

Efficacy of Silver nanoparticle against pathogenic yeast: *Cryptococcus neoformans* in vivo

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Abstract:

The objective of this study was to clarify the antifungal efficiency of silver nanoparticles (SNPs) against one of pathogenic yeast, *Cryptococcus neoformans* that causes pulmonary cryptococcosis. Nostril experimental exposure of male albino rats to a dose of untreated yeast suspension and a dose of treated yeast with SNPs, amphotericin B, and SNPs combined with amphotericin B (gi, gii, giii and giv) respectively. Results show that SNPs have high antifungal activity against *Cryptococcus neoformans*, this is indicated by results of histological effects which show that the histological changes that emerged in lung tissues in control group (gi) had eased or disappeared and these tissues began to restore natural forms when treated with SNPs and antifungal (gi, gii, giv). Also, there is a significant increase in IFN- γ levels and (WBC, RBC, PLT) count in control group (gi) compared with treated groups (gii, giii, giv). This confirms the effectiveness of inhibitory of SNPs.

Key words: *Cryptococcus*, SNPs, pulmonary cryptococcosis, nanoparticles, antifungal effect.

الخلاصة

كان الهدف من هذه الدراسة هو توضيح الكفاءة المضادة للفطريات من لجزيئات الفضة النانوية ضد واحدة من الخميرة الممرضة، المستخفية المورمة التي تسبب المستخفيات الرئوي ان Nostril. التعرض التجريبي لذكور الجرذان البيضاء لجرعة من معلق الخميرة تعليق غير المعالجة وجرعة من الخميرة المعاملة بتلك الجزيئات مع amphotericin B، بينت النتائج أن جزيئات الفضة النانوية لها نشاط مضاد للفطريات عالي ضد المستخفية المورمة، وهذا ما تشير إليه نتائج الآثار النسيجية والتي تظهر أن التغيرات النسيجية التي ظهرت في أنسجة الرئة في المجموعة الضابطة (GI) قد خفت أو اختفت، وبدأت هذه الأنسجة تستعيد الأشكال الطبيعية عندما تعامل مع جزيئات الفضة ومضاد للفطريات (GI)، (GIII)، (GIV). أيضاً، هناك زيادة كبيرة في مستويات IFN- γ ، WBC، RBC، و PLT في المجموعة الضابطة (GI) مقارنة مع المجموعات المعالجة (GII)، (GIII)، (GIV)، وهذا يؤكد فعالية المثبطة لجزيئات الفضة النانوية.

الكلمات المفتاحية: المستخفية المورمة، جزيئات الفضة النانوية، مستخفية مورمة رئوية، جزيئات دقيقة، فعالية مضادة للفطريات.

Introduction:

Nanotechnology is an emerging and fast growing technology and has been potentially used in a wide assortment of commercial products worldwide. Nanomaterials have potential applications in the fields of medical and pharmaceutical Nano engineering. They are employed as therapeutic agents, in chronic disease diagnostics, and in sensors. The metal nanoparticles including silver and zinc oxide nanoparticles have gained considerable attention due to their unique antibacterial, antifungal, and UV filtering properties. The microbial

biosynthesis of nanomaterials using bacteria and fungi has been exhaustively studied by various researchers (Ritika *et al.*, 2014). There are very few reports related to yeast mediated biosynthesis of nanomaterials. Silver nanoparticles (SNPs) have potent antibacterial and antifungal activity and general anti-inflammatory effects (Chaloupka *et al.*, 2010). Regarding mycoses, NPs can be considered as potential antifungal agent. Today a number of synthetic fungicides have been found to cause adverse effects to human and the environment and for this reason many of them have been banned. It's difficult to control fungal growth because fungi have developed resistance to many conventional fungicides (Elad *et al.*, 1992).

In recent years, nanoparticles have received increasing attention due to their unique physical and chemical properties. Many studies have demonstrated antimicrobial efficacy against bacteria, viruses and eukaryotic microorganisms of various NP materials (Kumar *et al.*, 2008). However, the antifungal effect of silver NPs has received only marginal attention and just a few studies on this topic have been published. Recent studies revealed the effects of silver NPs on some species of fungi particularly candida genus. However, only few studies have been performed for the mentioned effects on other fungi. To the best of our knowledge,

there is no study carried out for other pathogenic yeasts such as *Cryptococcus neoformans*. In this study, we investigate the effects of silver NPs alone and in combination with fungal antibiotics as amphotericin B on *Cryptococcus neoformans*. Also this study determined INT-g levels and WBC, RBC and PLT count in serum of rat that is infected with yeast (treated and without treated with SNPs).

Material & Methods

Fungal strain:

Cryptococcus neoformans clinical isolate from patients with bronchial asthma. Yeast cells suspension of *Cryptococcus* prepared according to the method reported by (Gross *et al.*, 2000). Finally the number of yeast cells $1 \times 10^7 \text{ ml}^{-1}$.

Nanoparticle material:

Silver nanoparticles with size (80 nm) were purchased from (nanoshel company) and were used in this study in concentration (2mM) (Kelly *et al.*, 2006)

Animals:

Forty male albino type of *Rattus norvegicus* weighting 100-180 g. and 6-8 weeks in age were maintained controlled environment of temperature, humidity and light. They were fed a commercial rat chow and tap water.

Experimental infection :

Rats were divided into four groups (10 per group): (i)-group 1- infected with untreated yeast (25ml) (1×10^7 yeast cells/ml. (ii)-group 2- infected with treated yeast (25ml) with silver nanoparticles suspension at concentration (2mM) (Kelly *et al.*, 2006) (iii) group 3- infected with yeast treated with amphotericin B (1mg/kg) (Nan *et al.*, 2007) (iv) group 4- infected with yeast treated with nanoparticles and amphotericin B. All groups of animals were injected with a single dose of hydrocortisone before beginning the experiment of infection which weakens the immune system (Naj *et al.*, 2006) Animals leave until the signs of fatigue, weakness, loss of appetite as evidence of the fungal incidence, then, all animals were killed by spinal dislocation, lung, and blood specimens were collected.

Histopathology test:

The lung was removed and fixed in 10% of formaline solution, tissue was embedded in paraffin and cut into sections which stained with hematoxyline and eosin stain (Bancroft and Stevens, 2006)

Determination of IFN γ -concentration in rat serum:

The level of interferon γ in rat serum was determined by using Elisa method and the test was carried out according to the instructions by the company processed for the kit.

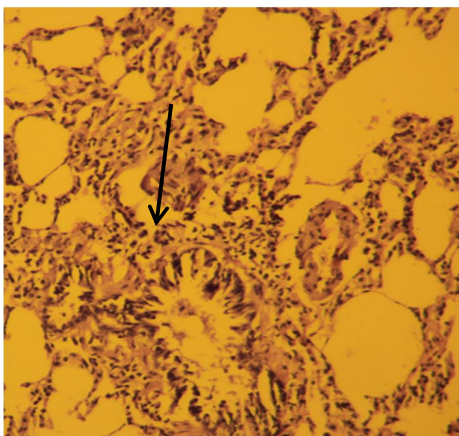
Routine blood tests to determine the effect of silver on blood components:

Orbital blood was collected and blood indices were determined for erythrocytes (red blood cells) (RBCs), leucocytes (white blood cells) (WBCs) and platelets (PLTs) using an automatic hemocytometer.

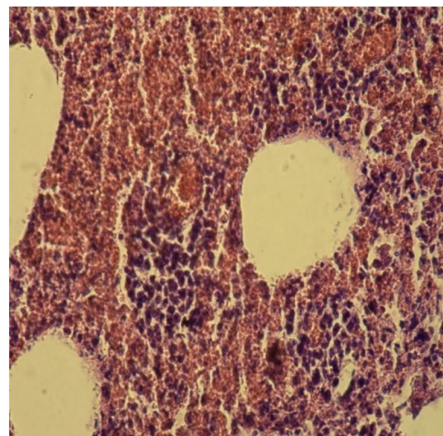
Results:

Histopathological effects:

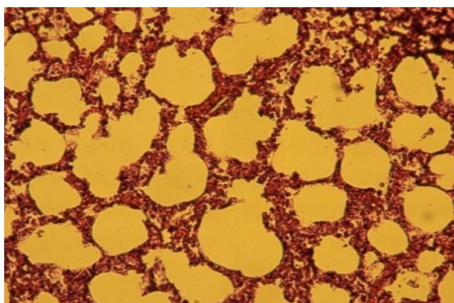
Results of histopathological effects show different histological changes in the lung tissue in group (i) which was infected with untreated yeast. These effects included laceration of the wall between the alveoli composed emphysema with presence of thrombus and *Cryptococci* are found in the necrotic tissue Fig (1) these effects less when treated the yeast with silver nanoparticles Fig(2), show almost normal tissue of the lung and these effects are considered ideal result if compared with histologic effects when treated with the yeast with antifungal amphotericin B. Fig(3). Also there are some histological effects of yeast on the lung tissue, in case of treating the yeast with SNPs and amphotericin B. (Fig.4), which is considered mild compared with the effects caused by the non-yeast treatment in the first group.



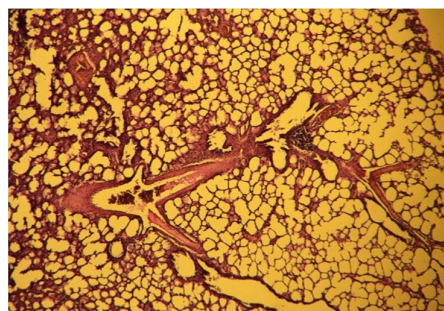
Fig(1)section of the lung group(i)shows group laceration f the wall between the alveoli composed emphyosema, showed spherical vein organisms *Cryptococcus neoformans* (←) H & E x100)



Fig(2)section of the lung tissue (ii) shows normal pulmonary bronchioles and pulmonary (H & E x100)



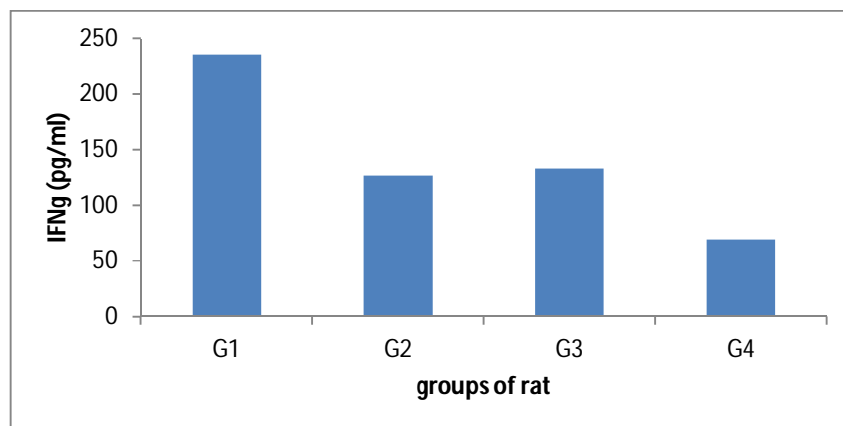
Fig(3)section of lung tissue group(iii) show Simple infiltration of inflammatory installation of cells between Alveoli Pulmonary its natural form bronchioles .(almost normal (H & Ex400



Fig(4) section of lung tissue group(iv) restoration of a typical the fabric (H & Ex100)

IFN g-concentration in rat serum:

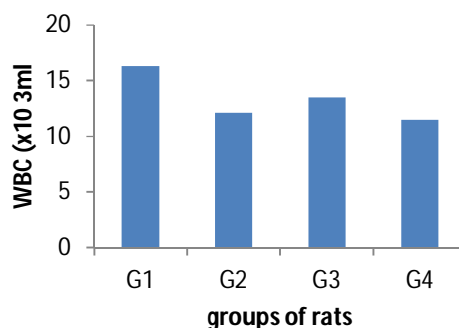
The production of IFN-g by natural killer (NK) cells and T cells , an important component of the early host defense reaction against infection (Dunn and North, 1996 ;Scharton and Scott, 2000) Fig(5)show asignificant elevation in IFNg.level between groups of rats test ($p < 0.005$),but no statistical difference was seen between group(ii) and group(iii).



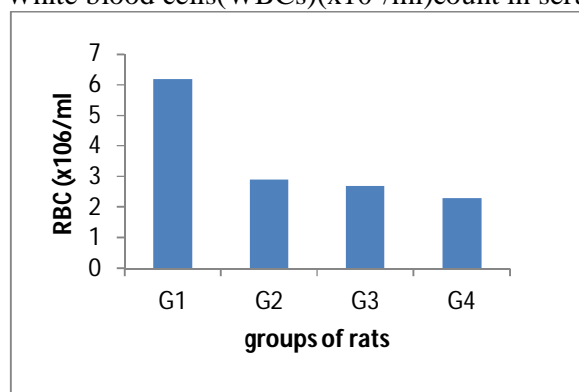
Fig(5)IFNgamma level in serum of rat

Blood component analysis:

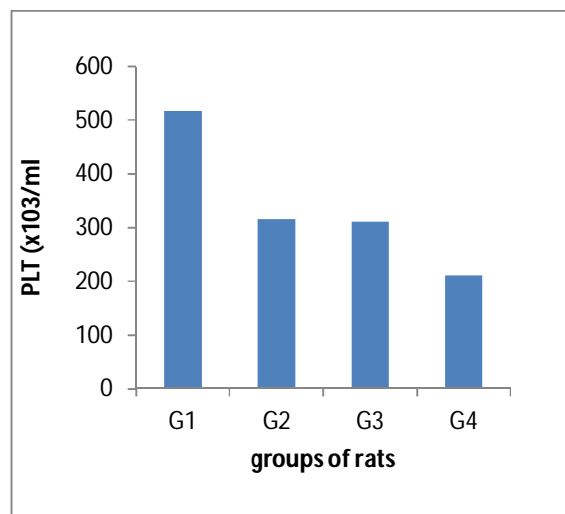
In state of investigating the adverse effects different treatments various indices were measured for blood component (Fig.6,7,8),WBC,RBC and PLT showed significant increase in case of group(i) which is injected with untreated yeast compared with other groups .Also , they show asignificant value between other groups of rats test ($p < 0.005$)



Fig(6) White blood cells(WBCs)($\times 10^3$ /ml)count in serum of rat



Fig(7) Red blood cells(RBC)($\times 10^6$ /ml) count in serum of rat



Fig(8)platelet (PLT)(x10³/ml) count in serum of rat

Discussion:

When we highlight the results of acquired may because confirmed the effectiveness of nanoparticles in growth of one of the opportunistic yeasts that caused a series of serious illnesses , Cryptococcal infection can occur in individuals with normal immunity but is most common in the immunocompromised host (Wheat *et al.* , 2002; Kerkering *et al.* , 1996) .Previous studies have shown that SNPs exhibit antimicrobial activity against some of fungal species, such as *Trichophyton spp.* and *Candida spp.* (Musarra *et al.* , 2010; Kaviya *et al.* 2011)

In our work ,we observed that experimental infection of the rat with yeast that is treated with 2mM of silver nanoparticles decrease of histological effects compared with untreated yeast(groupi) ,these results are similar to the previously reported by Kelly *et al.* , 2013; Kaviya *et al.* ,2011).

The antimicrobial activity of silver nanoparticles depended on size of it,smaller sized of particles more effects in microorganisms and this due to their higher surface area to volume ratio(Morones *et al.* ,2005). the superior characteristic of silver nanoparticles due to the large surface area of it which provides better contact with the microorganisms .Also the silver nanoparticles release silver ions directly into the microorganisms which inhibit the growth activity(Rai *et al.* , 2005)The results of IFN-gama show that increasing in interferon gama level to protection responses and against cryptococcal infection, (groupi) this is similar to (Levitz and Eleanor, 2000; Doyle and Murphy, 2005)

Host resistance to microbial pathogens includes accumulation of appropriate inflammatory cells at the site of infection at an appropriate time. In cryptococcal infection, mononuclear leucocyte such as macrophages and lymphocytes, rather than neutrophils, are important (Murphy, 2006; Floyd *et al.* , 2007)

. Although chemokine's are directly and selectively associated with these cellular inflammatory responses, many other proinflammatory cytokines include , IFN-g (Kozel,2003) .

Results above are reflected in the blood analysis data where treated with yeast had a significant effect on all the tested parameters . WBC,RBC and PLT counts were elevated when animals were treated with yeast group i, but a lesser degree in other groups when treated with SNPs and SNPs and amphotericin B which have the largest effect .This means that ,the utilization of silver in nanoparticles form is a new strategy for treating of fungal infection especially cryptococcal infection .Also our study shows that SNPs can be used with amphotericin B as an alternative for the antifungal agent amphotericin B.

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