European Journal of Biotechnology and Bioscience
Online ISSN: 2321-9122, Impact Factor: RJIF 5.44
www.biosciencejournals.com
Volume 5; Issue 1, January 2017; Page No. 66-68

The effect of low power diode laser on the growth of *Aspergillus spp*.

[1] Dr. Hadeel M Younis, [*2] Hiba Y Khalaf, [3] Nahla K Assad [4] Abdul Nasir

[1] Medical microbiology/College of Dentistry, Tikrit University, Iraq
[2] Medical microbiology /College of veterinary medicine, Tikrit University, Iraq
[3] College of Dentistry, Tikrit University, Iraq
[4] Department of Molecular Science and Technology, Ajou University South Korea

Abstract
*Aspergillu*s is a filamentous fungus opposite to yeast which is single cells. Fungi reproduce by forming tiny spore which can easily be airborn. Some pathogenic *aspergillus* species can cause serious disease in humans and animals. *Aspergillus* can produce mycotoxins these are often found in contaminated foodstuff and are hazardous to hay and s are easily airborne and we are normally breathe in 100-200 spores daily. The most common causative invasive disease are *aspergillus fumigatus* and *aspergillus flavus.* While the most causing allergic disease are *aspergillus fumigatus* and *aspergillus clavatus* (4). Aspergillosis is a group of disease caused by *aspergillus* which produce symptoms includes fever, cough, chest pain,or breathlessness occur in many other illness. Laser is a device that generate light by a process called stimulated emission. The acronym laser stands for light amplification by stimulated emission of radiation. Semiconducting lasers are multilayer semiconductor devices that generates a cohere beam of monochromatic light by laser action. A coherent beam resulted which all of photons are in phase. This study was carried out to: 1) isolate and diagnose of *Aspergillus spp.* and 2) evaluate the effect of low-power diode laser on growth of *Aspergillus spp*. fifty samples were taken from skin scraping and hair. The samples were collected from human after cleaning the area with 70% ethanol to remove contaminants such as bacteria. There are three examination for analysis the specimens**:** direct examination (direct microscopy), culture, staining by lacto phenol cotton blue (L.P.C.B.). The present study showed that 50% of isolates are aspergillus spp from all collected clinical specimens which are mature within 3 days. Also the present study showed that decrease in number of aspergillus spp. after exposure to laser for 4, 8, 16, 32, and 64 minutes. In conclusion: Exposure low power diode laser light was inhibited the growth of *Aspergillus spp.* after 32, 64 minutes.

Keywords: antiulcer activity, *hyoscyamus albus*, *umbilicus rupestris*, ethanol, methanolic extract

1. Introduction
Fungi belong to their own special kingdom as they differ from both plants and animals. Fungi are eukaryotic cells, it play an essential role in both nitrogen and carbon cycle by breaking down dead organic matter which allows nutrient to be cycled through the ecosyst. Fungi feed by absorbing nutrients from the organic material that they live in. They digest their food before they absorb it by secreting acids and hydrolytic enzymes [1]. *Aspergillu*s is a filamentous fungus opposite to yeast which is single cells. Fungi reproduce by forming tiny spore which can easily be airborn. Some pathogenic *aspergillus* species can cause serious disease in humans and animals. *Aspergillus* can produce mycotoxins these are often found in contaminated foodstuff and are hazardous to hay and s are easily airborne and we are normally breathe in 100-200 spores daily. Some species withstand heat e.g. *aspergillus fumigatus* (pathogenic) these are commonly found in compost [1] Aspergillus species generally are found in soil, air, water, food, compost, decaying vegetable, fire proofing materials, bedding, pillows, ventilation, air condition systems and computer van [1]. The most common causative invasive disease are *aspergillus fumigatus* and *aspergillus flavus.* While the most causing allergic disease are *aspergillus fumigatus* and *aspergillus clavatus* [3]. Aspergillosis is a group of disease caused by *aspergillus* which produce symptoms includes fever, cough, chest pain, or breathlessness occur in many other illness. Usually only patients with already weakened immune system or who suffer from other lung conditions are susceptible [4]. Those most at risk include some cancer and leukemia patients, those under chemotherapy treatment, and transplant patients [2]. There are three major forms of disease in human which includes:

a) Allergic aspergillosis (effects asthma, cystic fibrosis, and sinusitis patients),
b) Acute invasive aspergillosis, and
c) Disseminated invasive aspergillosis [2, 5].

Aspergillus mycotoxins are chemical products of fungi that have capacity to damage animal's health and contaminates crops. Repeated aflatoxin ingestion in man has been linked to liver cancer. These toxins produced by *aspergillus parasiticus* and *aspergillus flavus* which are commonly found to contaminate corn, peanuts, and other crops used for animal feedstuff. High temperature and humidity increase chance of contamination with *aspergillus*. In 1960 there are 100000 Turkey infected with Turkey X syndrome in south England, they died from liver damage after consumption of peanuts contaminated with *A.flavus* [2, 6].

Laser is a device that generate light by a process called stimulated emission. The acronym laser stands for light amplification by stimulated emission of radiation. Semiconducting lasers are multilayer semiconductor devices that generates a cohere beam of monochromatic light by laser

action. A coherent beam resulted which all of photons are in phase [7]. Such factors will be dictate whether the resulting effects will be [8].

i) **photochemical:** in which generation of free radicals and single oxygen that may either cause direct cellular toxicity or indirect killing of tumor cells by damage to subtending vasculature.
ii) **Photothermal:** in which denaturation of cell constituents, vaporization of cells.
iii) **Photo ablative:** direct breaking of chemical bounds.
iv) **Photo mechanical**: formation of plasma followed by its explosive dissipation and generation of shock waves [8, 9]. Laser light differs radically from ordinary light because lasers have many characteristics which includes:
    1) **Monochromatic light:** Each type of laser produces light in a very narrow band of wave lengths, which varies according to the particular type of laser,
    2) **Collimated light:** Laser produces a collimated or parallel beam of light, which does not diverge significantly over very long distances. This produces a very powerful light, the intensity of which can be increased even further by using a focusing mechanism to narrow the beam,
    3) **Coherent light**: This means that all light waves are in phase within the beam with the peaks in a ligament [10]**.** Diode laser is an electrically pumped semiconductor laser in which the active medium is formed by a p-n junction of a semiconductor diode [1, 2].

Aim of Study

This study was carried out to**:**

1) Isolate and diagnose of Aspergillus *spp.* and
2) Evaluate the effect of low-power diode laser on growth of *Aspergillus spp.*

Materials and Methods

1) The sample

fifty samples were taken from skin scraping and hair. The samples were collected from human after cleaning the area with 70% ethanol to remove contaminants such as bacteria [13, 14].

2) Specimens analysis

There are three examination for analysis the specimens**:**

a) **Direct examination (direct microscopy):** It served only as a screening test for the presence or absence of fungi but could differentiate among the pathogens**.** Skin scraping hair, etc., fragments were immersed directly in the fluid on a slid and a cover glass was applied, the slid was heated to digest the keratin material for the appearance of fungal cell more clearly. Direct potassium hydroxid (KOH) examination of infected hair to show endothrix or ectothrix type of hair invasion. Skin scraping and other were examined to see hyphae and arthrospore.
b) **Culture**: It was valuable to direct microscopy and was essential at least in all infections. Sabouraud dextrose agar (SDA) and potato dextrose agar (PDA) were used to grow aspergillus fungi [15, 16].
c) **Staining by lacto phenol cotton blue (L.P.C.B.):** cultures were examined directly when begin to grow and form conidia (spores).Place a drop of L.P.C.B. on a clean glass slid with bent dissecting needle, remove a small portion of a colony from the agar surface and place it on the drop of L.P.C.B. sever with a cover slide and examine under the microscope with power 10x and 40x magnification [16].
d) **Exposure to diode laser light:** power diode laser was employed in the present study with an emitting light wave length of 532nm in collimated beam with a diameter of 5mm [1]. According to the procedure of Wilson *et al* [18]. each isolate of *aspergillus* was grown in sabourauds dextrose broth at 25 C for 24hrs.,then it was harvested by centrifugation and resuspended in equal volume of 0.9%(w/v)normal saline and exposed to laser light for 2,4,8,16,32, and 64 minutes.

Results

1) Microscopical morphology

Was studied by direct examination KOH preparation which showed that the hyphae was septate, unbranched conidiophores at a tip, and a swollen vesicle.

2) Culture

The present study showed that 50% of isolates are aspergillus spp from all collected clinical specimens which are mature within 3 days. The cultural characteristics includes green, brown to black surface, cottony texture and the reverse side was brown.

3) The Effect of Time Exposure to Laser Light

The present study showed that decrease in number of aspergillus spp. after exposure to laser for 4, 8, 16, 32, and 64 minutes (Table 1).

Table 1: The effect of laser on isolated Aspergillus spp according to time of exposure. (No. of total exposed isolates =25).

Time (min)	No. of isolates
	Laser
4	25
8	19
16	10
32	4
64	0

Discussion

Direct microscopy and culture of sample are necessary to identify the etiologic agent. Direct microscope examination of clinical sample was rapid method and subsequently culture identification of organisms. In this study found that directly examined clinical sample with KOH preparation was highly positive. The present result was in agreement with the finding of Ajello and Hay, which considered the direct examination as a screening for the presence or absence of fungi [19]. Culture was only method by which the causative agent can be identified. The high positive results obtained from our culture by the growth of fungus. The negative results in culture that agreed with Hafith [20]. In the present study, it was found the etiological agents were non dermatophytic filamentous Aspergillus spp. The present results were agreed with other results [3]. Also, the present study showed that the exposure of Aspergillus spp. to laser light decrease in growth according to exposure time. Recent studies prefer low power diode laser than high power laser for killing fungi and bacteria because the high power laser are: firstly, the light can't distinguish between microbial and mammalian cell and it would be

difficult to destroy the microbes without adjacent host tissue, secondly, those lasers emitting light with a wavelength in or close to U.V. region which may have mutagenic effect on host cells, finally, big size and expensive [7]. On other hand low power diode laser are small and light which lead to easy to handle, cheap, available and painless. For these reasons low power diode laser more prefer than helium neon gas laser. The recent studies showed that the low power diode laser are widely using in surgery [12] and it has come to be applied as a pain relief treatment [18].

Conclusion

Exposure low power diode laser light was inhibited the growth of Aspergillus spp. after 32, 64 minutes.

References

1. Samson RA, Houbraken J, Summerbell RC, Flannigan B, Miller JD. Common and important species of fungi and actinomyetes indoor environment. Microorganisms in Home and Indoor Work Environment. CRC. 2001; 287-292.
2. Schuster E, Dunn-Coleman N, Cabanes F. Ochratoxin A production by strains of asperigllus Niger var Niger. Appl Environ Microbiol. 1994; 60(7):2650-2.
3. Klich MA. Identification of common aspergillus species. Utrecht, the Netherlands, Central Bureauvoor Schimmel Culture. 2002.
4. Mulder CJ. Effect of aspergillus Niger proly endoprotease (AN-PEP) enzyme on the effect of gluten ingestion in patients with coeliac disease, 2009.
5. Varga J, Kocsube S, Toth B, Frisvad JC, Perrone G, Susca A, Meijer M, Samson RA. Aspergillus brasilliensis sp nov., a biseriate black aspergillus species with worldwide distribution. International Journal of Systematic and Evolutionary Microbiology. 2007; 57(8):1925. doi:10.1099/ijs0.65021-0.edit.
6. Samson RA, Houbraken JAMP, Kuijpers AFA, Frank JM, Frisvad JC. New ochratoxin A or sclerotium producing species in aspergillus section Nigri.Studies in mycology. 2004; 50:45-6.
7. Wilson M. Photolysis of oral bacteria and its potential use in the treatment of caries and periodontal disease. Journal of Applied Bacteriology.1993; 75:299-306.
8. Seligman DA, Pullinger AG, Solberg WK. The prevalence of dental attrition and its association with factors of age, gender, occlusion and TMJ symptomatology. Journal of dental research. 198; 67 (10):1323.
9. Nisengard RJ, Newman MG. Oral microbiology and immunology. 2nd ed. Philadelphia. W.B.Saunders Company, 1994.
10. Pape SA. Laser treatment of skin conditions update. Journal of continuing education for practitioners. 1997; 54 (10):724-728.
11. Abouyz Alwanas. Iraqi drug guide. 1st ed. Baghdad national board for the selection of drugs and control drug information burean.1990. 2.79. Hugo wb, and russelad. pharmacutical micrology.4th ed. Oxford. Black well scientific publication. 281-287.
12. Nagatani TS, Yoshida J. Experimer use of semiconductor diode laser for neoroendoscopic surgery. Minimum-Invasive-Neorosurg. 2013; 40(3):98-10.
13. Manciant F, Papini R. Isolation of keratinophilic fungi from the floors of private veterinary clinical in Haly. Vet. Res. Common. 2011; 162-6.
14. Elewiski BF., Large scale epidemiological study of the causal agents of onychomycosis: mycological findings from the multicenter onychomycosis study of terbinafine Arch.Dermatol.2010; 133(13):17-8.
15. Weitzman I, Summerball RC. The dermatophytes .Micrbial Rev 2012; 8:240-59.
16. Davise H. Lorne. Medically important fungi.3rd Edition, Washington, American Society for microbiology.1995; 163:219-51.
17. Farhan RK. Effect of diode laser and photochemical agents on some virulence factors of disinfectant-resistant pseudomonas aerogenosa isolated from wound. Thesis, University of Tikrit, 2003.
18. Igarashi H, Intomata K. Effects of low power gallium aluminum arsenide laser irradiation on the development of synapses in the neonatal rat nippocampus.Acta.Anal. 2011; 140:150-155.
19. Ajello L, Hay RJN (eds), Balows LA. Sumssman M. Microbiology and microbial infections. London: Arnold ;1998
20. Hafith R. Epidemiological and immunological studies in patients with dermatophytosis. Msc. Thesis. Al-Nahreen University, 2004.