ISSN: 0975 -8542

Journal of Global Pharma Technology

Available Online at: www.jgpt.co.in

RESEARCH ARTICLE

Investigation of Melanin in Dermatophytes that Isolated from Primary Schools Students

Abbas Abdel Hussein Mohi*, Majid Kadhem Aboud Al-Shibly

College of Education-University of Qadisiyah College of Education-University of Qadisiyah/Iraq.

***Corresponding Author: Abbas Abdel Hussein Mohi**

Abstract

The study was conducted on four types of dermatophytes, including *Trichophyton rubrub, T. mentagrophytes, Microsporium audouinii* and *M.canis*. For isolated samples from primary school students under the age of sixteen, samples were collected from different areas of tinea capitis, tinea corporis and then identified the fungal infections to distinguish it from, other infections, such as eczema and traditional sensitivity, the diagnosis was done by direct examination of 10% KOH and then SDACC to observe the fungal structures where Morphological Description was identified. The fungus stimulated the production of melanin dye using a PDA medium for the four species. Melanin was detected in two ways copper silver sulfide (AgCuS) and light microscopy where the melanin was presented in the fungal structures of the hyphae and Conidial. The second method was grown for twenty-one days. Melanin was extracted and the melanin concentrations and carefully were observed using the scanning electron microscopy.

Keywords: *Melanin; Primary school; and Dermatophytes*

Introduction

Melanin is one of the important natural pigments found in the collection of kingdoms of life and is characterized by being chemically stable and with a high molecular weight. 318.283 g / mol is formed by polymerization of phenolic or indole compounds which are negatively charged dyes. It is also characterized by its chemical stability because it cannot be dissolved with organic solvents or water, On the other hand, it has been found to have the ability to absorb high energy such as those resulting from ultraviolet radiation or energy from nuclear explosions[1,2,3]. Melanin also showed an ability to treat fungal infections. Studies indicated melanin's ability to resist fungal treatments compared with non-melanin fungi.

This was done when PKS1 (polyketide synthase) [4], was removed which is responsible for building melanin in *Wangiella dermatitidis*, such as Amphotericin B and Voriconazole, while *Histoplasmosis capsulatum* and *Cryptococcus neoformans* were found to be more resistant to these antagonists due to their ability to produce melanocytes[5]. The increase in fungal resistance can be attributed to the susceptibility of melanin to the association with these drugs which leads to impaired functioning .On the other hand; it was found that melanin was associated with the antioxidants Itraconazole and Ketoconazole. It inhibits its function. It also has the ability to bind with the polynes and Echinocandins, but this does not occur with the Azoles such as *Histoplasmosis capsulatum* and *Cryptococcus neoformans*. In the extraction of fungal antifungal such as Amphotericin B determines the final concentration in the drug [6] and these studies have indicated that inhibition of melanin that lead to an increase in the effectiveness of antifungal drugs as preliminary evidence which indicates that inhibition of melanin in infected mice led to a significant effectiveness to the antifungal in the treatment of infections [7].

Since melanin is a water-resistant dye with a negative charge and has the ability to bind to a wide range of materials, it is suggested that melanin fungi have a high capacity in

biological treatment. This association with toxic substances has been added. *Aspergillus niger* has been found to be able to bind with the ink in the water and thus permanently remove this color [8]. It is possible to isolate fungus associated with heavy elements in abandoned mines such as uranium, iron, and copper, which are usually found in lichen and fungus[9].

Materials and Methods

The study samples were collected from students with dermatophytes and were clinically diagnosed by specialized dermatologists. They included primary school students in Diwaniyah and Babylon governorates through consultation clinics in government hospitals or through private clinics of dermatologists. The study included 4 samples of skin lesions and injuries in The hair was examined directly using 10% KOH and transferred to the laboratory using sterile sliver cysts [10].

Samples from school children infected with SDACCG were incubated at 28-30˚C and observed after five days as fungi began to appear in the form of growing colonies. They were demonstratively diagnosed by monitoring growth of colonies and recording changes during incubation. We slept on a PDA medium to stimulate the production of pigments and the formation of cones, incubated for four weeks at a temperature of 28° C .

Direct Diagnosis

Direct examination was performed using 10% KOH. A small portion of the sample was placed on the clean glass slide. Add a 10% KOH solution and leave for 15-30 minutes to allow the tissue to be melted by KOH. Then the examination was performed to see the fungal yarn or reproductive spores. It left about an hour to allow tissue to be melted and then examined [11].

Morphological Description

The developing colonies were examined using lactophenol dye to see the structures of fungus and conidia using optical microscopy. The isolated fungal species, which differed in terms of colony shape, color which may be produced in contrast to planting plates, and the possibility of forming macroconidia or Microconidia, were identified or not[10].

Melanin Pigmentation

Butler method was adopted in the pigmentation of innate compositions as included .The fungal isolates were developed on a PDA medium and incubated at 28˚C for four weeks to stimulate dye production.

Take part of the developing colonies carefully without taking the center and put in a test tube size 1.5 ml. Washed with distilled water twice. Sediment bond in Sulfide silver copper (AgCuS) solution. Incubated at room temperature for a whole night.Wash with distilled water twice.Add 1% Sodium sulfate and incubate for 1 hour at 50˚C in the dark.Wash with distilled water twice.dried samples and suspended in a 20 ml solution of hydroquinone 170 mg, silver lactate 22mg and citrate buffer 0.1M, pH was set to 3.7.Incubation for 30-60 minutes at a temperature of 26 m.Added the 2000RT color fixer.Dry samples and placed on glass slides we add and examined under the light microscope to see the fungal melanin in fungal compositions [1].

Melanin Extraction

Melanin was extracted from four fungal isolates *M.gypseum, T. mentagrophyte, E.flocosum, T. rubeum*.by Wang *et al* (1996) method that was adopted as following: [12]

After the fungus were grown on a PDA medium for Twenty days, the conidia, were washed by using sterile BPS solution. The conidia were placed in a solution consisting of Sorbitol 1.0 M and Sodium citrate 0.1 M. The pH was adjusted to 5.5 by using hydrochloric acid (HCl) and 10 mg of Novozyme which was added and incubated at 30˚C for a full night. Then, Proteoplasts were produced, and collected by using a centrifuge 8000 cycles / min for 15 minutes. After that, they were washed by using sterile BPS for three times with centrifuge 8000 cycles / min for 15 minutes.

Add 4.0 M Guanidine thiocyanate to the product and incubate for a full night at the room temperature. Then, we collected melanin by centrifugation and washed the product for three times by using sterile BPS and Protanase K^+ 10 µL/ mL was added with a solution of 10mM from Tris and 1.0 mM of $CaCl_2$ and 0.5% (w / v) of SDS and pH control at 7.8 after that process the were incubated at 37˚C for a full night. The samples were washed by using centrifugal and BPS solution for three times .We collected the products by centrifugation and boiling by

using HCl 6.0 M for one `and half an hour at a temperature of 100° C.Samples were collected by using filtration papers No. 1.Then we washed the particles with distilled water for ten days to remove the acid residue. At last, they should be dried and examined by using electronic microscope.

Results and Discussion

Direct Inspection KOH 10%

Direct examination indicated the possibility of watching the fungal haemorrhages in new infections, while the chronic infections were characterized by the appearance of fungal cones, especially in hair injuries Figure (1).

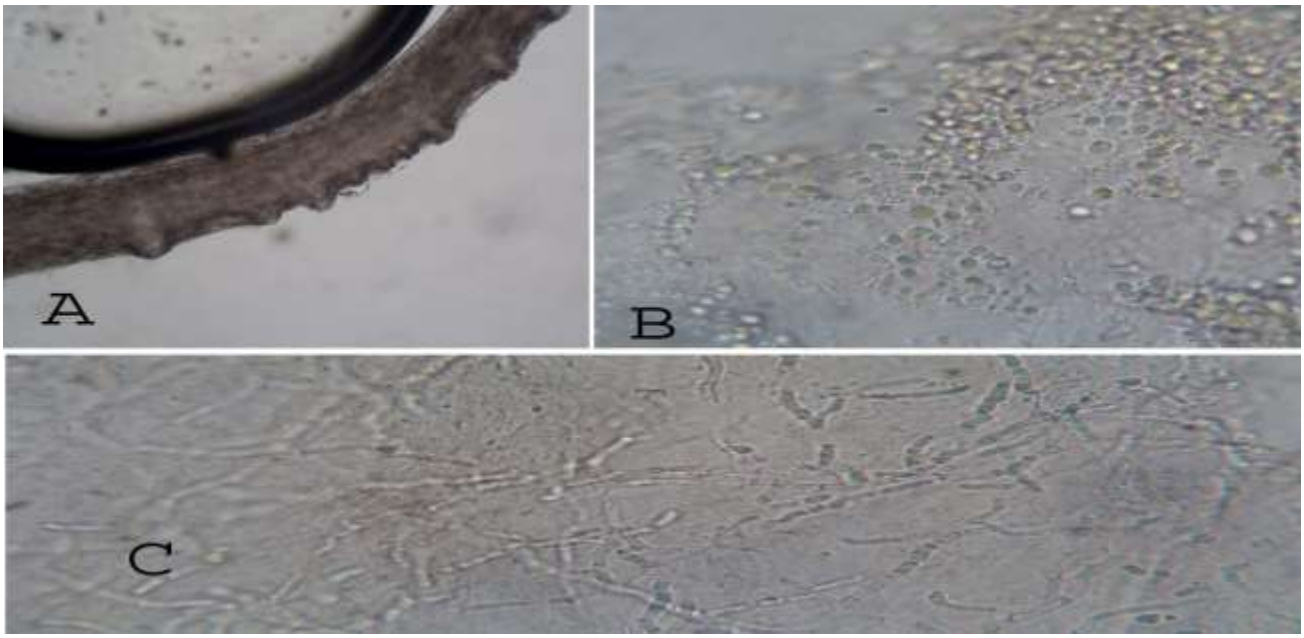

Figure 1: A- hair infected with fungal fungus Dermatophyton, B- fungal spores at the base of the infected hair, C- fungal infections after the dissolution of keratin found in epithelial cells

Diagnosis

The Dermatophytes were isolated on SDA medium with cycloheicomide, chloromafenicol and gentamycin which were used to prevent the growth of fungus Aspergillus sp, Penicillium sp and Mucor sp and prevent the growth of bacteria in both positive and negative gram stain species. M. audouinii and M. canis were isolated and also they were isolated by the two species, T.mentagrophytes and T rubrum.

Sample Preparation for Light Microscopy

The scan showed that melanin concentration is basically existed in cellular membranes, by using butler method as it is found in some cellular structures [13]. A high concentration of melanin is also found in both microconidia and macroconidia, which are mainly concentrated in their membranes. M .cains, T. mentagrophyte, T. rubeum, M. audouinii.

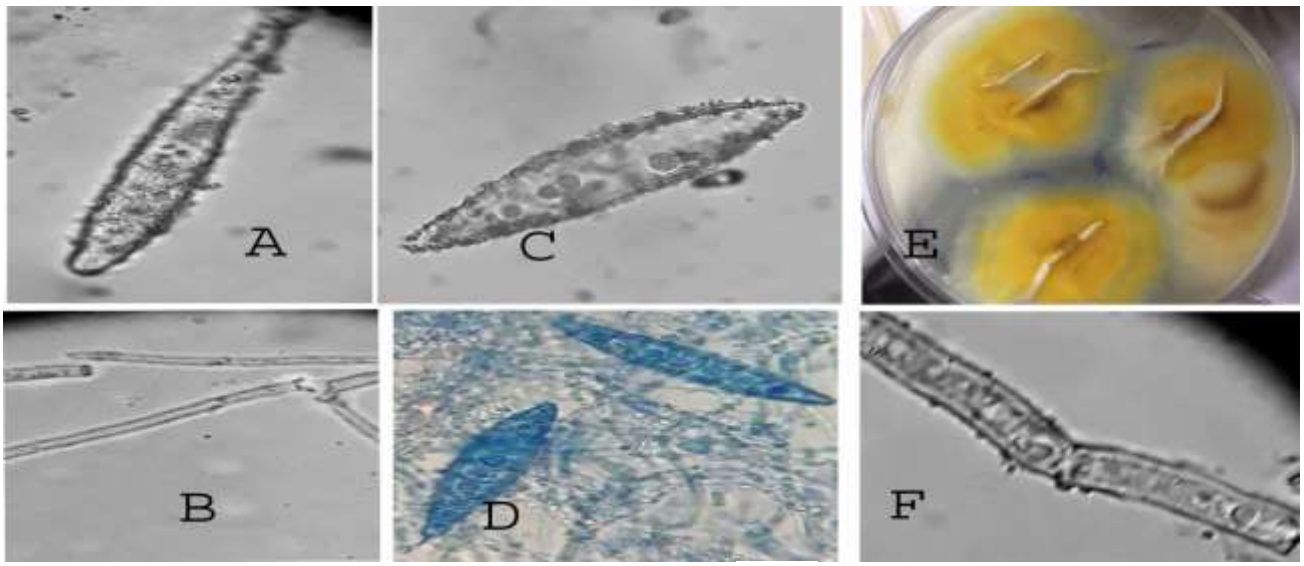

Figure 2: A: Trichophyton *rubrum* macroconidia. B : *T.rubrum* hyphae *Microsporium cains* (macroconidia) with

Silver lactat pigmentation, *E :M.cains* Grow on SDA at 28 ° C for 21 days,. *M. can is* macroconidia with lactophynol, *M. audouinii* hyphae with Silver lactat pigmentation

Scanning Electron Microscopy

After the melanin was extracted, the electron microscopy slides were introduced to review the melanin clusters, where melanin particles ranged between 157-156 nanometers that is confirmed the possibility of the presence of melanin and considered as a key ferocity factor in such fungi [14].

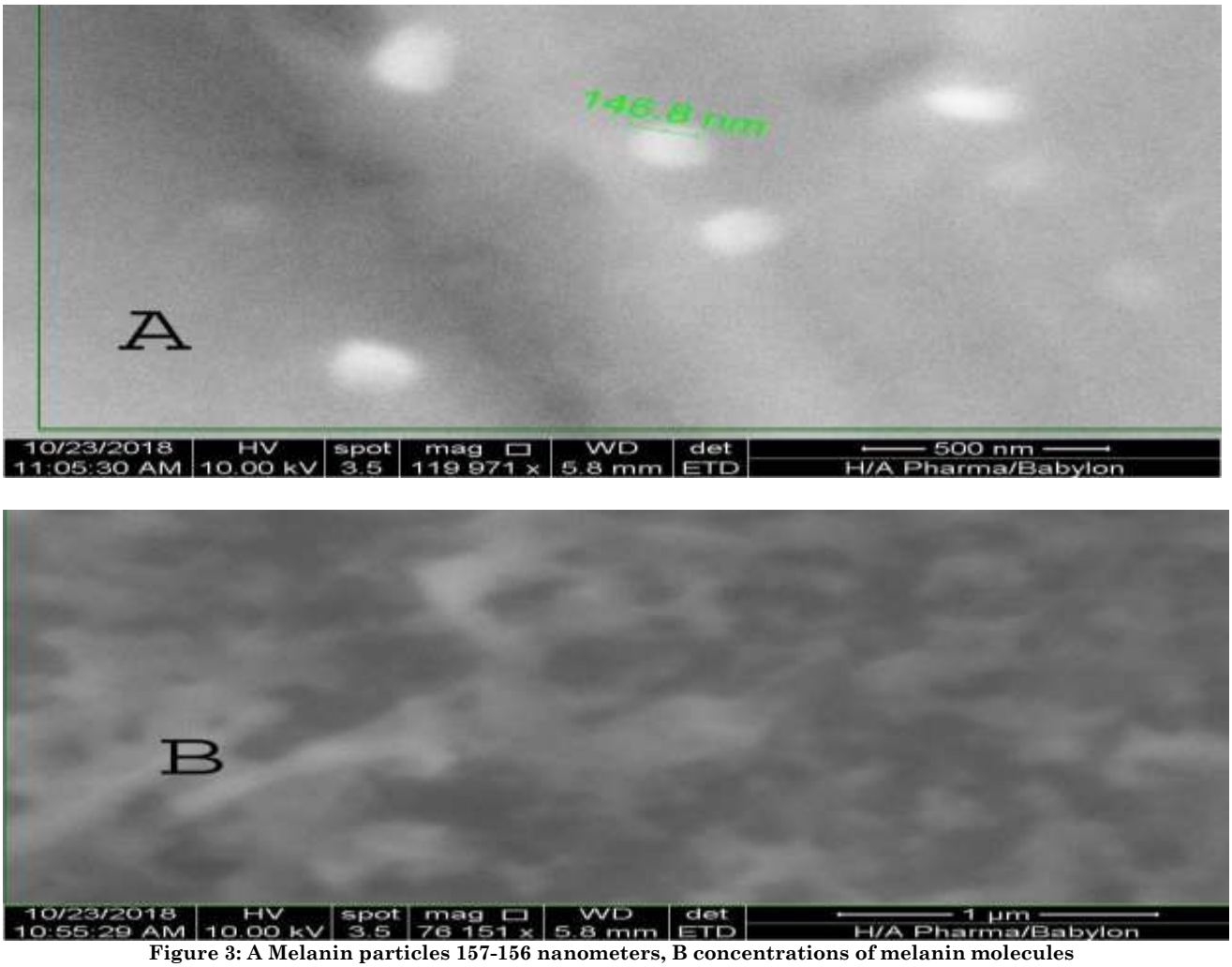

Figure 3: A Melanin particles 157-156 nanometers, B concentrations of melanin molecules

References

1. Butler MJ, Gardiner RB, Day AW (2005) Fungal melanin detection by the use of copper sulfide-silver. Mycologia, 97 (2): 312-319. doi:10.1080/15572536.2006.11832806

2. Hill HZ (1992) The function of melanin or six blind people examine an elephant. Bioessays, 14(1):49-56.

3. Jacobson ES (2000) Pathogenic roles for fungal melanins. Clin Microbiol Rev. 13(4):708-717.

4. Langfelder K, Streibel M, Jahn B, Haase G, Brakhage AA (2003) Biosynthesis of fungal melanins and their importance for human pathogenic fungi. Fungal Genet Biol., 38(2):143-158.

5. Paolo WF, Dadachova E, Mandal P, Casadevall A, Szaniszlo PJ, Nosanchuk JD (2006) Effects of disrupting the polyketide synthase gene WdPKS1 in Wangiella [Exophiala] dermatitidis on melanin production and resistance to killing by antifungal compounds, enzymatic degradation, and extremes in temperature. BMC Microbiol., 6(1):55.

6. Van de Sande WWJ, de Kat J, Coppens J (2007) Melanin biosynthesis in Madurella mycetomatis and its effect on susceptibility to itraconazole and ketoconazole. Microbes Infect, 9 (9): 1114-1123.

7. Rosas ÁL, Nosanchuk JD, Casadevall A (2001) Passive immunization with melanin-binding monoclonal antibodies prolongs survival of mice with lethal Cryptococcus neoformans infection. Infect Immun., 69 (5): 3410-3412.

8. Lamia K, N´ Ji G (2010) Aspergillus niger is able to decolourize sepia ink contained in saline industrial wastewaters. Desalin Water Treat, 20 (1-3): 144-153.

9. Turick CE, Ekechukwu AA, Milliken CE, Casadevall A, Dadachova E (2011) Gamma radiation interacts with melanin to alter its oxidation–reduction potential and results in electric current production. Bioelectrochemistry, 82(1):69-73.

10. Kidd S, Halliday CL, Alexiou H, Ellis DH (2016) Descriptions of medical fungi. David Ellis.

11. Harrington BJ, Hageage Jr GJ (2003) Calcofluor white: a review of its uses and applications in clinical mycology and parasitology. Lab. Med., 34(5):361-367.

12. Wang Y, Aisen P, Casadevall A (1996) Melanin and Melanin ghosts in Cryptococcus. Infect Immun., 64 (7): 2420-2424.

13. Henson JM, Butler MJ, Day AW (1999) The dark side of the mycelium: melanins of phytopathogenic fungi. Annu Rev Phytopathol., 37(1): 447-471.

14. Schweitzer AD, Howell RC, Jiang Z (2009) Physico-chemical evaluation of rationally designed melanins as novel nature-inspired radioprotectors. PLoS One, 4(9):e7229.

