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Effect of Zinc Oxide Nanoparticles and Vitamin C on Vaginal Infection Caused by *Candida albicans* and *Escherichia coli* in the Mouse Model

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ABSTRACT

Mixed vaginitis is the simultaneous presence of two or more types of pathogens. Zinc oxide (ZnO NPs) is commonly used in pharmaceutical products. Our study investigates the role of ZnO NPs and vitamin C (VC) in resolving mixed vaginitis. The NMRI mice were inoculated with a mixture of *Candida albicans* and *Escherichia coli*. Mice were classified into 8 groups: (1) control, (2) intact mice that received ZnO NPs, (3) intact mice that received daily injection of VC, (4) intact mice that received co- administration of ZnO NPs and VC, (5) infected, (6) infected treated with ZnO NPs, (7) infected that received daily injection of VC, and (8) infected mice treated with co- administration of ZnO NPs and VC. The antimicrobial activity was evaluated using broth dilution methods. Blood samples were obtained for hematological analysis. Vaginal tissue samples were separated and histopathological analysis was performed. Co-administration of ZnO NPs and VC improved the hematological profiles and vaginal architecture. Inhibitory concentration (IC- 50 and IC- 90) of ZnO NPs for the mixture of *C. albicans* and *E. coli* were 235.86 and 685.81 ppm, respectively. Co-administration of ZnO NPs and VC in mixed vaginitis management, Mixed vaginitis disrupted the structure of the vaginal epithelium. Consumption of ZnO NPs also caused adverse changes in the structure of the vagina, but co-administration of nanoparticles and VC completely improved the negative effects of infection and ZnO NPs in vaginal tissue, that these agents can be used in the management of mixed vaginitis.

1. Introduction

Mixed vaginitis is the simultaneous presence of two or more different types of pathogens, that cause an abnormal environment in the vagina. Due to the frequent incidence of *Candida* in the vagina, this yeast would likely present in this infection (Sobel et al., 2013). Bacteria and fungi face each other in different sites of the human body. There, they interact indirectly with the host response and directly with together (Krüger et al., 2019). Secreted molecules produced by these pathogens mediate interactions between fungi and bacteria. The changes in bacterial–fungal infection virulence depend on multiple factors such as the site of infection, the host, the microorganism type, the amount of the inoculum, and so on (Peleg et al., 2010). *Candida* is a commensal fungus that colonizes the vaginal mucosa and *Candida albicans* (*C. albicans*) is the most common species (Bandara et al., 2009). In *Candida* species, adhesions, morphological transition, and several transcription regulators implicate important roles in *Candida albicans* pathogenicity (De Brucker et al., 2015). Nair & Samaranayake showed that

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adhesion of *C. albicans* to various surfaces was increased by *Escherichia coli* (*E. coli*). In the next studies, they demonstrated that *E. coli* suppressed the adhesion of *C. albicans* to human epithelial cells (NAIR and SAMARANAYAKE 1996). *E. coli*, a gram-negative bacterium, lipopolysaccharides (LPS), motility, surface appendages, toxins, and so on affect its virulence (De Brucker et al., 2015). Nowadays, antimicrobial resistance is one of the major problems in the health system, representing the global challenge for public health. The increase in antibiotic-resistant microorganisms implies a lack of antimicrobial drugs to treat infectious diseases (Vivas et al., 2019). Metal-based nanomaterial has been widely studied for biomedical applications. Nanoparticles have non-specific microbial toxicity mechanisms which make difficult the development of resistance by pathogens and increases the spectrum of antimicrobial activity (Slavin et al., 2017). Zinc oxide nanoparticles (ZnO NPs) are identified as the safest nanoparticle in the pharmaceutical field by the Food and Drug Administration (Sánchez-López et al., 2020). ZnO NPs show attractive antimicrobial features that are employed in various pharmaceutical products (Pasquet et al., 2014). Many antimicrobial mechanisms of ZnO NPs were assumed including the attachment between *E. coli* cells and ZnO NPs is the main factor for antibacterial properties, hydrogen peroxide, which is produced from the surface of the nanoparticles, can penetrate via the bacterial cell membrane, generate some kind of damage, and inhibit the growth of the *E. coli* (Wang et al., 2012). ZnO NPs enhancement the permeability of the *C. albicans* cytoplasmic membrane and cell wall via binding to proteins and lipids. This function destructs the 3-D structure of *C. albicans* protein (Hosseini et al., 2020). In spite of the advantages of ZnO NPs against bacteria and fungi, some doubts create about their toxicity (Hobman and Crossman, 2015). ZnONPs usage has been associated with decreased count of red blood cells resulting from toxicity against them. Oxidative stress is seen in all-metal nanoparticles. Their toxic effects occur when they penetrate cells through biological pumps and ion channels (Chang et al., 2012). This feature leads to an inflammatory response induced in the cell by the production of reactive oxygen species (Hobman and Crossman). High levels of ROS generate detrimental effects in the DNA and result in the death of cells (Barad et al., 2017).

Vitamin C (VC), a water-soluble type of vitamin, can boost immunity and health in the human body, is efficient at inhibiting free radicals like aqueous peroxyl radicals, hydroxyl radicals, and superoxide anions (Gu et al., 2018). VC is known to protect membranes against injury and oxidation. VC has been reported to prevent intracellular oxidative stress induced by nanometals agents. Some research has shown that VC reduces intracellular lipid peroxidation caused by nanoparticles in cells and inhibits ROS production (Ahmad et al., 2012; Akhtar et al., 2010). VC not only prevents intracellular oxidative stress but also stops inflammation (Fukui et al., 2017). We investigated the antimicrobial effect of ZnO NPs on *E. coli* and *C. albicans*. Inhibiting the mixed vaginitis caused by *C. albicans* and *E. coli* species. Therefore, the effect of ZnO NPs and VC on mixed vaginitis in adult female mice was examined.

2. Materials and Methods

2.1. In vitro tests

2.1.1. Preparation of zinc oxide nanoparticles:

ZnO NPs used in this research were purchased from Armina Engineering company, Tehran, Iran with a size of 5 to 10 mm in the form of white powder. The authenticity of these nanoparticles was confirmed by the company using X-rays and electron microscopes (SEM and TEM). Dimethyl sulfoxide (DMSO) was used to prepare various dilutions of nanoparticles for antimicrobial tests and intravaginal inoculation in treated mice. The tubes containing the nanoparticles were vortexed for 10 minutes and sonicated for 15 minutes.

2.1.2. Microbial strains and culture condition

C. albicans ATCC 10231 and *E. coli* ATCC 25922 were purchased from the *Iranian* Research Organization for Science and Technology (*IROST*). Bacteria were cultured in Luria Bertani broth medium at 37°C. *C. albicans* was cultured in Sabouraud dextrose broth at 37 °C. After 24- hour incubation broth culture containing microorganisms were collected via centrifugation at 5000 rpm for 5 minutes.

Sedimented substances were washed twice with normal saline and diluted (standard 0.5 McFarland). The required turbidity for *E. coli* and *C. albicans* were 1×10^8 and 3×10^6 CFU/ mL, respectively. For the mixed model infection of *E. coli* and *C. albicans* the proportion 1: 10 was applied (Liu, Wu et al. 2014). Mixed vaginitis is caused by two different types of microbes, each of which affects the process of the infection. These types of infections are often misdiagnosed, and the treatments target only one type of these pathogens, either *E. coli* or *C. albicans*. Therefore, in this study, standard antibiotics were not used to treat this model of infection.

2.1.3. Disc diffusion susceptibility tests

The disc diffusion method was performed by swabbing *E. coli*, *C. albicans*, and their combination in standard 0.5 McFarland concentration, which was presented in the previous part, on Mueller-Hinton agar medium. Sterile paper discs (6 mm diameter) were impregnated with different concentrations of ZnO NPs (128- 2048 ppm) and placed on the surface of the agar medium. An impregnated disc with only sterile DMSO was used as a negative control. After 24- hour incubation at 37 ◦C, a zone of inhibition around the disc was observed and reported based on millimeters (Pasquet, Chevalier et al. 2014).

2.1.4. Broth dilution tests

The antimicrobial effects of ZnO NPs were tested by using the micro-dilution procedure. In this experiment, 100 µm of Muller-Hinton broth medium was added to all wells of the microplate. The ZnO NPs were diluted in nine concentrations (8 to 2048 ppm). The first well in each row was filled with 100 µl of the highest concentration of ZnO NPs. 100 µl of this nanoparticle was transferred to the second well and continues to well 9. Well 10 of each row was considered to control the growth of microorganisms (*C. albicans*. *E. coli*, and their mixture). The microplate was inoculated at 37 °C for 24 hours. The minimum inhibitory concentration (MIC) was the concentration of wells in which no turbidity was observed and considered as MIC (1024, 256, and 512 ppm for *E. coli*, *C. albicans*, and the mixture of them, respectively). To determine the minimum bactericidal (fungicidal) concentration (MBC, MFC) of nanoparticles, two wells before the well of MIC were cultured on Muller-Hinton agar medium and incubated for 24 hours. After that, those plates without microbes were considered MBC (or MFC). Optical density (OD) of these plates was read at 600 nm and inhibitory concentration 50 and 90 percent (IC-50 and IC- 90) was reported in the presence of nanoparticles (Elshikh et al., 2016).

2.2. *In vivo tests*

2.2.1. Groups and infection

Female mice were tested with compliance of the local ethics committee of Razi University (IR.RAZI.REC.1399.051), Iran. Forty NMRI mice (5 mice per group) of about 8 weeks old and 27–30 g weight was purchased from Pasteur Institute, Iran. The mice were given water, fed on a commercial pellet diet, and kept in plastic cages in a 22± 2 ◦C, 55% relative humidity with 12-hour light and dark cycle. After a week of acclimation, the mice were randomly divided into eight groups: Group 1 was set as a control and healthy mice, group 2 was not infected and only received 1024 ppm of ZnO NPs for two weeks (ZnO group), group 3 was not infected and only received 20 mg/ Kg of VC for two weeks (VC group), group was not infected and received simultaneous intravaginal inoculation of ZnO NPs (1024 ppm) and intraperitoneal injection of VC (20 mg/ Kg) for two weeks (ZnO + VC group), in group 5 mice were infected with 50 µl of mixed microbial suspension (1: 10 of *E. coli* and *C. albicans*) and were not received any treatment (I group) (Sobel, Subramanian et al.), group 6 was infected mice that received intravaginal inoculation of ZnO NPs (1024 ppm) for two weeks (I+ ZnO group), group 7 was infected mice that received intraperitoneal injection of VC (20 mg/ Kg) for two weeks (I+ VC group), group 8 was infected mice that received simultaneous intravaginal inoculation of ZnO NPs (1024 ppm) and intraperitoneal injection of VC (20 mg/ Kg) for two weeks (I+ ZnO + VC group). Two weeks after treatment, the mice were sacrificed. The blood sample was obtained from their heart. The vaginal tissues were collected.

2.2.2. Blood collection

Nanoparticles have strong antimicrobial properties and are widely used for treatment. The results of various research have shown that these materials have cytotoxic effects and the smaller their size, the greater the toxicity caused by them. The results show that nanoparticles can enter the bloodstream after consumption and then interact with plasma proteins, coagulation factors, platelets, red and white blood cells (Chang et al., 2012 and Srivastav and Kumar, 2013). Mice positioned on its back, the abdomen was cleaved, and the heart tissue visualized. Using a two ml syringe, 0.5 ml of blood was obtained from the left ventricle. Blood samples were stored in tubes containing anticoagulants. Then blood factors such as white blood cells (WBCs), red blood cells (RBCs), Hemoglobin (HB), hematocrit (HCT), Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelet (PLT) were evaluated using a cell counter device.

2.2.3. Histopathology

Vaginal tissues separated from mice were fixed in 10% formaldehyde. For histology study we applied dehydration with graded ethanol, clearing with xylene, infiltration with paraffin, and embedding. The vaginal tissues were cut into thick pieces about 5 μm via microtome. These sections were stain with hematoxylin and eosin (H & E) (Sultana, Qureshi et al. 2019).

2.3. Statistical analysis.

Descriptive statistics, mean, and standard deviation were used to present the data. Data were analyzed using Excel and SPSS version 26. Differences values and means were compared using one-way analysis of variance (ANOVA) and the significance level was set at $p< 0.05$.

3. Results

3.1. Results of In vitro tests

3.1.1. Antimicrobial activity by disc diffusion susceptibility test

In this experiment, the disk diffusion method was used to investigate the effect of ZnO NPs on growth inhibition of *E. coli*, *C. albicans*, and the combination of them. In this test, nanoparticles were prepared at concentrations of 128, 256, 512, 1024, and 2048 ppm. DMSO was used for dilution at these concentrations. According to the results of this experiment, ZnO NPs had the most inhibitory effect on *C. albicans* and the least effect on *E. coli*. In this test, the antibiotic discs of gentamicin and fluconazole were used for *E. coli*, *C. albicans*, respectively. A disc impregnated with DMSO was also used as a negative control in the test, which showed that it had no inhibitory effect on the microbes in the experiment. The results of this test were shown that the most inhibitory effect of ZnO NPs was at 2048 ppm, which was 17.05±0.28, 22.45±0.35, and 18.3±0.28 mm for *E. coli*, *C. albicans*, and both combinations, respectively (Figure 1 and Table 1).

3.1.2. Antimicrobial activity measured by the broth dilution method

The microdilution method was used to evaluate the antimicrobial effect of ZnO NPs. The rate of inhibition under the influence of nanoparticles at concentrations of 8 to 2048 ppm was measured using the inhibitory concentration of 50% (IC-50), 90% (IC-90), MIC, and NBC (MFC). The inhibition of *E. coli*, *C. albicans*, and their mixture under the effect of ZnO NPs was calculated according to OD 600 nm. IC-50 represents a concentration of ZnO NPs that inhibits microbial growth by 50%, and IC-90 is a nanoparticle that inhibits 90% of microorganisms, as shown in Figure 2, 3. Based on the results of this experiment, the IC-50 levels for *E. coli* ATCC 25922, *C. albicans* ATCC 10231, and for the *E. coli* & *C. albicans* mixed model were 499.49, 83.15, and 235.86 ppm, respectively. The IC-90 values for *E. coli* ATCC 25922, *C. albicans* ATCC 10231, and mixed model of them were 1346.95, 338.74, 685.81 ppm, respectively. The MIC values of *E. coli* ATCC 25922, *C. albicans* ATCC 10231, and mixed model of them were 1024, 256, and 512 ppm, respectively. The MBC (MFC) values for *E. coli* ATCC 25922, *C. albicans* ATCC 10231, and mixed model of them were 2048, 512, and 1024 ppm, respectively. The results of this test showed that in these three tests, *E. coli* was less sensitive to ZnO NPs than *C. albicans* and the mixed infection caused by the mentioned bacteria and yeast (Table 2).

3.2. Results of In vivo studies

3.2.1. Hematological profile

In the present study, the toxicity due to the consumption of ZnO NPs was evaluated by examining blood parameters. The results showed significant changes in the amount of blood factors. As shown in Table 2, WBC, HCT, MCH, and MCHC counts in infectious groups and groups receiving nanoparticles were significantly different from the control group, and groups treated with VC and a combination of nanoparticles and VC were similar to the group. RBC counts in VC, EC+ ZnO NPs, and EC+ VC+ ZnO NPs groups were similar to the control group. Other groups were significantly different from the control group. HB in VC+ ZnO NPs and EC groups were significantly different from the control group and other groups were similar to the control group. MCV counts in EC+ VC and EC+ VC+ ZnO NPs groups were similar to the control group. Other groups were significantly different from the control groups. PLT in VC group was significantly different from the control group and other groups were similar to the control group (Table 3).

3.2.2. Histological Study

Vaginal tissue sections were studied under microscopic. Leukocytes, hyperplasia, inflammation, hyperemia, epithelium surface, and connective tissue were examined. The characteristics of mice vaginal tissue in VC, ZnO NPs +VC, and I+ ZnO NPs +VC groups were normal and similar to the control groups and other groups. However, in other groups, compared to the control group, significant changes were seen in vaginal tissue, as shown in Figure 3. The results of histological studies showed that the co-administration of ZnO NPs and VC can be effective in treating a mixed vaginal infection caused by *E. coli* and *C. albicans*. Also, this treatment could eliminate the possible toxic effects of ZnO NPs in treated mice (Figure 4).

Table 1. Diameter measurements of inhibiting the growth of microorganisms under the influence of different concentrations of ZnO NPs (ppm)

ZnO_2 Concentration	*E. coli*	*C. albicans*	*E. coli & C. albicans*
256	0	16.7±0.28	14.25±0.35
512	7.75±0.35	17.2±0.28	16.2±0.28
1024	9.2±0.28	18.25±0.25	17.15±0.21
2048	17.05±0.28	22.45±0.35	18.3±0.28

Table 2. Inhibiting effects of ZnO NPs (ppm) on the growth of microorganisms

	E. coli ATCC 25922	*C. albicans* ATCC 10231	*E. coli & C. albicans*
IC- 50[1]	499.49	83.15	235.86
IC- 90[2]	1346.95	338.74	685.81
MIC[3]	1024	256	512
MBC/ MFC[4]	2048	512	1024

[1] Concentration of ZnO NPs that inhibits microbial growth by 50%
[2] Concentration of ZnO NPs that inhibits microbial growth by 90%
[3] Minimum Inhibitory Concentration
[4] Minimum Bactericidal (Fungicidal) Concentration

Table 3. Effects of ZnO NPs, VC, and combined ZnO NPs and VC administration on blood factors in infected mice with E. coli and C. albicans

	Groups							
	Control	ZnO[1]	VC[2]	ZnO+ VC[3]	I[4]	I+ ZnO[5]	I+ VC[6]	I+ ZnO+ VC[7]
WBC ($\times10^3/\mu l$)	5.65± 0.07	7.7± 0.14*	5.55± 0.21	5.75± 0.07	3.7± 0.57*	6.25± 0.07*	4.35± 0.07*	5.8± 0.14
RBC ($\times10^6/\mu l$)	8.76± 0.01	8.11± 0.31*	8.91± 0.13	8.98± 0.22	9.63± 0.19*	8.53± 0.12*	9.25± 0.22	8.94± 0.08
HB (g/dl)	14± 0.14	14.8± 1.13	14.35± 0.21	14.95± 0.21	14.95± 0.21	14.85± 0.35	14.75± 0.35	14± 0.14
HCT %	43.05± 0.21	37.37± 2.65*	44.51± 1.97	44.95± 0.64	50.85± 1.34*	44.3± 0.99*	46.05± 0.92	44.05± 0.64
MCV (fl)	49.25± 0.35	53.75± 2.19*	54.85± 0.35*	52± 0.57	53.6± 0.71*	53± 0.01*	50.8± 0.57*	51.15± 1.34
MCH (pg)	15.55± 0.35	16.15± 0.21	16.25± 0.21*	16.85± 0.35*	15.15± 0.35	15.8± 0.14	15.89± 0.42	16.5± 0.14*
MCHC (g/dl)	32.5± 0.71	32.8± 0.14	34.25± 1.48*	35.9± 0.71	29.65± 0.78*	30± 0.28*	31.25± 0.35*	33.89± 0.14
PLT ($\times10^3/\mu l$)	673.5± 2.12	688.5± 7.78	494± 2.36	708.5± 14.85	656± 8.49	752± 15.56	628.5± 17.68	681.5± 3.54

[1] ZnO: The group of healthy mice that received ZnO NPs in IC- 90 concentration,
[2] VC: The group of healthy mice that received VC,
[3] ZnO+ VC: The group of healthy mice that received co-administration of ZnO NPs and VC,
[4] I: The group of mice that infected with both of E. coli and C. albicans,
[5] I+ ZnO: The group of infected mice that received ZnO NPs in IC- 90 concentration,
[6] I+ VC: The group of infected mice that received VC,
[7] I+ ZnO+ VC: Group of infected mice that received co-administration of ZnO NPs and VC.
* (The significancy deference with control group P< 0.05).

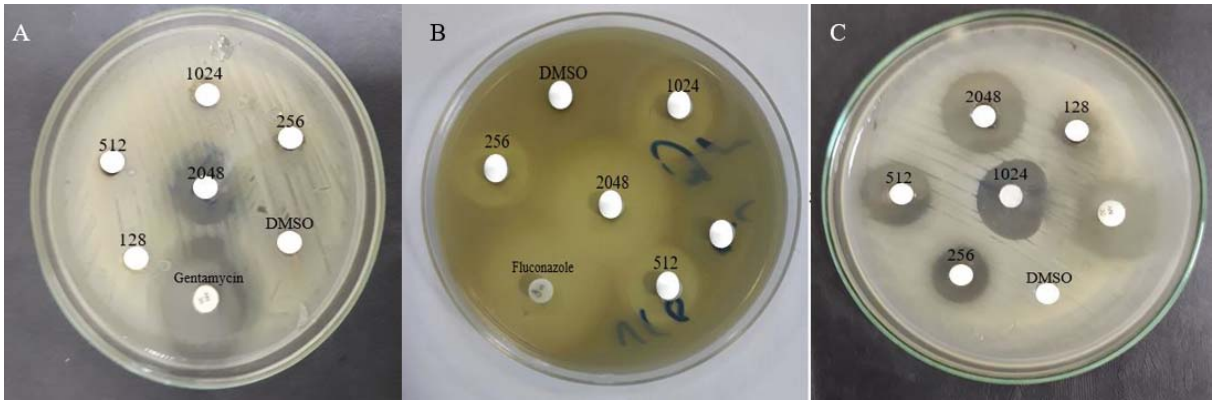

Figure 1. The zone of inhibition the growth of microorganisms under the influence of different concentrations (128- 2048 ppm) of ZnO NPs. A) E. coli ATCC 25922, B) yeast *C. albicans* ATCC 10231, and C) a combination of *E. coli/ C. albicans*.

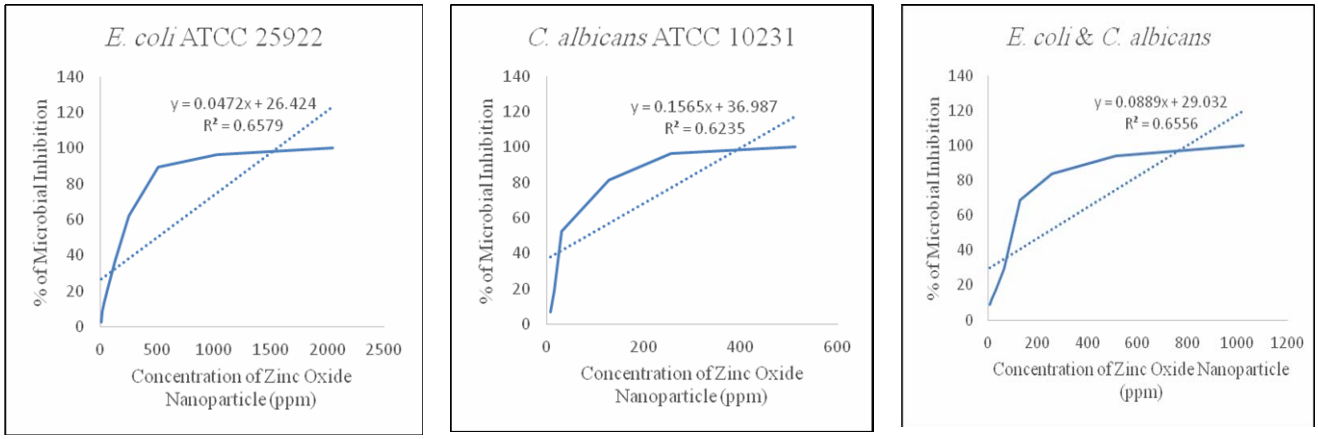

Figure 2. IC-50 and IC-90 values of *E. coli* ATCC 25922, *C. albicans* ATCC 10231, and mixed model of *E. coli* & *C. albicans* against ZnO NPs.

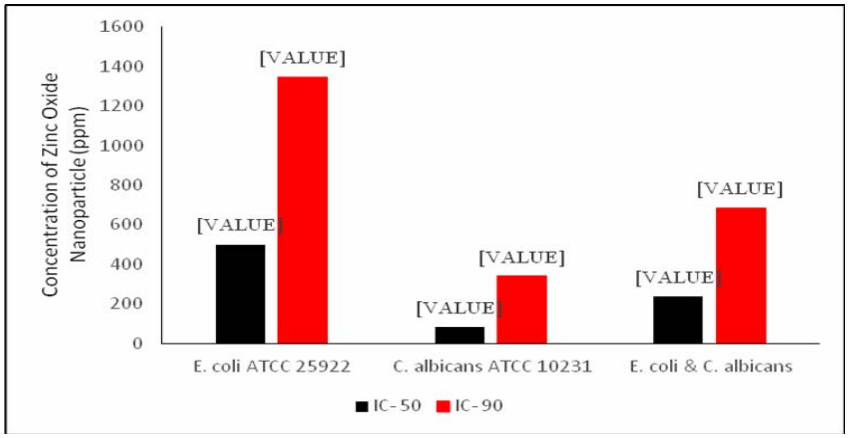

Figure 3. Comparison of IC-50 and IC-90 levels in *E. coli* ATCC 25922, *C. albicans* ATCC 10231, Combined model *E. coli* & *C. albicans* against ZnO NPs.

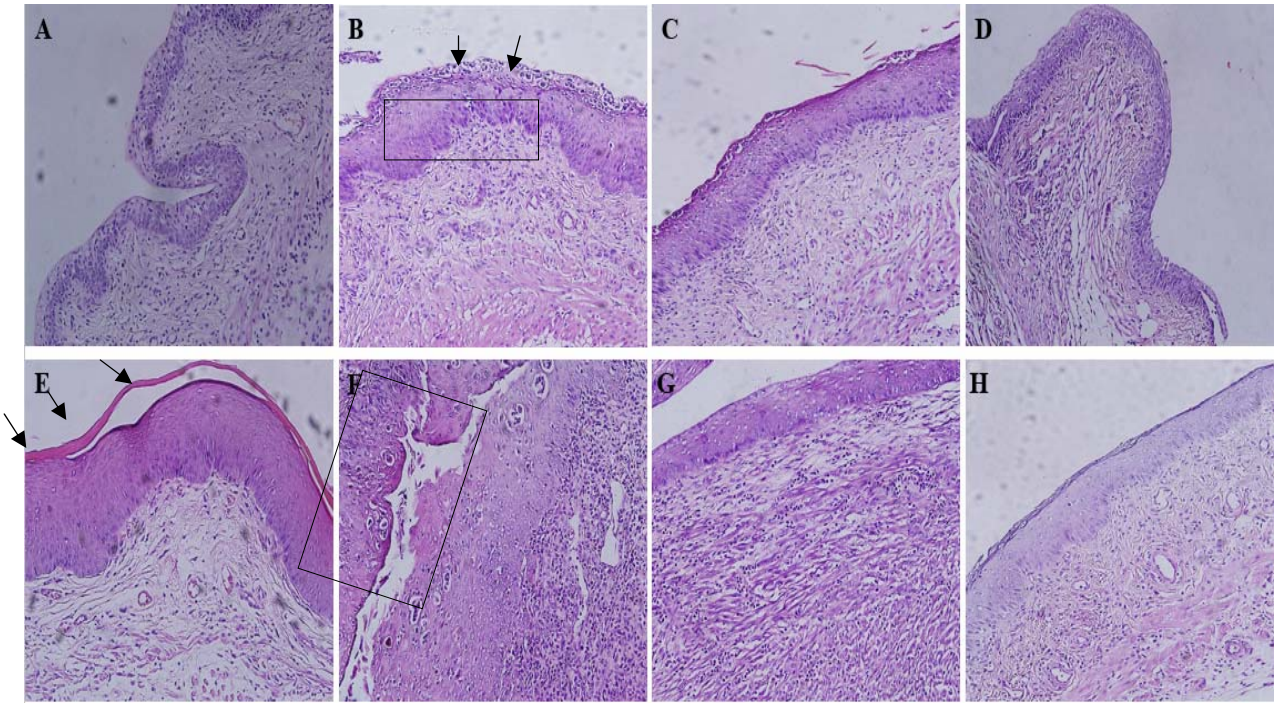

Figure 4. Tissue section examination of mice vagina after Hematoxylin and eosin staining; control group (A) was demonstrated normally feature, ZnO NPs group (B) was shown that hyperplasia, inflammation, and tissue connection was very disturbed, VC group (C) and ZnO NPs + VC group (D) was almost similar to the control group, I group (E) infected mice with the mixture of *C. albicans*/ *E. coli*, I + ZnO NPs group (F), Infected mice that treated with 1024 ppm of nanoparticles, I + VC group (G) Infected mice that treated with daily injection 20 mg/ kg of VC, and I + ZnO NPs + LC group (H) infected mice that treated with co-administration of ZnO NPs and VC. These mice had similar properties to the intact mice group.

4. Discussion

Although vaginitis is a very common reason for vulvovaginal signs, poor information is accessible on the prevalence of mixed vaginal infection (Sobel et al., 2013). The fungal-bacterial community has been characterized to exist in almost all ecosystems and contains microbial species from a broad variety of bacterial and fungal families (Deveau et al., 2018). *Candida* is a common yeast that colonizes the vaginal mucosae in humans. Studies indicated that *E. coli* had no remarkable effect on the *C. albicans* adhesion to polystyrene surfaces. They also showed that the adhesion of *C. albicans* to acrylic surfaces in the presence of bacteria, irrespective of the *E. coli* load on the area (Bandara et al., 2009). Various other research has subsequently had the same results, whereby mixed infection with *E. coli* and *C. albicans, E. coli* was able to decrease the viability of yeast over time, and bacterial endotoxin seemed to be necessary for this virulence (Peleg et al., 2010; Yang et al., 2016). Our studies showed similar results and demonstrated that *E. coli* had more destructive effects than a mixture of *C. albicans* and *E. coli*. Antibiotic resistance in pathogenic microorganisms is one of the most drastic threats to health today. So, alternative procedures for controlling antimicrobial-resistant pathogens are very important (Medina and Pieper, 2016). ZnO is widely applied in the biomedical field for several infections as an antimicrobial agent (Pasquet et al., 2014). Among the several metal oxides nanoparticles investigated for their antimicrobial properties, ZnO NP has been found to have optional toxicity to fungi and bacteria and only represent the fewest adverse effects on human cells, that introduce their prospective uses in pharmaceutical fields (Pasquet et al., 2014; Nazoori and Kariminik, 2018). Although ZnO NPs has been used in several research in human medicine, the therapeutic effect of ZnO NPs has not been examined against mixed vaginitis caused by *E.*

coli and *C. albicans*. Many studies have shown that ZnO NPs have potential antimicrobial activity against *E. coli* and *C. albicans*. Y. Liu et. al in 2009 investigated the antibacterial effect of ZnO NPs against *E. coli*. The size of their nanoparticle was 70 nm (Liu et al., 2009). The results of many experiments indicated that ZnO NPs led to complete inhibition of the growth of *E. coli* as the concentrations of nanoparticles increased. These outcomes proposed that ZnO NPs could be used as a potential antibacterial factor (Şahin et al., 2017; Shakerimoghaddam et al., 2017). Sahar MG Felemban et. al in 2020 tested the effect of various concentrations of ZnO NPs on C. albicanes. nanoparticles with 70 ± 15 nm diameter were examined for antifungal tests (Abd Suha and Ali, 2015). The results of several studies showed that the ZnO NPs as an effective agent on *C. albicans* (Abd Suha and Ali, 2015; Ficociello et al., 2018; Nazari, 2020). While production of nanoparticles and nanotechnology is growing extremely, investigations into the toxicological impacts and possible danger of nanomaterial to the environment and human health are still in their infancy (Elsaesser and Howard, 2012). Our studies show similar results that these nanoparticles have significant inhibitory and lethal effects on *E. coli*, *C. albicans*, and both combinations. This is the first study to investigate the antimicrobial effects of ZnONPs against multidrug-resistant mixed vaginal infection pathogens. The antimicrobial effects of ZnO NPs were measured by disk difusion and broth microdilution methods against standard species of *E. coli* and *C. albicans*. Our results showed that the most inhibitory effect of ZnO NPs was at 2048 ppm, which was 17.05±0.28, 22.45±0.35, and 18.3±0.28 mm for *E. coli*, *C. albicans*, and both combinations, respectively. The IC-50 level for *E. coli* ATCC 25922 was 499.49 ppm, for *C. albicans* ATCC 10231 was 83.15, and for the *E. coli* & *C. albicans* mixed model was 235.86 ppm. The IC-90 values for *E. coli* ATCC 25922, *C. albicans* ATCC 10231, and mixed model of them were 1346.95, 338.74, 685.81 ppm, respectively. The MIC values for *E. coli* ATCC 25922, *C. albicans* ATCC 10231, and mixed model of them were 1024, 256, 512 ppm, respectively. The MBC (MFC) values for *E. coli* ATCC 25922, *C. albicans* ATCC 10231, and mixed model were 2048, 512, 1024 ppm, respectively. Noxious effects on different systems and organs can be caused by long-term and short-term exposure to ZnO NPs. This nanomaterial significantly reduced the number of *C. albicans*. ZnO NPs Increased the rate of ROS that is depend on the concentration of ZnO NPs (Aslani et al., 2018).

Base on the histopathological examination, female mice intravaginally inoculated with ZnO NPs have shown microscopic changes in the vaginal tissue due to treatment with ZnO NPs. In this test, the vagina cured with ZnO NPs showed hyperemia, increased inflammation, hyperplasia, and epithelial destruction. Vaginal tissue in the infected group (Krüger et al., 2019) was almost similar to the groups that treated with lone nanoparticles. The tissue of the control group did not show any lesions. So far, no studies have been presented on the effects of ZnO NPs on the vagina, but few studies have been performed on the effects of ZnO NPs on other parts of mice bodies, indicating the destructive effects of these nanoparticles on tissues (Hosseini et al., 2019). Treatment with VC significantly improves the side effects induced by ZnO NPs but it has no effect on infection (Alkaladi, 2019). Histopathological examination of H&E stained sections from the vagina of VC and control groups showed normal appearance in the tissues. furthermore, the blood factors of the VC group were similar to the control group. Co-administration of ZnO NPs and VC showed the therapeutic effect on the adverse effect of vaginitis and ZnO NPs (Mohamed, El-fakharany et al. 2019). Our experiment also confirmed these results. In this study, in addition to the destructive effects of infection and ZnO NPs on vaginal tissue, the number of blood factors was also examined and the results are presented in the table 3. According to the results of this experiment, both nanoparticles and mixed infection cause significant changes in blood factors compared to the blood factors of mice in the control group. For example, the level of WBC in these groups significantly increased compared to the control group. In this study, we used VC as an antioxidant agent to eliminate the possible toxic effects of ZnO NPs. VC has antioxidant activities against the harmful effects of the free radicals.

In conclusion, mixed vaginal infection is a serious disease that requires accurate diagnosis and therapy. Treatment of these infections is complex and requires a mixture of medication

that target the fungi and bacteria at the same time. Evidence from this study showed that co-administration of ZnO NPs, as an antimicrobial agent, and VC, as an antioxidant factor, led to the treatment of the mixed vaginitis in mice. There was no evidence of treatment in vaginal infection by the single-use of either medicine. Interaction between ZnO NPs and VC resulted in the treatment of mixed vaginitis and eliminated the symptoms of infection.

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Conflict of interests: The authors declare that there is no conflict of interest.

Refereces

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