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Candida albicans characters, Pathogenesis and effect of ZnONPs in treatment of Candidiasis

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Fungi kingdom is separate kingdom characterized by the chitin in its cell walls, exist everywhere in the environment. Some of them are useful. Fungal diseases frequently come from common fungi found in the environment. In most localized fungal infections cases, the problem usually resolves after bacteria growing back. Localized fungal infections usually affect areas like skin, nails, vagina, mouth or sinuses. Candidiasis infection considered the fourth most common hospitalized acquired bloodstream infections with a predisposing factors like using of catheters, neonatal intensive care, major gut surgery, or liver transplantation. More than 12 Candida species can cause disease. *Candida albicans* that most dimorphic fungi that are human pathogens, show growth by budding into diseased tissues and, stay as filamentous mycelial fungi in the external environment, low temperature or pH promote the development of a budding yeast. Other substances like zinc, biotin, cysteine, and serum transferrin, stimulate yeast dimorphism. Biofilms thick can range from 25 to 450 µm and start to appear within the first 24 hours of colonization. This biofilm is composed of cellulose, Polynucleotides, Polypeptides, and polysaccharides. The biofilm also contains fibrinogen and fibronectin which are bound together by ligands. Vulvovaginal candidiasis is infection in the women vaginal wall. The yeast causes itchiness and a burning-sensation in the vagina tissues, a white cheesy discharge. Application depending on the usage of materials in the nanometer scale known as nanotechnology that permit engineering to control nanomaterial which have physiochemical properties and interacts with biological systems. In addition to its electrical alterations, morphological, magnetic, physical, structural and chemical properties of nanomaterials, which has characteristic comparable nano size with pioneering attractive applications in biological and technologically. Metal oxides with nanostructure have attracted considerable interest in many such as, zinc oxide (ZnO). Finlay resistance too many antifungal is becoming a large problem. New antifungal have to be developed to overcome this issue since only a limited number of antifungal are available. It is important to explore novel antifungal, which many replace the current protocols. Recently, nanoparticle material is using as new antifungal treatment that much different from conventional antimicrobial.

Keywords: yeast infection, Opportunistic, Candidiasis, Zinc Oxide Nanoparticles, *Candida Albicans*

INTRODUCTION

Fungi kingdom is separate from plants, animals, protests, and bacteria kingdoms. The main difference places fungi in a different kingdom is the chitin in its cell walls , unlike the cell walls of other organisms. Fungi exist everywhere in the environment. Some of them are useful, for example they present in foods, such as mushrooms and baker's yeast, and they have important roles as the basis of medication. Others

are not useful, such as mold on food, or spores that cause diseases. The Centers for Disease Control and Prevention (CDC) estimate that there are about 1.5 million known species of fungal organisms, like yeasts, rusts, smuts, mildews, molds, and mushrooms. But only about 300 of those are known to Couse people sick (Hawksworth, 2001; Garcia and Cosadevall, 2010 and Benedict, et a.l, 2019).

Fungal diseases frequently come from common fungi found in the environment. Most fungi are not risky, but some consider harmful to health. In some areas many people suffering from fungal diseases live in resource limited conditions, where diagnosis and treatment of these infections can be difficult. There is a shortage in the laboratory diagnose of fungal diseases, and have limited antifungal medications, which mean that patients who have fungal diseases aren't able to receive the life-saving treatments needed. Fungal Infections that affect only one area are known as localized, while those that affect many areas of the body are known as systemic infections (Sexton, et al, 2007)

Opportunistic fungal infections can affect anyone, including travelers, they pose a serious threat to people who have weakened immune systems, like those who have cancer, HIV, AIDS or other medical problems. These types of Opportunistic fungal infections like aspergillosis, candidiasis, and mucormycosis can spread rapidly to other organs, which sometimes can be fatal (Cogliati, 2013).

People with normal immune system can be affected by primary fungal infections. Some primary fungal infections are more common in certain geographic areas, which can cause serious health problems. Primary fungal infections like paracoccidioidomycosis, coccidioidomycosis, and histoplasmosis tend to develop at a slow rate. Often months or years may pass before a person seeks medical care. This fungal infections do not spread deeply at body organs, for most people with normal immune system (Martins, et al, 2014).

In damp places where mold is present, People have a higher chance of developing respiratory diseases, skin irritation, and other health problems. When normal balances are upset, localized fungal infections affect only one area of the body. Some kinds of antibiotics kill harmful bacteria, which can also kill helpful bacteria and mild overgrowing symptoms occur. In most localized fungal infections cases, the problem usually resolves after bacteria growing back. Localized fungal infections usually affect areas like skin, nails, vagina, mouth or sinuses (Martins, et al., 2014; Erdogan & Roa, 2015 and Mukherjee, et al., 2015).

Candidiasis infection considered the fourth most common hospitalized acquired bloodstream infections with a predisposing factors like using of catheters, neonatal intensive care, major gut surgery, or liver transplantation. More than 12 Candida species can cause disease, but in almost all patient groups and disease manifestations, *Candida albicans* is the most incidence (Pfaller & Diekema, 2007). In this research we will focus in Candida albicans that grow as harmless organism but can become pathogenic, invading the mucosa and causing significant damage.

Candida albicans:

Taxonomy*:* *C. albicans* was discovered by Langenbeck in 1839. Table 1 shows the taxonomy of *C. Albicans (NCBI:txid1356450)*

Table 1 : taxonomy of *Candida albicans*

Superkingdom	**Eukaryote**
Kingdom	Fungi
Subkingdom	Dikarya
Phylum	Ascomycota
Subphylum	Saccharomycotina
Class	Saccharomycetes
Subclass	Saccharomycetidae
Order	Saccharomycetales
Family	Debaryomycetaceae
genus	Candida
Species	albicans

Morphology

There are three different morphologies for human fungal pathogen, *Candida albicans*: yeast, pseudohyphae, and hyphae. More morphological forms occur during colony switching, for example, opaque phase cells are oblong, instead of the oval shape of yeast cells. Hyphae and Pseudohyphae are both elongated and occasionally there has been try to distinguish between them, because both are filamentous forms of the fungus. Switching ability between yeast, pseudohyphal, and hyphae morphologies is often count to be important for virulence, even though formal proof remains lacking. Both pseudohyphae and hyphae are invasive, when they grow in the laboratory, they invade the agar substratum. Various environmental conditions can induce a morphological switching from yeast to filamentous forms. The switching is spontaneous and reversible, though possibly controlled by regulatory gene expression (Odds,1988). In

general, either low temperature or pH promote the development of a budding yeast. Other substances like zinc, biotin, cysteine, and serum transferrin, stimulate yeast dimorphism.

Figure 1: invasive colony

Nevertheless, it is significant to understand that most dimorphic fungi that are human pathogens, show growth by budding into diseased tissues and, stay as filamentous mycelial fungi in the external environment, Thus, filamentous growth is not necessary coupled with tissue invasion, and genetic programs related with growth in vivo have no universal association with a certain growth morphology (Gow, et al, 2002).

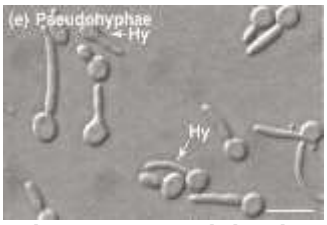


Figure 2: Pseudohypha

Figure 3: Yeast

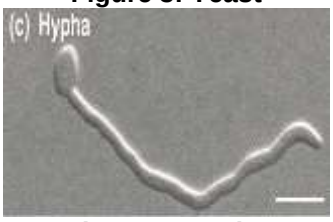


Figure 4: Hypha

Polymorphism:

According figure (1) *C. albicans* is a white and opaque cells, that can grow either as elongated ellipsoid cells with septa (pseudohyphae) in figure (2) or ovoid-shaped budding yeast as in figure (3) (Berman, et al, 2002). Forming chlamydospores that have a spore-like structures with thick-walls (Sudberym, et al, 2004). Pseudohyphae may switching to unclear chlamydospores (Staib, et al, 2007 and Soll, 2009).

Rendering of pH (< 6) *C. albicans* grow mainly as yeast form, while elevation of pH to (>7) induced hyphal growth as in figure (4) (Odds, 1988). Any stress factors such as; presence of N-acetylglucosamine, starvation, CO_2, fluctuations of temperature that enhance hyphal formation (Sudbery, 2001 and Albuquerque & Casadevall, 2012). *C. albicans* make quorum sensing molecules and high cell densities (> 10^7 cells ml^{-1}) such as; dodecanol, tyrosol and farnesol which enhance yeast growth (Hornby, et al, 2001; Chen, et al, 2004 and Hall, et al, 2011). While, low cell densities (< 10^7 cells ml^{-1}) enhance hyphal formation both dimorphism growth are essential for pathogenicity (Jacobsen, et al, 2012). The hyphae is the more invasive than yeast while, the smaller yeast has more effectiveness in dissemination (Saville, et al, 2003).

Yeast physiology

Yeast often lives inside the intestinal environment of the human body and usually causes no problems.

Figure 5: Oral candidiasis

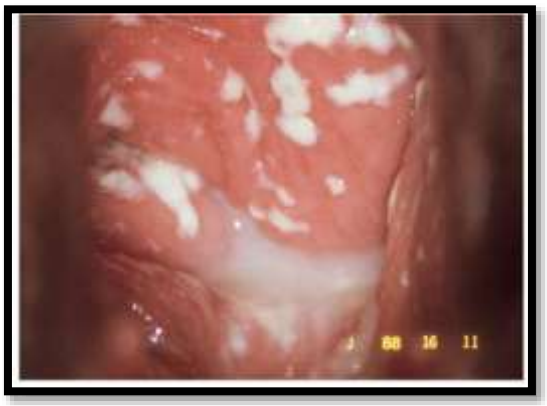


Figure 6: Oropharyngeal candidiasis

The yeast form is 10-12 µm, and is gram-positive but, due to several environmental agents, it converts to an invasive multicellular form known as "hyphae, pseudohyphae" from a unicellular yeast form and starting reproduce very rapidly. As soon as it spreads it construct a biofilm, these biofilms thick can range from 25 to 450 µm and start to appear within the first 24 hours of colonization. This biofilm is composed of cellulose, Polynucleotides, Polypeptides, and polysaccharides. The biofilm also contains fibrinogen and fibronectin which are bound together by ligands (Ghandra, et al, 2001).

The invasive candida grows as a smooth white colony, or as a rod like flat gray colony as in figure (5) & (6). These colonies may be smooth or wrinkled. As a method of adaptation, the same species of candida can switch between the phases. Another strain of candida making shape shifting, which produces seven different types of colonies. This converting ability is reversible and is inherited from one generation to the other. Candida hyphae reproduce sexually because its nucleus has two copies of the chromosomes to make two separate cells (Webb, et al, 1998).

Yeast is known as the unicellular phenotype of *candida albicans* before it becomes multicellular candida and classified into1000 species. It can be either anaerobic or aerobic and with no oxygen they convert sugars into carbon dioxide and ethanol to produce energy. Yeast can reproduce asexually or sexually, and within asexual reproduction the cell split as a new bud to make a new yeast cell without producing spores in the gut (Medical microbiology guide, 2016).

Culture Medium

Laboratory Diagnosis:

Sample depends on disease presentation. Common submitted specimen includes; urine in case of UTI (Christopher, 2015), vaginal discharge in case of vaginal thrush, CSF in case of suspecting meningitis, sputum when pneumonia is suspected or other sample from surface of mucosa (Repentigny, et al, 1985).

Microscopy and Staining:

According figure (7 & 8) the cells of candida yeast can be detected in unstained wet preparations or Gram stained preparations of sample. Candida Gram stained smears appears as gram positive budding cells (blastoconidia) and/or pseudohyphae with regular points of constriction (Donald, 2009)

Candida albicans totally grows on Sabouraud dextrose agar and other most routinely used bacteriological media. Pale creamy colored colonies commonly become visible after 24-48 hours incubation at 25-37°C. The colonies have a special yeast smell and the budding cells can be readily seen by direct microscopy in stained or unstained smears as in figure (9, 10 & 11). In Blood Agar, *Candida albicans* gives white, creamy colored colonies which can be misunderstand with Staphylococcus species. When analyzing the culture report of vaginal swab, extra care must take because the observing colony can be of *Candida albicans* instead of *Staphylococcus aureus* or vice versa (for rapid solution, gram staining must done and observing under microscope) (Nolte, 1982).

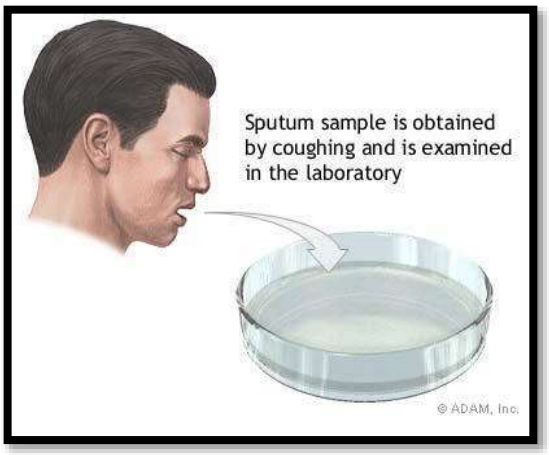


Figure 7: Dish for sputum sample

Figure 8: Urine container

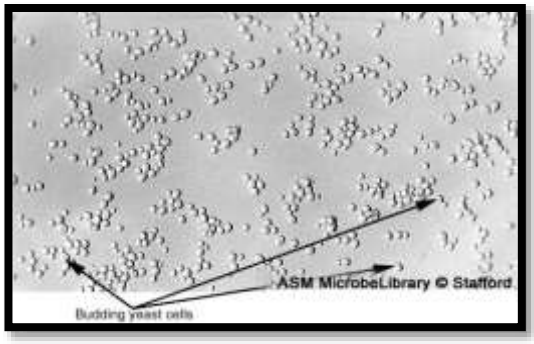


Figure 9: Candida yeasts film without dye

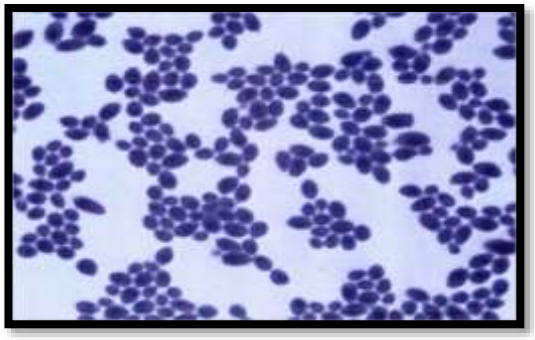


Figure 10: Gram positive yeasts

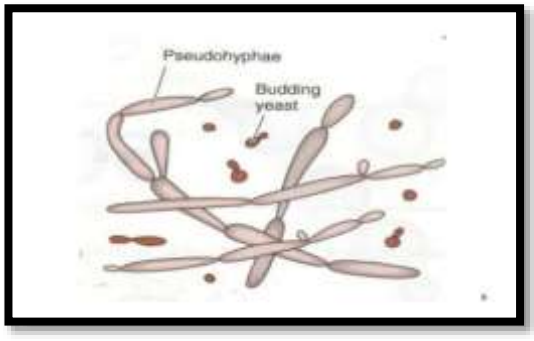
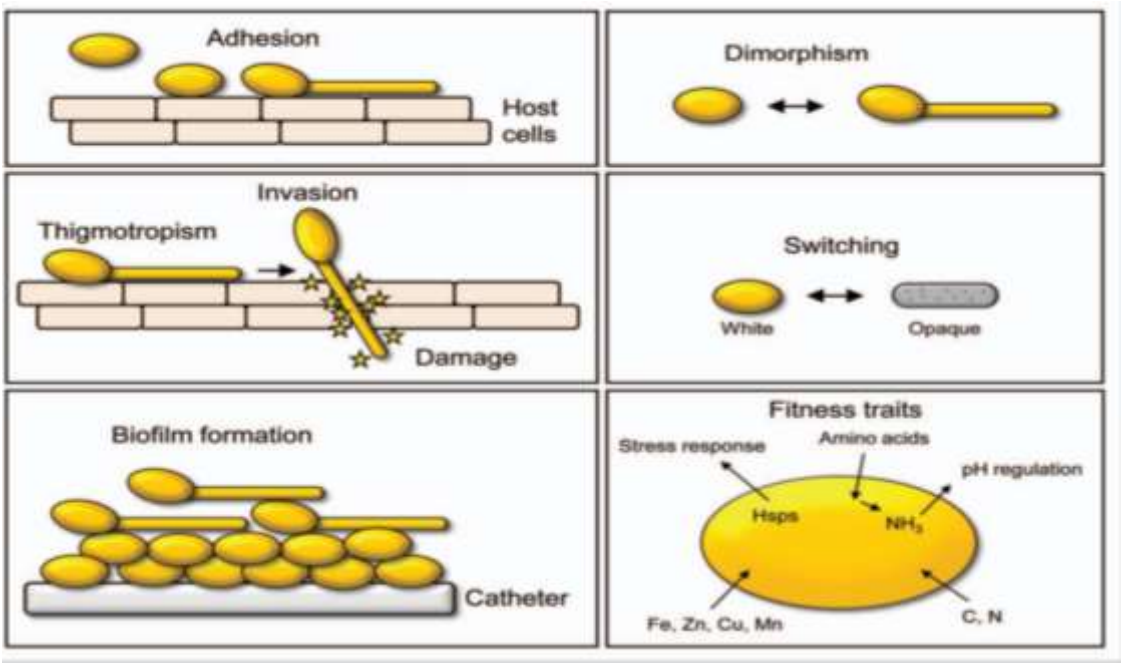


Figure 11: Pseudohyphae

Figure 12: Tube culture



1.5.1. Further tests from culture isolate:

Candida albicans can be recognize by an ordinary germ tube test as in figure (12) (Donald, 2009)

Candida albicans Pathogenesis

C. albicans can infect a verity host species by different virulence with several attributes number which depending on the changes on the morphological structure between yeast and hyphal structure, the expression of invasins and adhesins on the cell surface, thigmotropism, the formation of biofilms, phenotypic switching and the secretion of hydrolytic enzymes are considered virulence factors. On the other hand, *C. albicans* has great ability to adapt with any pH environmental changes, nutrition system, and any metabolic fluctuation, stress (Nicholls, et al, 2011).

Adhesins and invasions:

C. albicans characterized by specific (adhesins) proteins which facilitate adherence of C. albicans to abiotic surfaces of the host cells (Verstrepen, et al, 2006 and Garcia, et al, 2011). Adhesins consists of eight members (Als1–7 and Als9) which are the agglutinin-like sequence (ALS) proteins. The hypha associated adhesion is the most important gene specially the linked cell surface glycoproteins which called "glycosylphosphatidylinositol" (GPI) while, ALS3 gene developed during oral and vaginal infection (Cheng, et al, 2005; Phan, et al, 2007; Zakikhany, et al, 2007; Naglik, et al, 2011; Wachtler, et al, 2011; Zordan & Cormack, 2012 and Murciano, et al, 2012). Hwp1 is a hypha-associated GPI-linked protein that link the hyphae of C. albicans to mammalian host cells (Sundstrom, et al, 2002 and Zordan & Cormack, 2012). While, hwp1Δ/Δ play important role in oral epithelial cells in systemic candidiasis (Sundstrom, et al, 2002 and Nobile, et al, 2008). According to the morphological characters adhesion including; GPI-linked proteins (Ecm33, Iff4 and Eap1), cell-surface associated proteases (Sap10 & Sap9), non-covalent wall-associated proteins (a β-1, 3 glucanosyl transferase, Mp65, Phr1 and a putative β-glucanase) (Naglik, et al, 2011 and Zhu & Filler, 2010). Invasion of *C. albicans* has two complementary mechanisms: induced endocytosis mediated by Als3 and Ssa1 and active penetration mediated molecular mechanisms.

Biofilm formation

Biofilms is one of the most essential virulence factor of *C. albicans* formed on biotic or abiotic surfaces, epithelial cell surfaces (biotic) and dentures (abiotic) (Fanning & Mitchell, 2012). Yeast adherence forming biofilms gradually by yeast cells proliferation of hyphal cells in the upper side which accumulated on the extracellular matrix forming the biofilm complex which become

more antimicrobial resistant agents and host immune codes compared with planktonic cells (Finkel & Mitchell, 2011 and Fanning & Mitchell, 2012). This virulence referred to presence of the complex architecture of biofilms and the biofilm matrix and formation of mature biofilm (Uppuluri, el al, 2010). Hsp90 is the major heat shock protein which responsible for antifungal drug resistance (Robbins, et al, 2011).

Controlling of biofilm formation occur by transcription factors such as; Efg1, Bcr1, and Tec1 (Fanninf & Mitchell, 2012). In addition to these factors there is several regulator factors including; Rob1, Ndt80 and Brg1. Any deletion of these regulators; (TEC1, NDT80, BCR1, ROB1, BRG1 or EFG1) leading to defective in biofilm formation (Nobile, el al, 2012). On the other hand, there is some additional factors produced extracellular such as zinc-responsive transcription; Zap1 which has negative effect on the main component of biofilm matrix which known as "β-1,3 glucan" (Nobile, et al, 2009) while, glucan transferases (Phr1 & Bgl2), exo-glucanase (Xog1) and Glucoamylases (Gca2 & Gca1) have positively effect on the production of β-1,3 glucan (Nobile, et al, 2009 and Taff, et al, 2012). GCA1 & GCA2 usually controlled negatively by; Bgl2, Xog1, Zap1 and Phr1. When the biofilms formed from; XOG1, PHR1 or BGL2 usually be very susceptible to; fluconazole and other antifungal agent in vitro and in vivo (Taff, et al, 2012). Recently studies found that C. albicans biofilms has resistant to neutrophils and not reacted to oxygen species (ROS). C. albicans protected in the extracellular matrix by β-glucans (Xie, et al, 2012).

pH-sensing and regulation.

Human candidiasis strongly affected by host niche and any pH fluctuation from slightly acidic to alkaline such as; the slight alkalinity of human tissues and blood (pH 7.4) or acidic pH in the human digestive system which reach to (pH 2) while, the vaginal pH ranged from 4.78 to neutral media. Therefore, alkaline pH leading to severe stress to C. albicans causing pH-sensitive proteins malfunctioning and badly effect on nutrient acquisition (Davis, 2009). C. albicans is able to adapt to pH of the environment, alkaline environment and nutrient starvation inducing hypha formation (Vylkova, et al, 2011 and Mayer, et al, 2012). The molecular mechanisms beginning after uptake of amino acids and other amine containing molecules, including; polyamines, then C. albicans cleaves intracellularly by urea amidolyase Dur1,2 resulting ammonia Ato (ammonia transport outward) export proteins which causing alkalinization of the extracellular milieu, that induces the hyphal morphogenesis (Vylkova, et al, 2011). Therefore, *C. albicans* actively modulates extracellular that contribute its remarkable capacity to co-exist as a commensal, and to declare as a humans fungal pathogen.

Candidiasis Pathogenesis

Transmission:

Candida albicans is commonly pass from mother to her infant during childbirth, and exist as part of a normal human's microflora. The overgrowth of *candida albicans* cause symptoms of disease, like when there are imbalances for example, changes in the normal vaginal PH. People to people acquired infections generally occurs in hospital environment where immunocompromised patients get the infection from healthcare workers, studies display about a 40% incident rate (Fanelloa, et al, 2001).

Clinical features:

Candida albicans consists from three main kind of infections; Oropharyngeal candidiasis, Vulvovaginal (genital) candidiasis, Invasive candidiasis (candidemia).

Symptoms of oropharyngeal candidiasis:

Oropharyngeal candidiasis happen in the mouth and throat area, characterized by the formation of white patches on top of tongue and mouth, which called "thrush". Thrush can be removed, but the underlying tissue will be irritable and appear redness. This infected area will cause pain and eating difficulty (Medicine Health).

Symptoms of Vulvovaginal (genital) candidiasis:

Vulvovaginal candidiasis is infection in the women vaginal wall. The yeast causes itchiness and a burning-sensation in the vagina tissues. Furthermore, a white discharge" similar to white cheese" is typically present. Genital candidiasis infection is much more common in women, but men can also get it. Even though it is not considered one of the six transmitted disease, men are commonly infected after sex with a woman having that infection. Men symptoms involved rash, irritation on the head and surrounding tissue of penis (Goncalves, et al, 2016 and Benedict, et al, 2019).

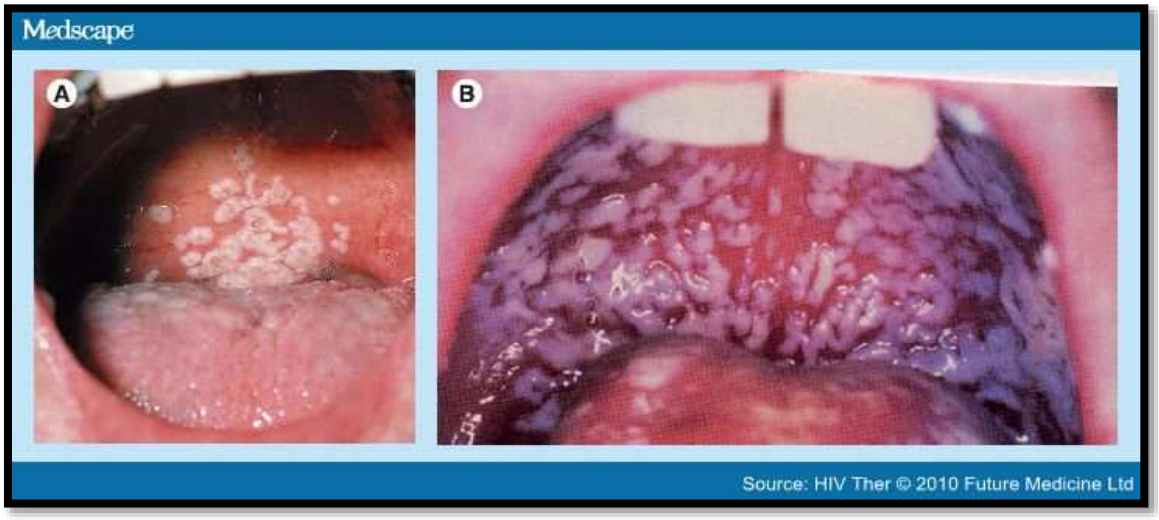


Figure 13: oropharyngeal candidiasis

Figure 14

Figure 15: Penile candidiasis

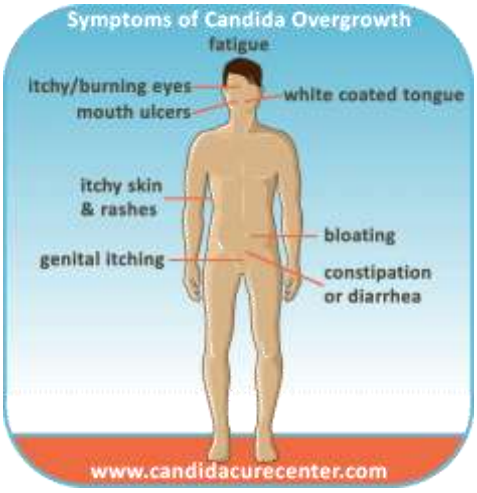


Figure 16: manifestations of candideamia

Figure 17: manifestations of microbial blood infection

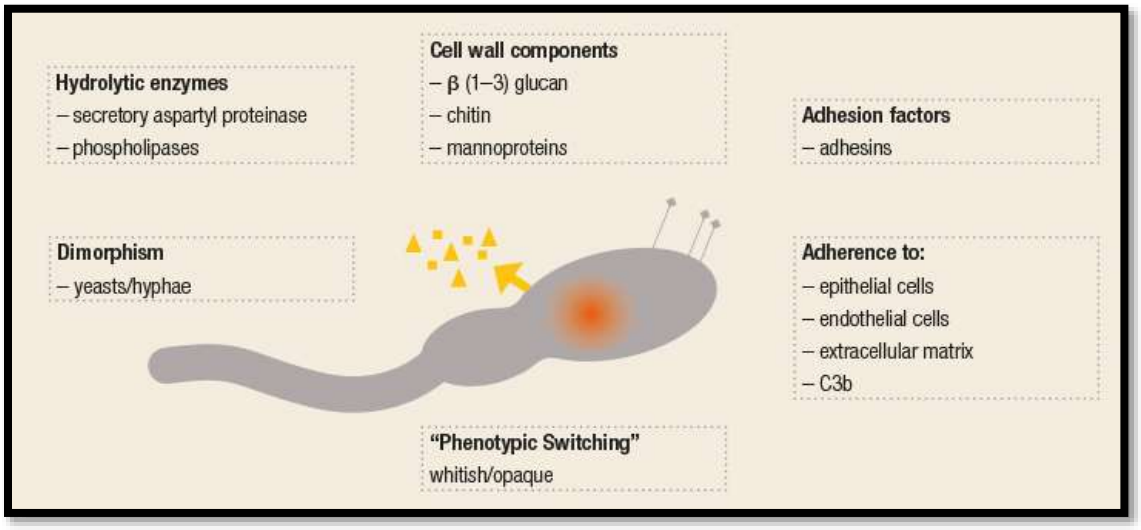


Figure 18: virulence factors of *candida albicans*

Invasive candidiasis (candidemia) Symptoms:

Invasive candidiasis is the infection of the bloodstream by *candida albicans*. This leads to candida invasion into organs over the body, like kidney, liver, brain, and other organs. Patients start suffering fevers, chills, fatigue, abdominal pains, and muscles ache. Generally, patients with weak immune systems are only endanger, however healthy people are liable to oral/genital candidiasis. Weak immune systems can be result from chemotherapy, transplantation, broad-spectrum antibiotics, and other causes (American Thoracic Society).

Mortality:

Figures (13 -18) declared that most patients with oral and genital candidiasis are recover after a treatment with antifungal drugs. Furthermore, candidemia infection is much more life-threatening. Recent studies showed that the mortality rate for candidemia patients' reached about 34%. In addition, this figure almost doubled when treatment is delayed for more than 48 hours, with a mortality rate 78% (Nguyen, et al, 1995).

virulence factors that make *candida albicans* harmful :

Candida albicans have fibronectin receptor which facilitates its adherence to the (fibronectin) at epithelium of the gastrointestinal or urinary tract as the following; Hydrophobic molecules that present on the surface of Candida also support the adhesion, Aspartyl proteases at *candida albicans* has shown more ability to make disease in animal models and Phenotypic switching in addition to presence of phospholipase play a role in pathogenesis of *candida albicans* (Frank, 1994).

Treatment

Candida albicans is generally treated with antifungal drugs. The antifungal drugs commonly used to treat candidiasis are topical nystatin, fluconazole, topical ketoconazole, and topical clotrimazole. For example, only one dose of fluconazole has been reported effective in treating 90% of vaginal yeast infection but, other types of yeast infections may require different treatments. For local treatment the vaginal suppositories or medicated douches is effective. Gentian violet can be used for breastfeeding thrush, but it can cause mouth and throat ulcerations in nursing babies, if used in large quantities as in figure (19 & 20) (CDC Genital Candidiasis, 2013).

In severe hospitalized patients infections, amphotericin B, caspofungin, or voriconazole may be used. *Candida albicans* can develop resistance to antifungal drugs, such as fluconazole. Consequently, the recurring infections may be treatable with other anti-fungal drugs, but resistance to these alternative agents may also appear. To overcome this resistance, it is vital to seek for novel antifungal, which may substitute the present control strategies. Recently, there is a growing interest in nanoparticle material, which different from the conventional therapy (Stoimenov, et al, 2005).

Nano-agents including nanoparticles of metal oxide which have unique characters including the high surface to volume ratio which have antimicrobial characters. Zinc oxide nanoparticles is one of the metal oxides nanoparticles which have a useful antibacterial and antifungal agents when used topically on affected surface. Zinc oxide Nano rod arrays diminished the growth of *Candida albicans* with stable action for two months (European Journal of Medicine, 2015).

Nanotechnology:

Application depending on the usage of materials in the nanometer scale known as nanotechnology that permit engineering to control nanomaterial which have physiochemical properties and interacts with biological systems. In addition to its electrical alterations, morphological, magnetic, physical, structural and chemical properties of nanomaterials, which has characteristic comparable nano size with pioneering attractive applications in biological and technological applications (Jain, et al, 2005 and McNeil, 2005).

Recently, the attention of nanoparticle (NP) materials increase due to their characteristic chemical and physical properties which differ significantly from their conventional counterparts (Stoimenov, et al, 2002) Recent studies have demonstrated antimicrobial activities of various NP materials, including silver (Kim, et al, 2008), copper (Cioff, et al, 2005), titanium dioxide (Kwak, et al, 2001) , and zinc oxide (Lia, et al, 2009).

Metal oxides with nanostructure have attracted considerable interest in many areas of technology (Sangeetha, et al, 2011) Among metal oxide nanoparticles, zinc oxide (ZnO) has received much attention in the recent past. ZnO nanostructures are the forefront of research due to their unique properties and wide applications (Rouhi, et al, 2013).

ZnO nanoparticles:

Zinc oxide is one of the nanomaterials which have considerable arising attention by their industrial and electronic potential applications (Baxter & Aydil, 2005). From a biological application, ZnO nanoparticles (NPs) being used in the sunscreen and cosmetic industry due to their ability to reflect, transparency, absorption of UV radiation, in addition to its uses as food additives (Nohynek, et al, 2007 and Nohynek, et al, 2008) ZnO NPs considered to use in next-generation biological applications including antimicrobial agents, bio imaging probes, drug delivery, and cancer treatment (Peer, et al, 2007 and Nair, et al, 2008).

There are different methods used for the synthesis of zinc oxide nanoparticles: direct precipitation, homogeneous precipitation, salvo thermal method, son chemical method, reverse micelles, sol gel method, hydrothermal, thermal decomposition, and microwave irradiation as in figures (21 & 22) (Kolekar, et al, 2013)

Figure 19: Vaginal douche

Figure 20: topical antifungal

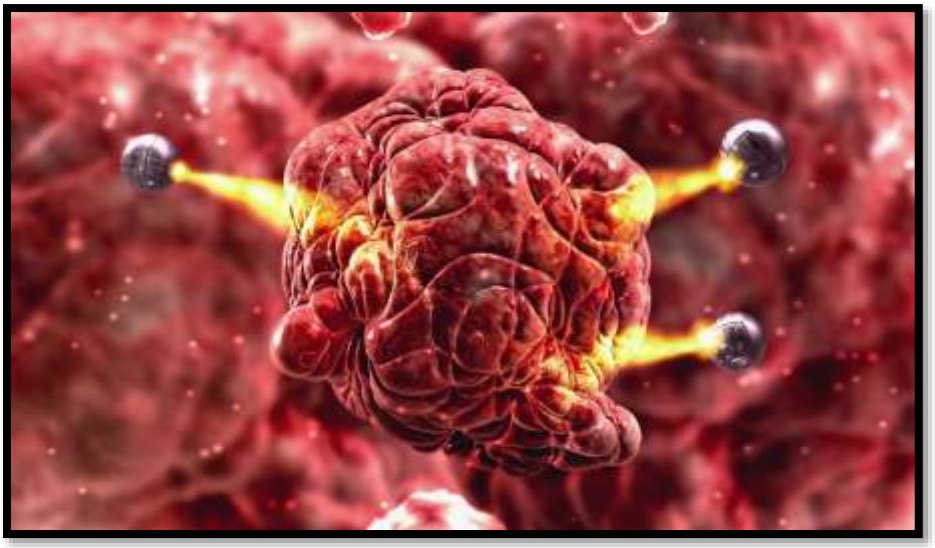


Figure 21: demonstration of nanotechnology

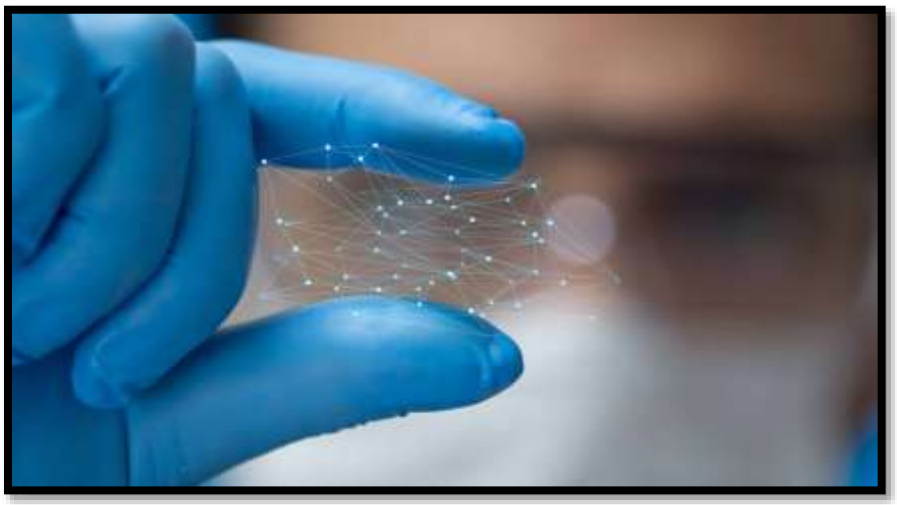


Figure 22: demonstration of nanotechnology

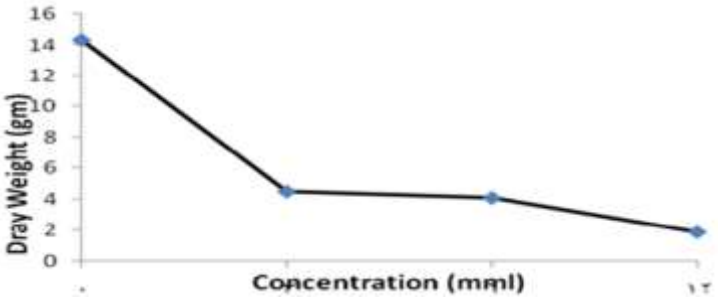


Figure (23): effect of ZnONP on dry weight of *Candida albicans* each point represents an average of triplicate measurements.

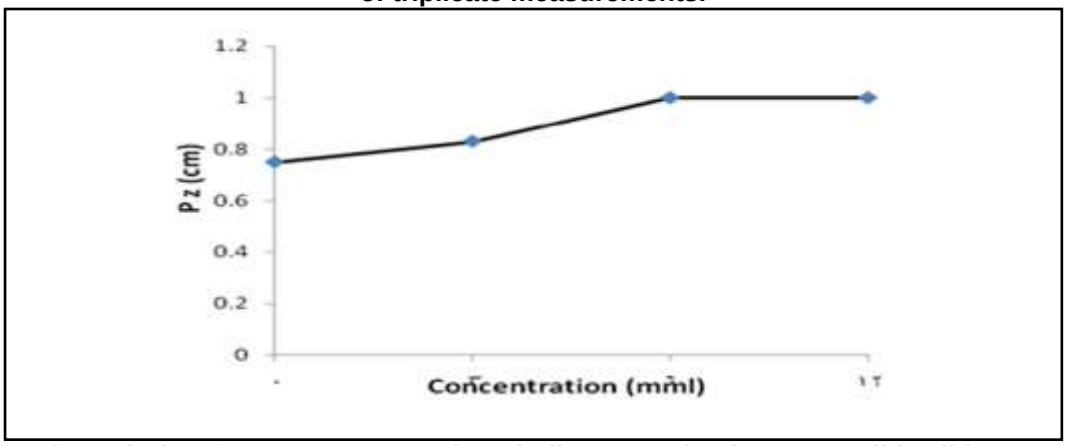


Figure (24): effect of ZnONP on phospholipase production of *Candida albicans*

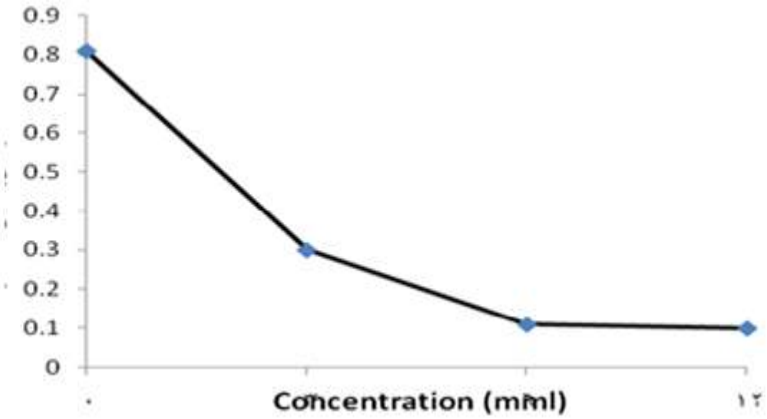


Figure (25): effect of ZnONP on lipase production of *Candida albicans*

The biological method of the synthesis of ZnO nanoparticles is gaining importance due to its simplicity, eco-friendliness and extensive antimicrobial activity (Gunalan, *et al,* 2012). The use of eco-friendly biosynthesized nanoparticles as an alternative to the chemically synthesized ones would help control chemical toxicity in the environment (Mahanty, *et al,* 2013).

The synthesis of ZnO nanoparticles using plants has been carried out using milky latex of Calotropis procera, Aloe vera extract (Salam, *et al,* 2014), Ocimumbasilicum L. var. Purpurascens, Parthenium hysterophorus L (Rajiv, *et al,* 2013), Citrus aurantifolia extract (Samat, *et al,* 2013), Plectranthus amboinicus (Sangeetha, *et al,* 2011).And used Nephelium lappaceum L. (rambutan) peel extract in the biosynthesis of zinc oxide nanocrystals (Yuvakkkumar, *et al,* 2015). The advantage of using ZnO nanoparticles is that they strongly inhibit the action of pathogenic microbes when used in small concentration (Applerot, *et al,* 2012). it is known that ZnO demonstrates significant growth inhibition of a

broad spectrum of bacteria. For example: Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhi, and Staphylococcus aureus (Lakshmi, *et al*, 2012).

The green synthesized ZnO has a stronger inhibitory effect than chemically synthesized nanoparticles in a study used the following bacterial strains: S. aureus,Serratia marcescens, Proteus mirabilis, Citrobacter freundii, and fungal strains: Aspergillus flavus, Aspergillus nidulans, Trichoderma harzianum, and Rhizopus stolonifera (Gunalan, *et al,* 2012).

Effect ZnO nanoparticles in *Candida Albicans*

Recently (Jasim, 2015) studies of Clinical Laboratory which investigated the antifungal activity of ZnONPs on opportunistic fungi (A. fumigatus, C. albicans) ZnONPs with size ≤ 50 nm and concentrations of (0, 3, 6 and 12mmll^{-1}) were used. Radial growth and dry weight, were used to estimate the inhibitory effects. Results showed nano-ZnO exerted activity on the radial growth and dry weight in addition of production of two enzymes in yeast. Thus, this study indicates ZnONPs have considerable antifungal activity.

The result of Dry weight of yeast Shown in the following figure that the addition of ZnONPs to the media that used for growth of the yeast, had a significant influence on the growth of it and increased this effect with increased of concentrations that used Also, the results showed that the yeast was more sensitive to ZnONPs than the fungus compared with control treatment (Jasim, 2015). The result of Phospholipase and lipase production by yeast are denoted in on inhibition, but effect on some enzymes produced which considered as one of virulence factors of this yeast. Where notes that the value of coefficient arise with increasing of concentrations. This means that decline in the production of phospholipase enzyme because the relationship between this coefficient and production of this enzyme is an inverse relationship. In other hand, is showmen that a sharp decline in the production of lipase enzyme, based on measuring of the diameter of colony of yeast with diameter of region that appear around of the yeast colony as in figures (23 – 25) (Jasim, 2015).

The present results demonstrate that the ZnO nanoparticles have antifungal effects on fungal and yeast growth. The antifungal activity reveals that the growth of *Candida albicans* were inhibited at concentrations of 3to12mmll^{-1}ZnONPs. Add to that, the inhibitory effect was not limited to the growth inhibition, but it was the influence of nanoparticles on some of virulence factors of the yeast. Phospholipase is one of Candida species virulence factors which has a significant role in the pathogenesis of infections and invasion mucosal epithelia. In addition, several studies have shown that clinical isolates of Candia have higher levels of extracellular phospholipase activity (Jasim, 2015).

CONCLUSION

Candida albicans is a prevalent fungal pathogen that infecting humans, while it has developed a virulence mechanisms that permit successful colonization and infection of the host under convenient predisposing conditions. It can expand as yeast cells form, pseudohyphae, and hyphae, this dimorphism is required for full virulence. *Candida albicans* has been of major concern to the scientific community for its pathogenic nature, especially for increasing immunocompromised patients worldwide.

There are numerous methods that can be adopted by hospitals or independent diagnostic laboratories to help in the identification of this fungi. Well identifying of Candida species is important in the treatment and management of the disease.

There are three major forms of *candida albicans* diseases: oropharyngeal candidiasis, vulvovaginal candidiasis, and invasive candidiasis with different drug of choice for each one. Notably, resistance too many antifungal is becoming a large problem. New antifungal have to be developed to overcome this issue since only a limited number of antifungal are available. It is important to explore novel antifungal, which many replace the current protocols. Recently, nanoparticle material is using as new antifungal treatment that much different from conventional antimicrobial.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

SMF: collection of the data, photography, drafted the manuscript. HAA: revised the manuscript. FA: revised the manuscript. NTE: Corresponding author of the manuscript, study designed, wrote the manuscript, drafted, revised the manuscript, and data analysis. All authors have read and

approved the final manuscript.

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