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Some Studies on the Effect of Zinc Oxide Nanoparticles against *Candida albicans*

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Candida albicans is one of the most popular opportunistic fungi, it is the fourth cause of the nosocomial infections affect different parts of body; vagina. Nanotechnology provides the possibility in designing modern drugs with such greater specificity of cell, although the metal oxides nanoparticles such as; zinc oxide nanoparticle (ZnO NPs) has safe and useful antimicrobial agents. The research aimed to; investigate the effect of different concentrations of ZnO NPs on; (the morphology of *Candida albicanes*, ultrastructure of *Candida albicans*, and molecular monitoring of the different effects of ZnO NPs on different *Candida albicans* metabolites and enzymes. Comparing with an antifungal drug). *Candida albicans* Isolate (No.: 10234) obtained, cultured on media Saboroud Dextrose Agar medium. ZnO NPs suspensions with NP size of 70 ± 15 nm were obtained and by aliquot (10 ml) of ZnO NPs suspension was diluted to make (1%, 3%, 5% & 7%) concentrations. Antifungal tests were performed on plates, then scanned by scanning electron microscopy, then performed protein scanning of the fungus and studied the enzymes activity. Results cleared the ZnO NPs as very effective on *Candida albicans* than antifungal even at its lowest concentration was 1%. The most powerful effect were detected in case of 7% ZnO NPs and the inhibition effect were relatively correlation with concentration of ZnO NPs by make deformity in cell structures, reduction of protein content and adversely effect on *Candida albicans* enzymes. The study recommended to perform further research to apply ZnO NPs in treatment of *Candida albicans* infection.

Keywords: Biological effects, Biochemical effect, SEM, Candidial Enzymes, Protein analysis, Antifungal agents

INTRODUCTION

Candida albicans is one of the pathogenic fungus to the human which is capable to infect both skin as well as membranes of mucous, sometimes reasoning such severe infections of systemic in the immunocompromised hosts. It is highly hard to control the process of growing the fungi since it has improved the resistance to various antifungal agents (Kangogo et al. 2009).

Candida infections may be seen in such different parts of body, such as the oral cavity, gastrointestinal tract, skin, and vagina. In such oral cavity, the infection of candida is usually visible like thrush, as well as white/yellowish cream as the patches which are on oral mucosa as well as tongue. These organisms' incidence emerges in order to increase age. That fungi may cause the characteristic infections which might be extremely serious as well as life-threatening (Calderone, 2012).

Candida albicans is considered as one of the most popular pathogens of the opportunistic fungal of the humans as well as it is the fourth cause of the nosocomial infections (Richards et al. 2008).

It is highly known that the nanotechnology is

the study of the very small structures that are having such size of 0.1 to 100 nm which serves as bridge between both the bulk materials and the atoms/molecules. The nano medicine is considered as a new, relatively, area of the science as well as technology (Nikalje, 2015).

Nanotechnology provides the possibility in designing modern drugs with such greater specificity of cell, in addition to the systems of drug-release which can act on such specific objectives selectively. Therefore, nanotechnology does allow the process of administrating of the smaller doses, which are more effective, and the process of minimizing the adverse effects (Patlosky & Zheng, 2006). Nanotechnology can be used in optimizing the formulation of drugs, increasing the solubility of drugs and changing the pharmacokinetics that are needed in sustaining the drugs' release, thereby the prolonging of the bioavailability (Chekman, 2008).

Although the metal oxides nanoparticles such as; zinc oxide nanoparticle (ZnO NPs) has gotten the maximum interest it is inexpensive to produce, and it is safe as well as it can be prepared in an easy way (Jayaseelan et al. 2012). ZnO NPs, is known as an inorganic white powder which cannot be insoluble in the water (Takahashi et al. 2007). ZnO NPs has been illustrated in various studies in order to be useful antibacterial agents and antifungal agents while it is used as a superficial coating on the materials as well as the textiles (Abramov et al. 2009 and Anbuvannan et al. 2015).

US FDA has enlisted zinc oxide (ZnO) as GRAS, as it is generally known as safe, as well as metal oxide (Pulit-prociak et al. 2016). ZnO NPs has many applications of biomedicine, such as drug's delivery, anticancer, antidiabetic, antifungal, antibacterial and agricultural properties. Even though the zinc oxide (ZnO) is highly used in targeting the drug's delivery, it has such limitations of the cytotoxicity that needs to be resolved (Ma et al. 2012).

The antimycotic effects of the zinc oxide nanoparticles which are having the average size of ~30±10 nm and ~50 ±10 nm is respectively examined on many pathogenic fungi (Wani & Saha, 2012). The powder of the zinc oxide (ZnO) is considered as an active ingredient in the applications of dermatology in the lotions, creams and ointments because of the antibacterial properties of zinc oxide (ZnO) (Magaldi, et. al., 2004).

According to Applerot *et al* (2009), the antibacterial activity of zinc oxide (ZnO) as well as the ability in inducing the reactive oxygen species (ROS) production arises with its particle size which is decreased. Consequently, the NPs of ZnO show such a greater antifungal effect.

The research aimed to; investigating the effect of different concentrations of ZnO NPs on the morphology of *Candida albicans*, investigating the effect of ZnO NPs by different concentrations on macroscopic morphology and ultrastructure of *Candida albicans*, and molecular monitoring of the different effects of ZnO NPs on different *Candida albicans* metabolites and enzymes. Comparing the different effects of ZnO NPs on *Candida albicans* with an antifungal drug.

MATERIALS AND METHODS

Candida albicans Isolate

(No.: 10234) which obtained from the central research laboratories in King Abdelaziz hospital, Jeddah, KSA. further tenfold dilutions were made up to 10^3 and 0.1 milliliter of each dilution was plated in triplicate over plates containing on different culture media Saboroud Dextrose Agar medium (Dextrose (Glucose)- 40 gm, Peptone- 10 gm, Agar- 15 gm, Distilled Water-1000 ml). An average three to five days of incubation at 37 °C (98.6 °F) examined daily for 5 days.

2.2. Determination of the antifungal activity of different concentration of ZnO NPs against *Candidia albicans*

2.2.1. Nanoparticle materials*:*

ZnO NPs suspensions with NP size of 70 ± 15 nm were obtained from previous study which was done by EL-Ghwas et al. (2019) in the use of cell-free culture filtrate for extracellular synthesis of zinc oxide nanoparticles (ZnO NPs). An aliquot (10 ml) of ZnO NPs suspension was diluted to make (1%, 3%, 5% & 7%) concentration. The composition of the NP-free solution was analyzed to be water and a dispersant, and its effect on *Candida albicans* growth was examined. The original ZnO NPs suspension (12 mol l^{-1}) and NP-free solution were then diluted with Sabaroud dextrose agar (SDA) **(**He et al. 2011).

2.2.2. Antifungal test:

Antifungal tests were performed by the agar dilution method with some modification. The autoclaved SDA media with ZnO NPs at concentrations of; 1%, 3%, 5% and 7% and a NP-free solution were poured into the Petri dishes

(9 cm diameter). The fungi were inoculated after the SDA media solidified, insertion of different ZnO NPs disc (1.4 cm) concentrations then inoculation of *Candida albicans.* The Petri dish with the inoculums incubated at 37 °C. The efficacy of ZnO NPs treatment was evaluated 24 hours by measuring the diameter the growth from the edge of each Petri dish. All tests were performed in triplicate and the values were expressed in centimeters (Fraternale et al. 2003).

2.3. Antifungal Activity

2.3.1. Electron microscope: The sample were fixated by gluteraldhyed 2.5% and dehydrated by serial dilution of ethanol with agitation using automatic tissue processor (Leica EM TP, Leica micrososystems: Austria). Then the sample drying using CO, critical point drier (Model: Audosamdri-815, Tousimis; Rockville, Maryland, USA). The sample coated by gold sputter coater (SPI-Module, USA).The sample was observed by scanning electron microscopy (model: JSM-5500 LV; JEOL Ltd –Japan**).**

2.3.2. Protein Extraction:

0.5 ml of 10% TCA (trichloro-acetic acid). Mix, vortex with the lyophilized powder and incubate 5 min at room temperature. Wash 3 times in 90% acetone, 20 mM HCL. Air drying. Grinding using 0.2 ml of SDS-PAGE sample buffer (1% SDS, 10% glycerol, 25 mM Tris-HCI pH 6.8, 1 mM EDTA, 0.7 M mercapto-ethanol). Boil for 2 min. Vortex for 1 min. Boil for another minute. Mixing with urea sample buffer (1%% SDS, 9 M urea, 25 mM Tris-HCl pH 6.8, 1 mM EDTA,0.7 M mercapto-ethanal). Boil for 2 min. Vortex for 1 min. Boil for another minute.

2.3.3. Enzyme activity: **Organism and Cultural Conditions:**

Stock cultures of *Candida albicans* was maintained on Sabouraud Dextrose Agar (SDA) at 37°C. Inoculum was prepared by adding sterile 0.9 % saline aseptically to the cultures grown on SDA plates for 48 h. The cell suspension was adjusted to approximately to 107 mL-1 and 1 mL of inoculum was added in each flask containing 50 mL of Sabouraud Dextrose broth media containing different concentration of Zn oxide nanoparticles) 0, 0.1, 0.3, 0.4, 0.5, 0.6, 0.7. (The cultures were incubated for 48 h at 25°C on a rotary shaker (100 rpm (Gunalan et al. 2012).

2.3.3.1. Preparation of Intracellular Crude Extract:

Cells were harvested from the media by centrifugation (7,000 rpm for 15 min) at 4°C, washed twice with 0.1 mM Tris-HCl buffer (pH 7.0) and centrifuged at 13,000 rpm for 30 min. Harvested cells were weighed to determine cell growth. The cells were ground with acid wash sand in pre-chilled mortar using 1.5 mL of Tris-HCl buffer for each gram of cell. The cell free homogenate was centrifuged at 13,000 rpm for 15 min at 4°C. The supernatant was used as the source of intracellular enzyme (Binn et al. 2009).

2.3.3.2. Lipase Activity:

1 .p-nitrophenol Curve was prepared in the following manner. Based on the molecular weight of p-nitrophenol of 139.11 Nouri, (2002) p-nitrophenol solution of 0.01 M was prepared by dissolving 14 mg powder of p-nitrophenol into 10 mL of distilled water. This p-nitrophenol solution is a stock solution for the preparation of the solution with smaller concentration. Concentrations used in this study were 300µM, 500 µM ,700 µM ,900 µM ,1100 µM ,1300 µM. Lipase activity in the culture was measured with p-nitrophenyl palmitate (p-NPP) as a substrate. First made Asolution) 15 mg pnpp dissolved into 5 mL of isopropanol (was added to 45 ml of B solution (0.05 g Gum Arabic and 0.2 mL of Triton X-100 are dissolved into 50 Mm Tris-HCl buffer pH 8.0), all materials homogenized until a final volume of 50 ml (Jalal et al. 2018). Lipase activity was measured by mixing 1.8 ml of substrate solution with 0.2 mL crude enzyme and incubated at room temperature for 15 minutes. The absorbance was measured using spectrophotometer with λ 410 nm .Activities per unit (U) on lipase activity was shown in 1 mol p-nitrophenol per minute in the treatment condition (Vitisan et al. 2013)

RESULTS

Evaluation of Effect of ZnO NPs on *Candida albicans*

Determination of addition of ZnO NPs on *Candida albicans* growth in different concentration was examined on SDA and declared on table (1) and figure (1) as following; in case of 1% the effect of ZnO NPs were; 1, 1.5, 1.22 ± 0.039 cm as; minimum, maximum, mean ±SE respectively. While, in case 3% ZnO NPs inhibition effect were; 1.8, 2.3, 2.04 ± 0.034 cm as; minimum, maximum,

mean ±SE respectively. Although 5% ZnO NPs were; 2, 2.4, 2.19 ± 0.027 cm as; minimum, maximum, mean ±SE respectively.

The highest inhibition effect were detected in 7% ZnO NPs as following; 2.3, 3, 2.61 ± 0.054 cm as; minimum, maximum, mean ±SE respectively. In case antifungal agent the inhibition zone measured about; 0.1, 1.4, 0.96 ± 0.088 cm as; minimum, maximum, mean ±SE respectively. While all control sample were zero values. All reported results at different concentrations of ZnO NPs, have significant inhibition effect on the growth of *Candida albicans.*

Table 1 : The Inhibition Effect of ZnO Nanoparticles in Different Concentrations on *Candida albicans*

	Minimum	Maximum	Means	±SE
1%	1	1.5	1.22[a]	0.039
3%	1.8	2.3	2.04[b]	0.034
5%	2	2.4	2.19[c]	0.027
7%	2.3	3	2.61[d]	0.054
Antifungal	0.1	1.4	0.96[e]	0.088
Control	0	0	0	0

Means followed by a different letter in the line are significantly different (*P<0.0001*)

Figure 1: The Effect of ZnO Nanoparticles Different Concentrations

The figure showed the inhibition zoon of the *Candida albicans* before exposure to ZnO NPs was 0 cm However, when exposure to *Candida albicans* for ZnO NPs 1%, the inhibition zoon after 24 hours increased to 1.22 cm, and so the inhibition zoon increased by increasing the concentration of ZnO NPs until it reached to 2.61 cm when *Candida albicans* are exposed to ZnO NPs 7%.

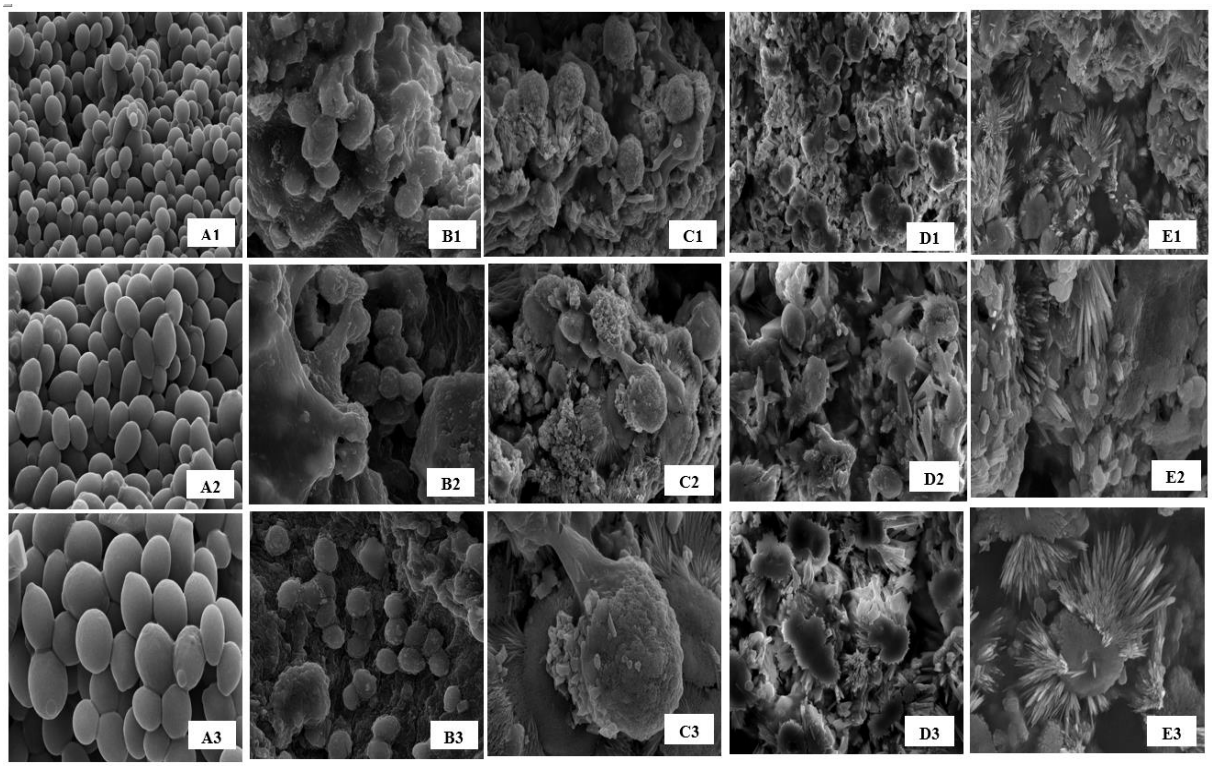


Figure 2: The effect of ZnO NPs on *Candida albicans*

A1, 2, 3: Represent *Candida albicans* cells without addition of ZnO NPs at focus X2500, X8000, X1500 respectively.
B1, 2, 3: Represent *Candida albicans* cells after addition of 1% ZnO NPs at focus X2500, X8000, X1500 respectively.
C1, 2, 3: Represent *Candida albicans* cells after addition of 3% ZnO NPs at focus X2500, X8000, X1500 respectively.
D1, 2, 3: Represent *Candida albicans* cells after addition of 5% ZnO NPs at focus X2500, X8000, X1500 respectively.
E1, 2, 3: Represent *Candida albicans* cells after addition of 7% ZnO NPs at focus X2500, X8000, X1500 respectively.

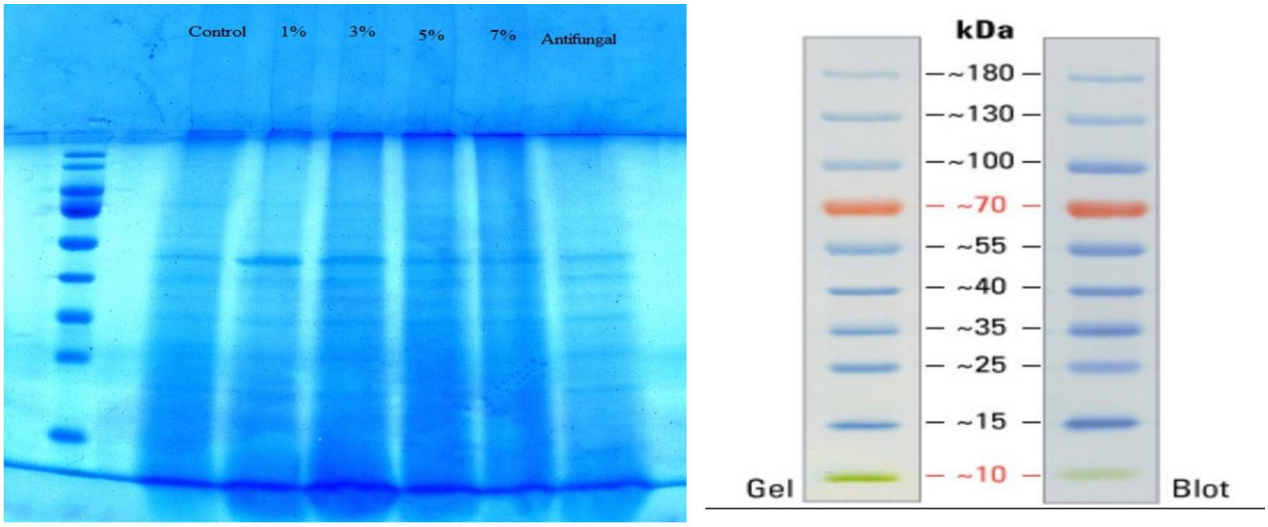
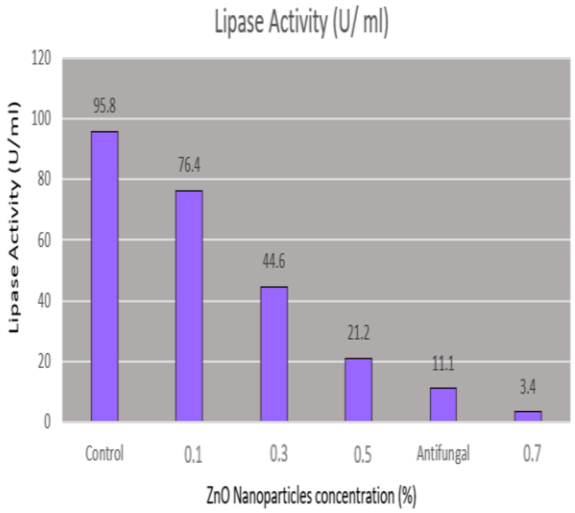


Figure 3: Protein profiles of *Candida albicans* isolates after exposing it to different ZnO NPs concentrations (1%, 3%, 5%, 7%) and antifungals

The Figure showed that the destructive effect of ZnO NPs against *Candida albicans* proteins increases with increasing ZnO NPs concentration. Whereas, the antifungal showed the greatest destructive power against the *Candida albicans* proteins. As the protein bundle appears more clearly at a concentration of 1%, then the bundle begins to fade with increasing concentration, until it appears less clear at a concentration of 7%. As for exposing *Candida albicans* to the anti-fungal, the bundle appeared less clear compared to ZnO NPs concentrations



ZnO Nanoparticles Conc.	Lipase Activity (U/ml)
0 (control)	95.8
0.1	76.4
0.3	44.6
0.5	21.2
Antifungal	11.1
0.7	3.4

Figure 4: The Effect of ZnO NPs Concentrations on lipase activity of *Candida albicans*
The figure showed that the activity of the lipase enzyme of Candida albicans before exposure to ZnO NPs was 95.8 U / ml However, when exposure to Candida albicans for ZnO NPs 1%, the enzymatic activity decreased to 76.4 U / ml, and so the enzyme activity decreased by increasing the concentration of ZnO NPs until it reached to 3.4 U / ml when Candida albicans are exposed to ZnO NPs 7%.

From the above-mentioned result cleared that the ZnO NPs were very effective on *Candida albicans* than antifungal even at it's lowest concentration was 1%. The most powerful effect was detected in case of 7% ZnO NPs and the inhibition effect were relatively correlation with concentration of ZnO NPs.

Figure (1) declared the inhibition effect of ZnO NPs in different concentration on *Candida albicans* were consistent with the results of many previous studies that recorded the high significant ability of ZnO NPs to influence the growth of *Candida albicans*, as well as the concentration-dependent effect.

3.2. The effect of Zinc Oxide Nanoparticles on the morphology of normal *Candida albicans*.

Figure (2) viewed the changed which occur by addition of different concentrations of ZnO NPs compared with the normal shape of *Candida albicans* by the Scanning Electron Microscope (SEM) pictures at 3 different focuses; X2500, X8000 & X1500. Figures (A1, A2, A3) showed that the *Candida albicans* appeared as; ovoid "yeast" cells with budding, the colony of *Candida albicans* appeared in the form of compact oval cells, Although the effect of treating by 1% ZnO NPs declared in figures (B1, B2, B3) which changed the shapes of the fungus cells and their colonies this change started by as shrinkage appeared in the usual oval shape, also, the previous stacking of cells in the colony was lost when exposing *Candida albicans* to ZnO NPs (1% or 1000 mmol or 10gm/l or 10mg/ ml) Then, by exposing the *Candida albicans* to higher concentrations of ZnO NPs, the deformation increased with increasing concentrations. Until the using of 5% and 7% concentrations, the oval shape of cells was completely disappeared and the colony cells became interfered with due to the severity of the deformation. These concentrations produce a distorting effect of 3%, 5% & 7% concentrations of ZnO NPs in comparable to that of an antifungal (figures groups; C, D & E) respectively.

3.3. The effect of Zinc Oxide Nanoparticles on the proteins of normal *Candida albicans*.

Our results of gel electrophoresis in figure (3) showed that the protein content in the bundle of the 1% ZnO NPs group was very large compared to the control group. This increase in protein content due to the low concentrations of ZnO NPs compared to the control group is an interesting thing that needs explanation. Then the protein content seemed to decrease with increasing the ZnO NPs concentration, which indicates an inverse relationship between the protein content and the concentration of ZnO NPs until we approach the concentration of 5%, it gives a close effect to the antifungal effect. As for 7%, it showed a destructive effect on the protein content in cells exceeding the effect of the antifungal.

3.4. The effect of Zinc Oxide Nanoparticles on the lipase activity of normal *Candida albicans*.

Current results in figure (4) revealed that the ZnO NPs effect clearly on produced of lipase enzyme in concentration, as when exposure to *Candida albicans* to ZnO NPs 1%, the enzymatic activity decreased to 76.4 U/ml, and so the enzyme activity decreased by increasing the concentration of ZnO NPs until it reached to 3.4 U/ml when *Candida albicans* are exposed to ZnO NPs 7%, while when exposure to *Candida albicans* to antifungal, the enzymatic activity decreased to 11.1 U / ml, which shows that the virulence ZnO NPs 7% was more severe than that of the antifungal agent towards the production of the lipase enzyme.

DISCUSSION

Candida albicans is a human pathogenic fungus that able to infect mucous membranes and skin, this pathogenic fungus usually causing severe systemic infections in immune compromised hosts (Abd and Ali, 2015). This fungus has developed resistance against antifungal agents, so it is difficult to control its growth (Jasim, 2015). To succeed in dealing with this problem, there is a need to find novel antifungals that could replace the present control strategies. Many nanomaterials such as; copper, silver, nickel, titania and zincite demonstrated antifungal properties. Especially, ZnO and MgO which have attracted interest as antimicrobial agents because of their stability and safety. Many studies have examined the effects of Nanoparticles with bacteria, but there was a shortage in studies that examined their effects on fungi. This due to the simplicity of bacterial testing systems comparing to fungal testing systems. Some studies have revealed that smaller ZnO particles have greater activity on different kinds of fungi and bacteria. However, under the influence of the same external agent, not all biological systems exhibit similar behavior (Yamamoto, 2001; Applerot et al. 2009 and Rosa-García et al. 2018).

Determination of addition of ZnO NPs on *Candida albicans* growth in different concentration

was examined on SDA and declared on table (1) and figure (1) as following; in case of 1% the effect of ZnO NPs were; 1, 1.5, 1.22 ± 0.039 cm as; minimum, maximum, mean ±SE respectively. While, in case 3% ZnO NPs inhibition effect were; 1.8, 2.3, 2.04 ± 0.034 cm as; minimum, maximum, mean ±SE respectively. Although 5% ZnO NPs were; 2, 2.4, 2.19 ± 0.027 cm as; minimum, maximum, mean ±SE respectively. The highest inhibition effect was detected in 7% ZnO NPs as following; 2.3, 3, 2.61 ± 0.054 cm as; minimum, maximum, mean ±SE respectively. In case antifungal agent the inhibition zone measured about; 0.1, 1.4, 0.96 ± 0.088 cm as; minimum, maximum, mean ±SE respectively. While all control sample were zero values. All reported results at different concentrations of ZnO NPs, have significant inhibition effect on the growth of *Candida albicans*.

From the above-mentioned result cleared that the ZnO NPs were very effective on *Candida albicans* than antifungal even at its lowest concentration was 1%. The most powerful effect was detected in case of 7% ZnO NPs and the inhibition effect were relatively correlation with concentration of ZnO NPs.

The ability of ZnO Nanoparticles to inhibit *Candida albicans* growth ranged between 1-3 cm in all the plates but, the ability of high concentrations of ZnO NPs to inhibit *Candida albicans* growth was greater than the low concentrations capacity. It is striking here that the ability of ZnO NPs in all its concentrations to inhibit the growth of *Candida albicans* were more powerful than the ability of the antifungal agent.

Figure (2) declared the inhibition effect of ZnO NPs in different concentration on *Candida albicans* were consistent with the results of many previous studies that recorded the high significant ability of ZnO NPs to influence the growth of *Candida albicans*, as well as the concentration-dependent effect.

Similar result were found by Abd and Ali, (2015) whom studied the sensitivity of *Candida albicans* to different concentrations of ZnO NPs and found that *Candida albicans* were sensitive to all concentrations (0.01, 0.05, 0.1, 0.5, 1, 3 and 5.8) mg/ml of the ZnO NPs which revealing a highly significant difference in all concentrations. Another study performed by El-Diasty et al. (2013) to evaluate the in vitro inhibitory effect of ZnO NPs on the growth of dermatophyte isolates. They found that the antifungal activity depends highly on the concentration, and the minimal fungicidal concentration of ZnO NPs was 0.01 mg/ml. Another study performed by Jasim, (2015) investigated the antifungal activity of ZnO NPs on *Candida albicans* using concentrations of (0, 3, 6 & 12 mmol/l^{-1}), he found a significant decreased in the radial growth of the fungus at different concentrations, especially at (6,12 mmol/l^{-1}) and the inhibitory effect of ZnO NPs increase with prolonged period of incubation and concentration. Jalal et al. (2018) assessed antifungal activity of ZnO NPs against *Candida* species on SDA plates, using 0.062–1.0 mg/ml concentrations, and they observed a significant reduction in the growth of *Candida* species at highest concentration (1.0 mg/ml). Lipovsky et al. (2011) tested the ability of ZnO NPs to affect the viability of *Candida albicans* and observed a concentration-dependent effect of ZnO NPs on the viability of *Candida albicans*. They found that the minimal fungicidal concentration of ZnO NPs was 0.1 mg ml^{-1} which caused an inhibition over 95% on *Candida albicans* growth. While, Karimiyan et al. (2015) recorded complete inhibition on *Candida albicans* growth by addition of 200 µg/mL ZnO NPs concentration. Singh and Nanda (2013) concluded that ZnO NPs had complete inhibition activity against *Candida albicans* at a concentration of 6.25µg/ml.

The difference in the inhibitory ability of ZnO NPs or minimal fungicidal concentration between our study and the various previous studies can be attributed to the difference in the incubation time for the colony or the difference in the source of the candida, according to our study the minimum concentration was somewhat high (1% or 10mg/ml),

The effect of particle size of ZnO NPs on viability of *Candida albicans*, depend on the smaller particles of nanoparticles which had better antifungal activity than larger particles (Ma et al. 2012 and Nouri, 2002). This potency referred to the easier of ZnO NPs entrance through the cell wall due to its tiny size, thus ZnO NPs able to inhibit the fungal cells performance which caused the inhibition of fungal growth even the fungal cell death (Padalia & Chanda, 2017). In their turn Lipovsky et al. (2011) suggested that anticandidal activity of ZnO NPs could be due to intracellular production of many free radicals such as; singlet oxygen, hydroxyl radicals, nitric oxide radical, and superoxide radical which may pass through the nuclear membrane and cause DNA damage, that could causes irreversible chromosome damage or cell death (Taufiq et al. 2018).

Figure (2) viewed the changed which occur by addition of different concentrations of ZnO NPs

compared with the normal shape of *Candida albicans* by the Scanning Electron Microscope (SEM) pictures at 3 different focuses; X2500, X8000 & X1500. Figures (A1, A2, A3) showed that the *Candida albicans* appeared as; ovoid "yeast" cells with budding, the colony of *Candida albicans* appeared in the form of compact oval cells, Although the effect of treating by 1% ZnO NPs declared in figures (B1, B2, B3) which changed the shapes of the fungus cells and their colonies this change started by as shrinkage appeared in the usual oval shape, also, the previous stacking of cells in the colony was lost when exposing *Candida albicans* to ZnO NPs (1% or 1000 mmol or 10gm/l or 10mg/ ml) Then, by exposing the *Candida albicans* to higher concentrations of ZnO NPs, the deformation increased with increasing concentrations. Until the using of 5% and 7% concentrations, the oval shape of cells was completely disappeared and the colony cells became interfered with due to the severity of the deformation. These concentrations produce a distorting effect of 3%, 5% & 7% concentrations of ZnO NPs in comparable to that of an antifungal (figures groups; C, D & E) respectively.

SEM has been used successfully to assess morphological changes of microbial cells induced by ZnO NPs in many previous studies. As He et al. (2011) whom investigated the antifungal activities of ZnO NPs against two *Botrytis cinerea* and *Penicillium expansum* and found that ZnO NPs inhibited *B. cinerea* growth by deforming the structure of fungal hyphae and inhibited the germination of *P. expansum* conidia completely and suppressed its conidial development. Rosa-García et al. (2018) tested radial growth of *C. gloeosporioides* and found that ZnO NPs and MgO NPs caused deformation or inhibited sporulation through swelling the spores of *C. gloeosporioides* strains, they also observed structural deformation and melanization to the hyphae of fungi, as the NPs caused hyphae deformation via swelling or vacuolar expansion. The difference in the way of distorting the fungal morphology in different studies could be innate tolerance of each fungus to ZnO NPs (He et al. 2011).

The difference in the effect of ZnO NPs exposure between different organisms may be due to the difference in the structure of their bodies and their cell walls. Thus, they give different reactions to exposure to ZnO NPs.

The mechanism of the inhibitory effect of ZnO NPs on microorganisms is not understood completely yet (He et al. 2011). Diverse toxicity mechanisms have been proposed by previous studies. Some studies suggested that the integration of ZnO NPs into microbial cells may causes continuous release of membrane proteins and lipids, resulting in changes in the permeability of microbial cells membrane (Amro et al. 2000; Sawai & Yoshikawa, 2004 and Brayner et al. 2006). While other study suggested that ZnO NPs may impact functions of cell, and then cause the increase in contents of carbohydrate and nucleic acid. Nucleic acid increase could be due to stress response of fungal hyphae. While, carbohydrates increase could be a result of the self-protection mechanism versus the ZnO NPs (Fraternale, et. al., 2003). Others observed higher carbohydrates in *Candida albicans* after addition of nanoparticles (Kim et al. 2008 and Kim et al. 2009). Kim et al. (2009) suggested that nanoparticles has antifungal activity against *Candida albicans* through their damage of the cell membrane structure and inhibition of the normal budding as a result of membrane integrity destroying. But in our study, the SEM picture shows increasing in deformations of *Candida albicans* morphology, with increasing ZnO NPs concentration, which supports, the observations regarding damaging of cell membrane structure due to ZnO NPs.

This study also used gel electrophoresis in figure (3) showed that the protein content in the bundle of the 1% ZnO NPs group was very large compared to the control group. This increase in protein content due to the low concentrations of ZnO NPs compared to the control group is an interesting thing that needs explanation. Then the protein content seemed to decrease with increasing the ZnO NPs concentration, which indicates an inverse relationship between the protein content and the concentration of ZnO NPs until we approach the concentration of 5%, it gives a close effect to the antifungal effect. As appeared in case of 7% ZnO NPs, which showed a destructive effect on the protein content in cells exceeding the effect of the antifungal.

The result concluded that low concentrations of ZnO NPs increase the protein content significantly, but large concentrations reduce the protein content. This may be a mechanism of adaptation by fungal cells with the newcomer in the case of few concentrations by increasing the protein content, but when the concentration of ZnO NPs increases significantly, the cells are destroyed and become unable to deal with these concentrations by increasing the protein content. In a previous study, Gunalan et al. (2012) found that there was increasing in the amount of

proteins that released from the cells of fungal strains (*Aspergillus nidulans, Aspergillus flavus, Rhizopus stolonifer,* and *Trichoderma harzianum*) along with increasing contact period and concentration of ZnO NPs. The difference in the results between our study and this study may be due to the difference in the types of the fungus that been used, and the difference in the conditions between the studies.

It should be noted that, in case of *Candida albicans*, the proteins responsible for formation fungal hyphae which considered as an essential components for the major virulence strategy of this fungi as well as expressions of these proteins and their interactions that performed various cellular functions, inducing pathogenesis, and adaptation to adverse conditions (Das et al. 2019).

Cell wall proteins plays a main role in maintaining structural integrity and in adherence mediating, whether to microbes or host, these proteins may have enzymatic functions such as proteolysis in biofilms. In addition to that proteins played a vital role in cells interactions, in the biofilm and the production of extracellular of matrix material (Chaffin, 2008).

The inhibitory effect of ZnO NPs was not limited to the inhibition of fungal growth, but it expanded to reach some of virulence factors of the yeast including lipase enzyme, extracellular lipase have been suggested to the possible virulence factors for *Candida* spp. (Jasim, 2015).

Current results in figure (4) revealed that the ZnO NPs effect clearly on produced of lipase enzyme in concentration, as when exposure to *Candida albicans* to ZnO NPs 1%, the enzymatic activity decreased to 76.4 U/ml, and so the enzyme activity decreased by increasing the concentration of ZnO NPs until it reached to 3.4 U/ml when *Candida albicans* are exposed to ZnO NPs 7%, while when exposure to *Candida albicans* to antifungal, the enzymatic activity decreased to 11.1 U / ml, which shows that the virulence ZnO NPs 7% was more severe than that of the antifungal agent towards the production of the lipase enzyme. These results accordant with Jasim, (2015) who found that the ZnO NPs effect clearly on produced of lipase enzyme in concentration (3, 6 and 12 $mmoll^{-1}$).

In addition to, Jalal et al. (2019), found that suggested that biosynthesized ScAgNPs suppressed strongly the secretion of hydrolytic enzymes (viz. lipases, phospholipases, hemolysin, and proteinases) by Candia spp., they found that ScAgNPs at 500 µg/mL suppress the production of lipases by 69.4% in *Candida albicans*.

Different results obtained by Barros et al. (2019) whom investigated the surface interactions between lipase from *Candida antarctica* fraction B (CALB) and gold nanoparticles (AuNPs), they found that the catalytic activity of CALB was maintained, although at a lower percentage (≥ 80%). The difference between our results and the results of this study may be due to the difference in the type of nanoparticles that have been used in both studies

Microbial extracellular lipases have many roles including; lipids digestion for nutrient acquisition, synergistic interactions with other enzymes, nonspecific hydrolysis because of additional phospholipolytic activities, adhesion to host cells and tissues, self-defense mediated through lysing competing microflora, and initiation of inflammatory processes by affecting immune cells (Gácser et al. 2007).

From the above-mentioned result cleared that the ZnO NPs were very effective on *Candida albicans* than antifungal even at its lowest concentration was 1%. The most powerful effect was detected in case of 7% ZnO NPs and the inhibition effect were relatively correlation with concentration of ZnO NPs. This potency referred to the easier of ZnO NPs entrance through the cell wall due to its tiny size, thus ZnO NPs able to inhibit the fungal cells performance which caused the inhibition of fungal growth even the fungal cell death. SEM picture shows increasing in deformations of *Candida albicans* morphology and damaging of cell membrane structure with increasing ZnO NPs concentration. The results concluded also that addition of ZnO NPs by low concentrations leading to increase in protein content while high ZnO NPs concentration showed a destructive effect on the protein content of *Candida albicans* cells structures exceeding the effect of the antifungal. ZnO NPs effect clearly on produced of lipase enzyme in concentration (3, 5 and 7 $mmoll^{-1}$).

CONCLUSION

In this study *Candida albicans* has been exposed to different concentrations of Zinc Oxide Nanoparticles (1%, 3%, 5%, 7%) (10 mg/ml, 30 mg/ml, 50 mg/ml, 70 mg/ml), these concentrations showed antifungal effects on fungal growth, structure and secretions, as it impacted the *Candida albicans* in various aspects, whether it is shape, protein content, or its lipase activity. While, high concentrations of it (5%, 7%) have more

inhibition effects on the fungus, comparable to or greater than the antifungal effect.

CONFLICT OF INTEREST

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

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AUTHOR CONTRIBUTIONS

This study has been approved by the Animal Rights and Ethical Use Committee of Jeddah University and New valley university. SMF: shared in laboratory examinations, photography, revised the manuscript. HAA: shared in study design, revised the manuscript. NTE: Corresponding author of the manuscript, study design, shared in laboratory examination, drafted and revised the manuscript, and data analysis. All authors read and approved the final manuscript.

REFERENCES

Abd, S. T. and Ali, A. F. (2015). Effect of Zinc Oxide Nanoparticles on *Candida albicans* of Human Saliva (in vitro study). European Journal of Medicine, 10(4): 235-244.

Abramov, O., Gedanken, A., Koltypen, Y., Perkas, N., Perelshtein, I., Joyce, E. & Mason, T. (2009): Pilot Scale Sonochemical Coating of Nanoparticles onto Textiles to Produce Biocidal Fabrics. In Surface and Coatings Technology 204: 718-722.

Amro, N. A., Kotra, L. P., Wadu-Mesthrige, K., Bulychev, A., Mobashery, S., & Liu, G. Y. (2000). High-resolution atomic force microscopy studies of the *Escherichia coli* outer membrane: structural basis for permeability. Langmuir, 16(6): 2789-2796.

Anbuvannan, M., Ramesh, G., Viruthagiri, N. & Shanmugam, N. (2015). Synthesis, characterization and photocatalytic activity of ZnO nanoparticles prepared by biological method, Spectrochim. Acta A Mol. Biomol. Spectrosc, (143): 304–308.

Applerot, G., Lipovsky, A., Dror, R., Perkas, N., Nitzan, Y., Lubart, R., & Gedanken, A. (2009). Enhanced antibacterial activity of nanocrystalline ZnO due to increased ROS-mediated cell injury. Advanced Functional Materials, 19(6), 842-852.

Barros, H. R. D., Santos, M. C., Barbosa, L. R., Piovan, L., & Riegel-Vidotti, I. C. (2019). Physicochemical Study of the Interaction between Gold Nanoparticles and Lipase from Candida sp. (CALB): Insights into the Nano-Bio Interface. Journal of the Brazilian Chemical Society, 30(10), 2231-2242.

Binn, J., Gunasekaran, M. and Mohan, N. (2009). Influence of Cultural Conditions on Lipase Production in *Candida albicans.* Asian Journal of Biotechnology, 1: 118-123.

Brayner, R., Ferrari-Iliou, R., Brivois, N., Djediat, S., Benedetti, M. F., & Fiévet, F. (2006). Toxicological impact studies based on *Escherichia coli* bacteria in ultrafine ZnO nanoparticles colloidal medium. Nano letters, 6(4), 866-870.

Calderone, R. (2012): Candida and Candidiasis. Washington: ASM Press.

Chaffin, W. L. (2008). *Candida albicans* cell wall proteins. Microbiol. Mol. Biol. Rev., 72(3), 495-544.

Chekman, IS. (2008). Nanopharmacology: Experimental and clinic aspect Ukrainian. Lik Sprava (3- 4): 104-109.

Das, S., Bhuyan, R., Bagchi, A., & Saha, T. (2019). Network analysis of hyphae forming proteins in *Candida albicans* identifies important proteins responsible for pathovirulence in the organism. Heliyon, 5(6): 01910-16.

El-Diasty, M.E., Ahmed, M.A., Okasha, N., Mansour, F.M., El-Dek, S.I., El-Khalek, A.H. And Youssif, M.Y. (2013). Antifungal Activity of Zinc Oxide Nanoparticles Against Dermatophytic Lesions Of Cattle. Romanian J. Biophys., 23(3): 191–202.

El-Ghwas, D., Mazeed, T., El-Waseif, A., Al-Zahrani, H., Almaghrabi, O. and Elazzazy, A. (2019). Factorial Experimental Design for Optimization of Zinc Oxide Nanoparticle Production. Current Nanoscience, 15: 1- 11.

Fraternale, D., Giamperi, L. and Ricci, D. (2003). Chemical Composition and Antifungal Activity of Essential Oil Obtained from In Vitro Plants of Thymus *mastichina L.* Journal of Essential Oil Research. 15(4):278-281.

Gácser, A., Stehr, F., Kröger, C., Kredics, L., Schäfer, W., & Nosanchuk, J. D. (2007). Lipase 8 affects the pathogenesis of *Candida albicans*. Infection and immunity, 75(10): 4710-4718.

Gunalan, S., Sivaraj, R., & Rajendran, V. (2012). Green synthesized ZnO nanoparticles against bacterial and fungal pathogens. Progress in Natural Science: Materials International, 22(6): 693-700.

He, L., Liu, Y., Mustapha, A. & Lin, M. (2011). Antifungal activity of Zinc oxide nanoparticles against *Botrytis cinerea* and *Penicillium expansum*. Microbiol. Res., 166(3): 207- 215.

Jalal, M., Ansari, M. A., Ali, S. G., Khan, H. M., & Rehman, S. (2018). Anticandidal activity of bioinspired ZnO NPs: effect on growth, cell morphology and key virulence attributes of Candida species. Artificial cells, nanomedicine, and biotechnology, 46(1): 912-925.

Jalal, M., Ansari, M. A., Alzohairy, M. A., Ali, S. G., Khan, H. M., Almatroudi, A., & Siddiqui, M. I. (2019). Anticandidal activity of biosynthesized silver nanoparticles: effect on growth, cell morphology, and key virulence attributes of *Candida* species. International journal of nanomedicine, 14: 4667-70.

Jasim, N. O. (2015). Antifungal activity of Zinc oxide nanoparticles on Aspergillus fumigatus fungus & *Candida albicans* yeast. J. Nat. Sci. Res., (5): 23-27.

Jayaseelan, C. Rahuman, A., Kirthi, V., Marimuthu, S., Santhoshkumar, T., Bagavan, A. (2012). Novel microbial route to synthesize ZnO nanoparticles using Aeromonas hydrophila and their activity against pathogenic bacteria and fungi, Spectrochim. Acta A Mol. Biomol. Spectrosc. (90): 78–84.

Kangogo, M., Revathi, G. & Wanyoike, M. (2009). Phenotypic characterization of *candida albicans* from clinical sources in Nairobi Kenya, African Journal of microbiology research. 3(9): 475-477.

Karimiyan, A., Najafzadeh, H., Ghorbanpour, M., & Hekmati-Moghaddam, S. H. (2015). Antifungal effect of magnesium oxide, zinc oxide, silicon oxide and copper oxide nanoparticles against *Candida albicans*. Zahedan Journal of Research in Medical Sciences, 17(10): 1-10.

Kim, K. J., Sung, W. S., Moon, S. K., Choi, J. S., Kim, J. G., & Lee, D. G. (2008). Antifungal effect of silver nanoparticles on dermatophytes. J. Microbiol. Biotechnol., 18(8): 1482-1484.

Kim, K. J., Sung, W. S., Suh, B. K., Moon, S. K., Choi, J. S., Kim, J. G., & Lee, D. G. (2009). Antifungal activity and mode of action of silver nano-particles on *Candida albicans*. Biometals, 22(2), 235-242.

Lipovsky, A., Nitzan, Y., Gedanken, A., & Lubart, R. (2011). Antifungal activity of ZnO nanoparticles—the role of ROS mediated cell injury. Nanotechnology, 22(10):1-10.

Ma, H., Williams, P.L & Diamond, S.A. (2012). Ecotoxicity of manufactured ZnO nanoparticles– a review, Environ. Pollut., (172): 76–85.

Magaldi, S., Essayag, C. & Caoriles, C. (2004). Well diffusion for antifungal susceptibility testing, Int. J. Infectious Diseases., (8): 39–45.

Nikalje, A. (2015). Nanotechnology and its Applications in Medicine. Med chem, (5): 2-10.

Nouri, S. (2002). Adsorption of p-nitrophenol in Three Different Activated Carbons at Different ph. Journal of Sciences, Islamic Republic of Iran 13(3): 241-247.

Padalia, H., & Chanda, S. (2017). Characterization, antifungal and cytotoxic evaluation of green synthesized zinc oxide nanoparticles using *Ziziphus nummularia* leaf extract. Artificial cells, nanomedicine, and biotechnology, 45(8), 1751-1761.

Patlosky, F. & Zheng, C. (2006). Nanowire sensors for medicine and life sciences, Nanomed, (1): 51–65.

Pulit-prociak, J., Chwastowski, J., Kucharski, A. & Banach, M. (2016). Applied surface science functionalization of textiles with silver and zinc oxide nanoparticles, Appl. Surf. Sc., (385): 543–553.

Richards, S., Oliver, G. & White, C. (2008). Micafungin activity against *Candida albicans* with diverse azole resistance phenotypes J. Antimicrob. Chemother (62): 55- 349.

Rosa-García, D., Susana, C., Martínez-Torres, P., Gómez-Cornelio, S., Corral-Aguado, M. A., Quintana, P., & Gómez-Ortíz, N. M. (2018). Antifungal activity of ZnO and MgO

nanomaterials and their mixtures against *Colletotrichum gloeosporioides* strains from tropical fruit. Journal of Nanomaterials, 1-10.

Sawai, J., & Yoshikawa, T. (2004). Quantitative evaluation of antifungal activity of metallic oxide powders (MgO, CaO and ZnO) by an indirect conductimetric assay. Journal of applied microbiology, 96(4), 803-809.

Singh, P., & Nanda, A. (2013). Antimicrobial and antifungal potential of zinc oxide nanoparticles in comparison to conventional zinc oxide particles. J Chem Pharm Res, 5(11), 457-463.

Takahashi, Y., Yoshikawa, A. & Sandhu, A. (2007): Wide bandgap semiconductors: fundamental properties and modern photonic and electronic devices. Springer. 357-65.

Taufiq, A., Ulya, H. N., Utomo, J., Hidayat, N., Susanto, H., Mufti, N., & Soontaranon, S. (2018). Structural, Optical, and Antifungal Characters of Zinc Oxide Nanoparticles Prepared by Sol-gel Method. In Journal of Physics: Conference Series, 1093(1):012001-10.

Vitisant, T., Leelaruji, W., Chulalaksananukul, S., Wattayakorn, G., dan Chulalaksananukul W, (2013). A High-Activity Lipolytic Yeast Isolated From Sichang Island, Thailand. Maejo Int J. Sci. Technol, 7: 96-105.

Wani, H. & Saha, A. (2012). A unique and profound effect of MgO and ZnO nanoparticles on some plant pathogenic fungi, J. Appl. Pharmaceutical Sci., 2(3): 40–44.

Yamamoto, O. (2001). Influence of particle size on the antibacterial activity of zinc oxide. International Journal of Inorganic Materials, 3(7): 643-646.