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Comparison Effect of Clotrimazole and Silver Nanoparticles on Epithelial of Vagina in the Treatment of Vaginal Candidiasis in Mice

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Vaginal candidiasis is one of infections occurred in the vagina and cause by Candida that is kind of yeasts. This disease transferable between male and female and cause disease in genital tract in both of them. Studies reported secondary infertility by this infection in genital tract and to be associated with low birth weight and premature birth. Major diseases in candidaisis caused by candida albicanse species that is opportunistic yeast in form of simple superficial infection to acute systemic infection and has been the fourth leading cause of death with infection disease. This disease is becoming resistant to commonly used drugs. With the development of nano technology, nanoparticles have become one of the promising and useful antibacterial agents. Silver nanoparticles (SNPs) have anti fungal, anti viral and anti bacterial properties. Therefore, in this study after microbiological analyses on Candida albicans species that obtain from the Iranian Research Organization for Science and Technology and patient, antifungal susceptibility of clotrimazole and SNPs on two Candida albicans strains-including standard and pathogenic-were evaluated by the broth microdilution method for determining MIC(minimum inhibitory concentration) and using sabaro dextros agar for determining MFC(minimum fungicidal concentration). MIC and MFC of SNPs and clotrimazole were administered to mice that have vaginal C. albicans infection. Our results revealed treatment with clotrimazole decrease the Cellular connections in cornify layer in vaginal epithelial that weren't observed in treatment with SNPs and negative control groups. Therefore according to our finding. Silver nanoparticles have antifungal activity and is also very effective against vaginal candidiasis caused by candida albicans and didn't have harmful effects on vaginal epithelium in comparison with clotrimazole, can be used in the treatment of this disease.

Key words: silver nanoparticle, clotrimazole, candida albicans, vaginal candidiasis, mice

Vagina is a fibro muscular organ in the female reproductive system and it's epithelium is a mucosal tissue that has a boundary between inside and outside of the body, for result of this situation is prone to different diseases that can be acute and spread to other parts of the body. Vaginal candidiasis is the most common female genital tract infections caused by candida albicans and after vaginosis caused by anaerobic bacteria in the second place. 75% of women experience this disease once in the life time. It may be as simple to acute or recurrent.[5]. this disease transferable between male and female and cause of infection in genital tract in both of them[6].This infection effect on sperm parameters such as motility , lead to apoptosis and effect on attach to oocyte. it also has a negative effect on the quality of oocyte zona pellucida[7,8]. The acute of this infection can disease the uterus and fallopian tube that cause infertility[9] and to be associated with low birth weight and premature birth.)thesis)

In recent decades opportunistic infections caused by yeasts have been widespread.[1]. Major part of the normal flora of the skin and mucosal surfaces are yeasts and when immunity of the body for any reason be suppress, opportunity fungal infection be appear in form of superficial, coetaneous, mucosal or systemic. Yeasts infection such as candidiasis is the most opportunistic fungal infection. This disease can be acute, sub acute and chronic in skin, nails, vaginal mucosa, bronchus, lung, gastrointestinal. sometimes outbreak to liver and heart.[2].The most common cause of candidiasis infection is candida albicans[3]. That is polymorphism and can grow in rounded shapes, blastupori germination, short or long pseudohyphea or hyphea[4].

Clotrimazole is one of the most common drugs in treatment of fungal infection. research was reported that candida species become resistant to common drugs[10].

Silver nanoparticle (SNPs) is silver atoms in size of 1 to 100 nm. Except their use in industries engineering such as catalytic, optical devices for electronic applications, the nanoparticles due to their antimicrobial activity apply to disinfection and medical devices[11]. SNPs have antifungal, antivirus and antibacterial activity, that now a days a lot of research reported antimicrobial activity of silver nanoparticle [12].

Materials and Methods:

Microbiological analyses for C. albicans species

C. albicans species were studied. standard strain of candida albicans (ATCC-10231) was obtain from the Iranian Research Organization for Science and Technology and pathogenic strain was obtain from patient.

At the first for monitoring and testing for detection of candida albicans in the sterile condition Petri on medium culture sabaro dextrose agar that prepared from equa laboratory.

Then a colony was cultured recently was coloration by two methyilene blue and observed under the microscope.

To create germ tube, mixed a colony in 1 cc of animal serum and incubated four hours at 37 ° C. After that time put amount of suspension on glass slide and observed under the microscope. In this condition, candida albicans germ tube very high at about 3 times the diameter of main cell that other species can't make it[13].

Prepared Materials Testing:

SNPs were prepared with a diameter of 20 nm in solution at 2000 ppm concentration. Dilution to using solvent with dimethyl sulfoxide diluted (1%) was obtain from Merk Germany. Dilution serial were provided of method serial dilution in 1000, 500, 250, 125 concentration. During the process were kept in dark glasses and away from sunlight.

For prepared 0.5 McFarland suspension of candida alicans, mixed a colony in 2 cc of normal saline and optical absorption measured by spectrophotometer. the number was in the range of 0.08-0.12.

The pure powder of clotrimazole was solvent with dimethyl sulfoxide(1%) at concentration of 10, 5, 2.5, 1.25 ppm.

In this experiment we used 8 weeks old NMRI mice. they cage in animal house under 12 hours of darkness, 12 hours of light in 20-25 ° C temperature with full access to enough food and water.

In this survey used microdilution method for evaluate the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of SNPs and clotrimazole and were conducted by the following steps.

At the first 100 µl of sabaro dextrose broth poured in all wells of the micro plate in sterile condition. Then 100 µl of the highest concentration of SNPs -1000 ppm- were added to the first well of the first horizontal row in the micro plate(now in this well have 200 µl solution). After mixing, 100 µl lifted and added to next well of the first row(second well), again after mixing lifted 100 µl and added to the next well(third well). This process continues until tenth well and one hundred micro liters of tenth well throw away until all wells have same volume. This process done for all concentration of SNPs and clotrimazole in all row of micro plate.

Then 10 µl of fungal suspension were added to all wells except twelfth vertical row.

Eleven vertical rows involved medium and fungal suspension and was considered as positive control. vertical twelfth rows only have medium culture and was considered as a negative control.

Optical absorption at wavelength 405-450 was read by ELISA reader. The door of micro plate was placed and incubated in 37 ° C for 72 hours. Optical absorption every 24 hours were read to control the growth. After 72 hours if the number of optical absorption of one well compare with first day didn't increased, which means that the fungi didn't grown and antifungal effected on their. If in a well the number of optical absorption increased, the fungi were grown and may be the turbidity of colony visible.

The first well of the horizontal rows that hasn't changed in number of optical absorption was considered as MIC or minimum inhibitory concentration.

To determine the MFC , took 10 µl of MIC well and wells after and before it, conveyed to sabaro dextrose agar plate and incubate at 37 ° C for 48 hours. After that time in which plate the fungi didn't grown, that concentration considered as MFC.

For three days 0.1 mg estradiol mixed with 20 ml sesame oil injected intraperitoneal. vaginal smears were taken to prove the absence of fungal disease in mice and confirmed the estrus phase in all of them, in addition symptoms such as discharge or swelling shouldn't be observed.

Then the mice were divided into 12 groups of five. Four negative control groups without any infection received, distilled water, clotrimazole, 125 ppm and 250 ppm concentration SNPs. Three groups were infected by pathogenic strain and treatment with 1.25ppm clotrimazole, 125 ppm and 250 ppm concentration of SNPs and the next three groups infected by standard strain and receive the same concentration as pathogenic strain. Finally two positive control groups infected with two strains of C. albicans and didn't treated.

For infected disease groups insemination 20 ml 0.5 McFarland fungal suspension to vaginal. after fixing the symptoms of the infected in vaginal the treatment was started. Once s day 20 ml of drugs solution insemination vaginal in mice.

After treatment of vaginal infection and complete remove symptoms, mice were killed by cervical dislocation and vaginal tissue were extraction. For histomorphology the epithelium of vaginal were section and were staining with hematoxylin and eosin stain.

Findings:

Different concentration of SNPs and clotrimazole on pathogenic and isolated strains of candida albicans were examined. The results showed that the concentration of 125 ppm SNPs has a inhibitory effect on growth of fungal and the concentration of 250 ppm has a fungicidal effect on fungal. Even that for clotrimazole, both MIC and MFC were 1.25 ppm.

The effect of two different concentrations of SNPs and clotrimazole vaginal epithelium in mice was investigated. The results show the epithelial which treat with clotrimazole disrupts in comparison of negative control groups. the vaginal epithelium which treat with SNPs that no or less damage to the tissue of the epithelium. It took seven days for treatment with clotrimazole and 250 ppm concentration of SNPs. treated with 125 ppm of SNPs tooks 10 days.

Results and Discussion:

Clotrimazole is a one of azole group of drug that widely used for treatment of fungal infection, especially candidiasis. Research show the resistant of candida species to these drug and recurrence of infection created by resistant strain[14].

Due to an increase in fungal infection and being resistant to the drugs, finding a new and effective medication is important. The seame research also showed the silver nanoparticle antimicrobial activity.

Shirvastava and colleagues in 2010 examined the effect of antimicrobial activity of SNPs on bacteria that show drugs resistant and show that signification antimicrobial effect of SNPs on bacteria[15].

lara and his colleagues in2007 surveyed as the synthesis of colloidal nanosilver antibacterial, large parts of the bacteria were killed by treatment with SNPs. In addition low concentration of SNPs inhibit the growth of the a major live bacteria in comparison with control leads[16].

Nozari and et al in 2014 were assessed the effect of SNPs alone on candidates and combination with conventional drugs under laboratory condition. Concluded that the synergistic effect of SNPs with common drugs will be more effective on strains of candida albicans and reduce drug resistant[17].

Kim and colleagues in 2007 research on effect of SNPs on species candida albicans and showed that in therapeutic dose didn't being toxic to humans and also can inhibit the growth and proliferation of candida albicans in the laboratory condition[18].

In comparison of healthy vaginal epithelium and negative control, treatment with clotrimazole decrease the Cellular connections in cornify layer in vaginal epithelial that weren't observed in treatment with SNPs and negative control groups and have more stability in the vagina for treatment.

Mucosal layer of vaginal consists of two parts, one of them scaling, this is the protective function and prevents adhesion the variety of pathogenic microorganisms and growth biofilm. Another part attached to the substrate and cover the vagina. During keratinization, cells loss connected with each other and secretion contents in the cell to around of the cell. This secretion has lipid property and coated the cells to protective against penetration invading pathogens.

Given that clotrimazole isolated horny layer from substrate and cell adhesion of the horny layer is much less than normal, is the possibility of invading pathogens, while SNPs didn't damage to the epithelium of the vagina after treatment and kept integrated the cells as like as healthy epithelium vaginal.

According to research and this survey in the future , SNPs can be used as a medicine in the treatment of condidiasis vaginal.

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