered WBCs, few micro blood capillaries were seen, the germinal epithelium on the surface of tunica albuginea were degenerated Figure. 1.

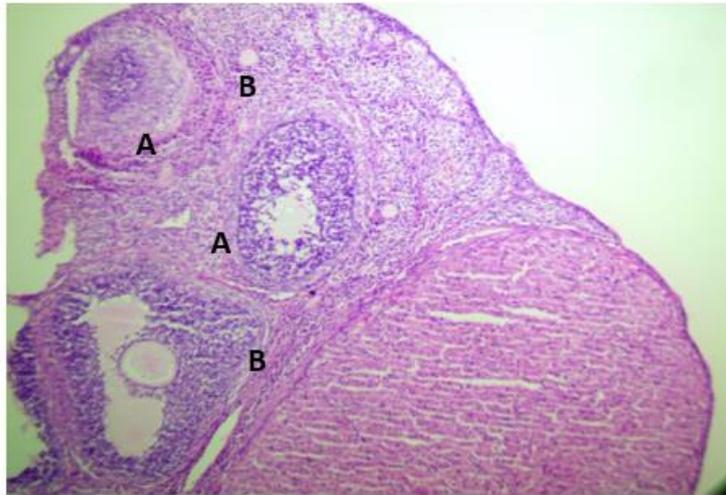


Figure 2. medulla: the ovarian follicular (A), dense connective tissue (B). (H&E X40)

**Group B:**

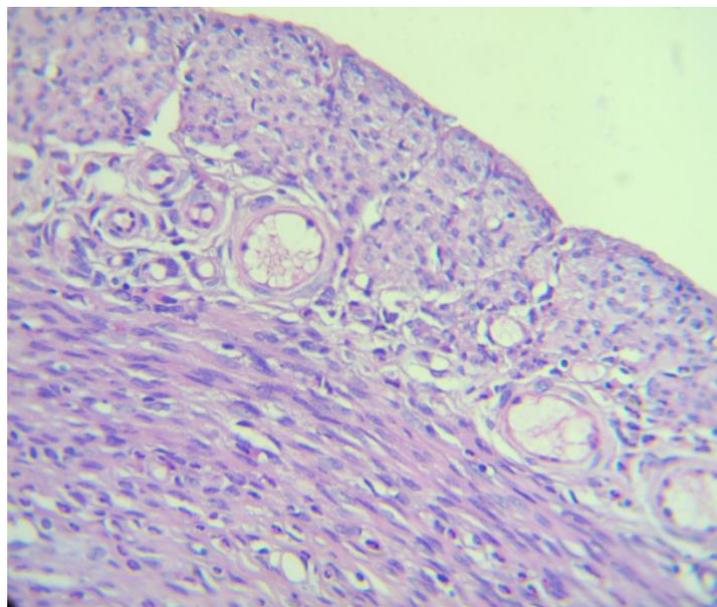


Figure 3. Degenerated germinal epithelium (A) collagen bundles with fibroblasts (B) Blood vessels empty from blood (C) smooth muscle fiber (D) (H&E X 40).

The ovary was covered by degenerated germinal cells and the parenchyma of cortex was occupied with collagen bundles and smooth muscle fibers various blood vessels of different size were present empty from the blood and surrounded by WBCs and macrophages Figure 3.

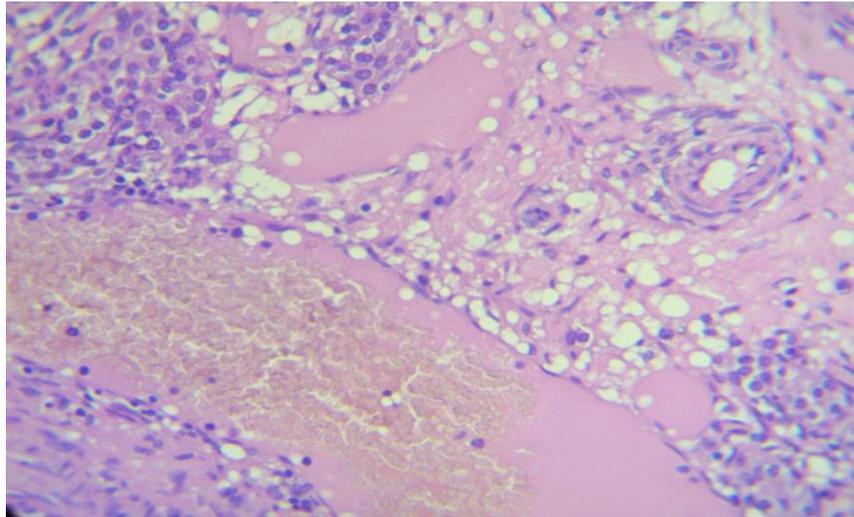


Figure 4. Medulla of ovary, Hemolyzed blood with fat droplets in the blood vessels (A), focal aggregation of WBCs (B) around blood vessels (H&E X40).

The medulla of ovary was demonstrated with many large blood vessels filled with hemolyzed blood with fat droplets in the blood and there was focal aggregation of WBCs in the connective tissue of medulla and around blood vessels Figure 4.

**Group C:**

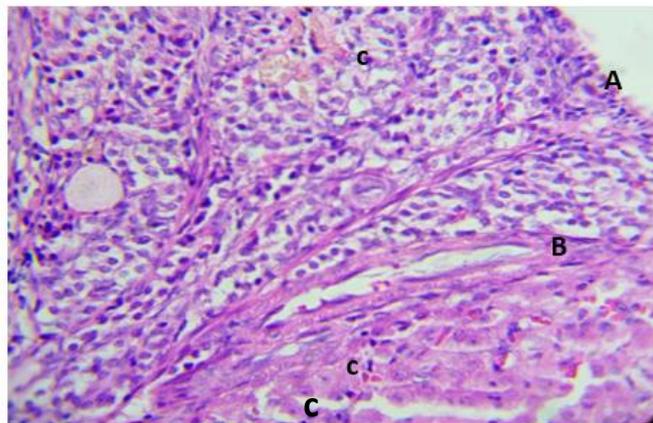


Figure 5. germinal epithelium (A), primordial oocyte (B), Strands of collagen fibers and smooth muscle fiber. (H&E X40)

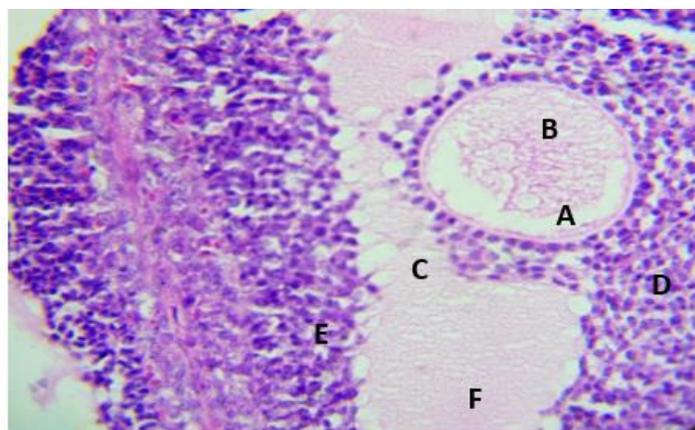


Figure 6. Graafian follicular oocyte (A), Zona peluceda (B), corona radiate (C), cumulus oopherus (D), thecal cells (E), Folicuular fluid (F) ( H&E X40).

**Group D:**

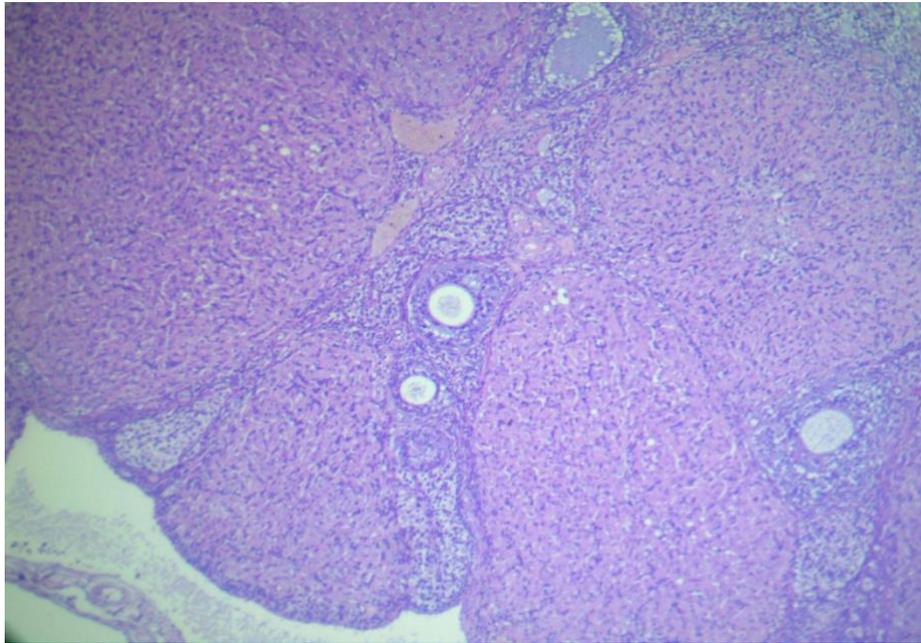


Figure 7. Germinal epithelium (A) primary follicle (B) secondary follicle (C) corpora lutea (D) ( H&E X 10).

The ovarian cortex had primary and secondary oocytes with its follicular cells, also many corpora lutea were present as spherical masses, the surface of ovary was covered by germinal epithelium of one row of epithelial cells figure 7.

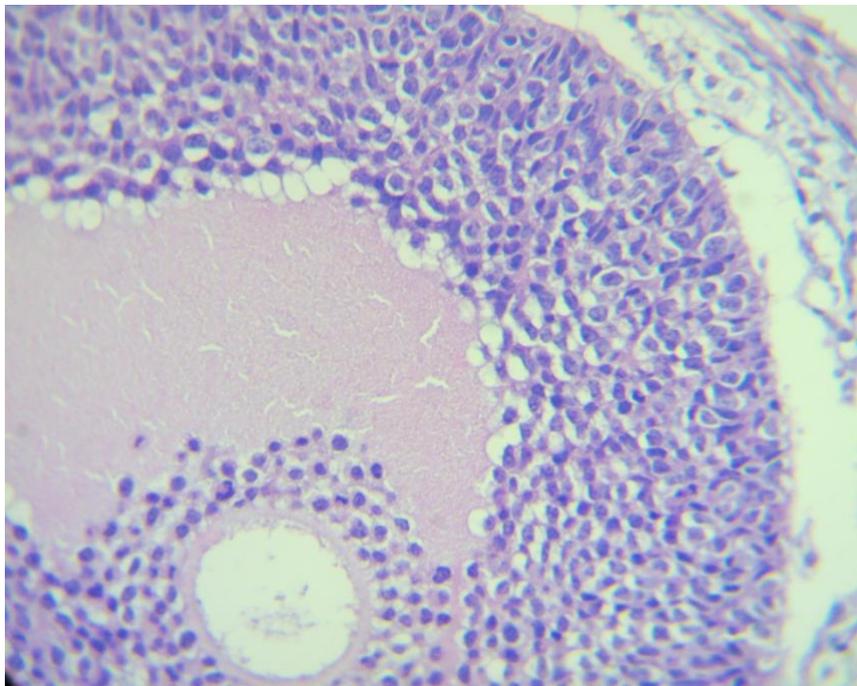


Figure 7. Graafian follicle, follicular cells (A), antrum with follicular fluid (B), Cumulus oophorus (C) Zona pellucida (D), Oocyte with dissolution of cytoplasm and loss of nucleus (E) (H&E X 40).

The Graafian follicle was formed by multiple rows of follicular cells, and present of follicular fluid in the antrum, the oocyte was present with dissolution of cytoplasm and nucleus of it figure 8.

## Conclusion

The results of this study suggest that exposure to *candida albicans* causes damage resulted from oxidative stress. Prescription of zno and coconut oil can prevent damage due to the elimination of free radicals and increase of the body's antioxidant system activity. it is recommended that the effects of different doses of zno on damages caused by EMF in ovarian tissue to be examined in future studies.

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