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EFFECT OF DIODE LASER-ACTIVATED ANTIMICROBIAL AGENTS (LAAAS) ON PATHOGENIC BACTERIA

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ABSTRACT

Laser-activated antimicrobial agents (LAAAS) are drugs that have no antimicrobial activity in the dark but can be activated by laser of an appropriate wavelength. The effect of photosensitizers (silver nanoparticle, Methylene blue Dye) was studied by the presence and absence of laser on two types of gram positive (*Staphylococcus aureus*) and negative (Pseudomonas *aeruginosa*) bacteria. The work was in several stages where the first stage was without the use of laser, which is divided in turn to the effect of dye only, the effect of nanoparticles only, the joint effect of dye and nanoparticles. The second phase was on the same format used previously but added to the effect of low power diode laser. Our results showed that the effective antibacterial of silver nanoparticle; (methylene blue) and combined between them were differenced, P. *aeruginosa* more sensitivity than *Staphylococcus aureus*, so AgNPs was more effective against gram positive bacteria than gram negative bacterial strain.

Keywords: Diode laser, LAAAS, silver nanoparticle, Methylene Blue, *Staphylococcus aureus*, Pseudomonas *aeruginosa*

INTRODUCTION

The bactericidal activity of silver nanoparticles against the pathogenic, multidrug-resistant (MDR) as well as multidrug-susceptible strains of bacteria was studied by many scientists, and it was proved that the silver nanoparticles are the powerful weapons against the MDR bacteria such as Pseudomonas aeruginosa, ampicillin-resistant E. coli, erythromycin-resistant Streptococcus pyogenes, methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant Staphylococcus aureus (VRSA) (Rai et al., 2012). These unique properties of nanoparticles have a large surface area, which increases their association with microorganisms (Christensen et al., 2010). as well as their ability to interact with the wall and the cellular membrane, leading to a change in cellular permeability and uncontrolled transfer of phosphate through the plasma membrane. The driving force of the proton, inhibition of ATP manufacturing, as well as its interaction with amino acids, especially the groups of thiol (SH-group) - as well as inhibiting the effectiveness of the enzyme by linking to the active location of the enzyme. Also disrupt the flow of energy which affects the respiratory chain and inhibition of cytochromes (Chaloupka et al., 2010).

Methylene blue has been shown to cause photo damage to the outer membrane, cell wall, ribosomes and nucleic acids of bacteria (Wainwright, 2010). At increasing concentrations, methylene blue may aggregate and form dimers, which may cause a shift in the absorption maximum of the photosensitizer. It has been proposed that dimerization is further induced at the bacterial cell surface due to electrostatic interactions between methylene blue and negatively charged polymers on the bacterial cell surface, and that these dimers are also involved in cell photo damage, as well as monomeric species (Usacheva, et al., 2003).

The effect of laser in the killing and inhibition of bacteria is a series of reactions and chemical and physical-optical changes by activating the biological processes in cells and living tissues in the process of biochemical stimulation (Ali, 2005). The most important biological processes that occur when the interaction of laser beams with cells is to increase the process of phagocytosis, which is the process of eating strange objects such as bacteria and fungi and dead and damaged cells, and the process of phagocytosis when stimulating the cells of white blood cells by laser (Vladimirov, 2004).

Experimental work

Dye: Methylene blue is a heterocyclic aromatic chemical compound with molecular formula (C16H18ClN3S) and molecular weight Mw = 355.89g/mol made in a Fisher Scientific International Company (United Kingdom). Methylene blue (MB) is a cationic Phenothiazine dye that has Physical Form Dark blue-green in the oxidized state, colorless in reduced form (leuko methylene blue), soluble in water, chloroform; sparingly soluble in ethanol, it was melting point 100-110°C, and the maximum absorption peak at 661 nm (Miclescu, et al., 2010). Methylene blue prepared with concentration 1×10-5 mol/liter. Nano particle: Ag Nanoparticle: The properties of Ag Nanoparticle illustrated in Table 1.

Table 1: Properties of silver nanoparticle.

Chemical formula	Nano Ag
Particle Size	50 nm
Purity	99.99 %
Appearance	Gray Powder
Company	Nanjing Nano Technology co, ltd

Laser system: the laser system used for the irradiation consists of a laser device (laser diode was used), The CW diode laser emitting laser light at 650 nm (visible light), with maximum power 500 mwatt, this laser is manufactured in the Chinese huonje company

RESULTS

The antibacterial activity of silver nanoparticles AgNPs; dye (methylene blue) and synergistic both them against two pathogenic bacteria of gram-positive bacteria (Staphylococcus aureus) and gram-negative bacteria (Pseudomonas aeruginosa), by broth micro dilution method.

Table 2: Determination antibacterial activity of different concentration of silver nanoparticles (AgNPs) only; dye (methylene blue) only and their synergistic effect against *S. aureus.*

Material study	Different concentration of three material study(µg/10 ml D.Water)			
	5000	2500	1250	625
	Optical Density (O.D)			
Ag NPs	0.526	0.399	0.354	0.359
Methylene blue	0.566	0.522	0.388	0.394
AgNPs + methylene blue	0.515	0.566	0.552	0.359

Table 3: Determination antibacterial activity of different concentration of **silver nanoparticles (**AgNPs) only; dye(methylene blue) only and their synergistic effect against *P. aeruginosa.*

Material study	Different concentration of three material study (µg /10 ml D.Water)			
	5000	2500	1250	625
	Optical Density (O.D)			
Ag NPs	0.521	0.372	0.339	0.314
methylene blue	0.566	0.588	1.107	1.015
AgNPs + methylene blue	0.542	0.500	0.471	0.353

The figures and images below show the effect of the diode laser on the bacteria.

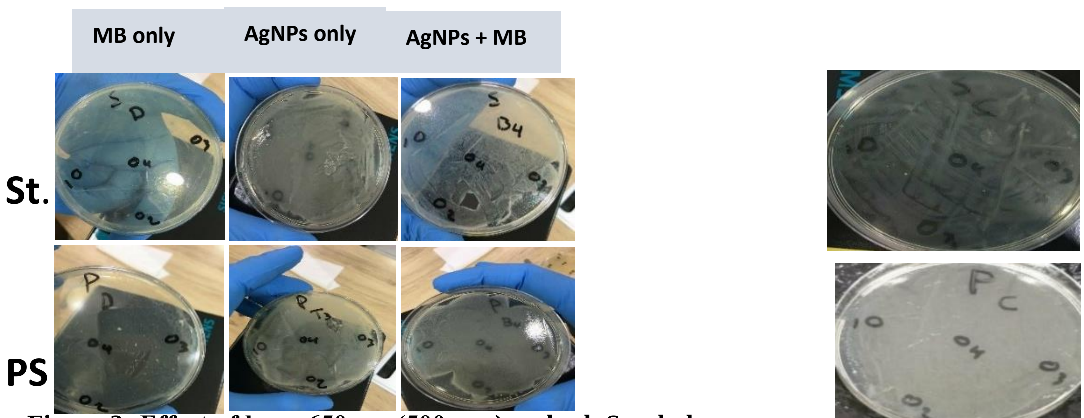
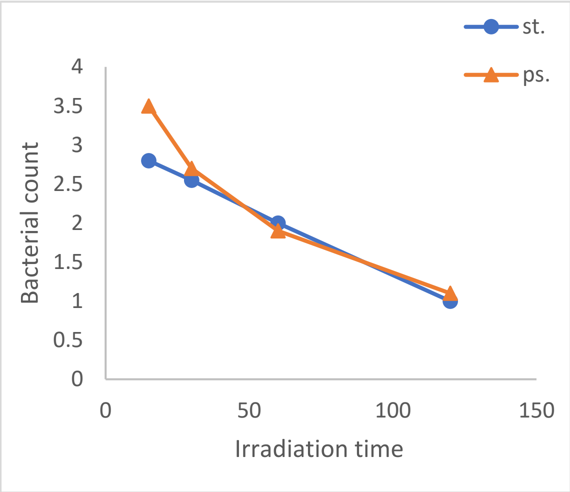


Figure 3: Effect of laser 650 nm (500 mw) on both Staphylococcus *aureus and Pseudomonas aeruginosa, after* at 15 second; 30 second; 1 minutes and 2 minutes *of irradiation.*



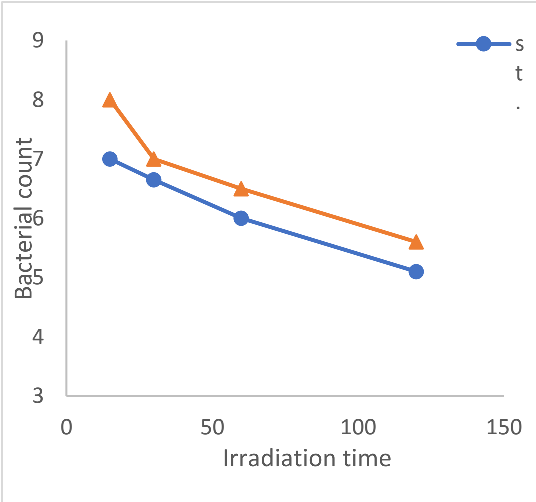


Figure 5: Effect of laser light 650 nm (500 mw) with treatment of nanoparticles (AgNPs) and methylene blue on both Staphylococcus *aureus and Pseudomonas aeruginosa.*

DISCUSION

The antibacterial activity of silver nanoparticles AgNPs; dye (methylene blue) and synergistic both them against gram-positive bacteria (*Staphylococcus aureus*) was illustrated in Table (2) which is showed the lowest concentrations that inhibitory effect of AgNPs against *Staph. aureus* were observed with lower concentration of 1250 µg/ml as 0.354, followed by 625µg/ml as 0.359, whilst the optical density for this bacteria was 0.399 by concentration as 2500 µg/ml as well as the 1250µg /ml methylene blue caused reduction in bacterial density as 0.388 followed both concentration 5000 and 2500µg/ml by reduced optical bacterial density as 0.566 and 0.522 respectively, Interestingly, results showed that when combination both methylene blue and AgNPs, against *Staph. aureus* was observed with lower concentration of 625µg/ml as 0.359 followed by 2500µg/ml as 0.566 of bacterial density.

In table 3 the results showed that 2500 and 1250 µg/ml treatments of AgNPs decreased bacterial optical density as 0.372 and 0.339 respectively , whilst 625µg/ml decreased bacterial optical density as 0.314 , but treatments with methylene blue 5000 and 2500µg/ml caused reduction in optical density (0.566 and 0.588) respectively, Interestingly for *P. aeruginosa* exposure to concentration of both AgNPs and methylene blue 625µg/ml were greater inhibited of bacterial optical density as 0.353,but 2500and 12500µg/ml respectively caused reduced optical density as 0.400 and 0.471 respectively. Our results showed that the effective antibacterial of AgNPs; dye (methylene blue) and combined between them were differenced, P. *aeruginosa* more sensitivity than *Staphylococcus aureus*, so AgNPs was more effective against gram positive bacteria than gram negative bacterial strain, as well as AgNPs may differ in size of nanoparticle and from one bacterial species to others, so differences in the composition of the cell wall and structure membrane (Madigan *et al.*, 2006). In contrast, high activity against *S. aureus* were observed in earlier studies (Kim *et al.*, 2007, Fayaz *et al.*, 2010, Dar *et al.*, 2013), differential sensitivity between *Staphylococcus aureus and Pseudomonas aeruginosa strain* may be related to the composition of cell wall of *Staphylococcus aureus and Pseudomonas aeruginosa strain* (Inphonlek *et al.*, 2010), as well as Ramgopal *et al.,* (2011) explained the difference susceptible between gram-negative and gram-positive bacteria due the differences in the morphology between them, so outer polysaccharide membrane of gram-negative bacteria carried lipopolysaccharide components, which makes cell wall impermeable to the lipophilic solutes, but gram-positive bacteria should more sensitivity, because it has only outer peptidoglycan layer, that don't effective permeability barrier (Sunitha *et al.,* 2013), also the silver nanoparticles caused increased antibacterial activity due its small size and allowed them to easily interacted with the others bacterial particles so in gram negative bacteria as *P. Aeruginosa* , potent bactericidal activity of silver may be due to the strong interactions between negatively charged components of cell wall and cationic compounds (Gurunathan *et al.*, 2014). Anthony *et al.*, (2014) showed 10µg/ml of AgNPs decreased cell viability completely, and reported of Hwang *et al., (*2012) that appearance the nanoparticals AgNPs, that adhere to the cell wall of bacteria and destroying bacterial cell membrane, causing kill bacteria, as well as the AgNPs may be pass by cell wall of the bacteria to oxidize surface proteins on plasma membrane and consequently caused disturb the cellular homeostasis (Nel *et al.*, 2006, Sondi *et al.*, 2004), also Sondi *et al.*, (2004) reported nanoparticals AgNPs may be attach to surface of cell membrane and caused disturb functions as respiration and permeability (Sondi *et al.*, 2004). Rai *et al.,* (2012) in his study showed silver nanoparticles has bactericidal activity against many important pathogenic bacteria such as multidrug-resistant (MDR) bacteria as *Pseudomonas aeruginosa; Escherichia coli*; vancomycin-resistant *Staphylococcus aureus* (VRSA) and methicillin-resistant *Staphylococcus aureus* (MRSA). Combination between AgNPs and methylene blue caused increased antimicrobial activity and increased levels of cell death (Gurunathan *et al.*, 2014), because the groups of active functional methylene blue as amino and hydroxyl groups may be react with larger surface area of nanoparticle (AgNPs) by the chelation (Fayaz etal., 2010). Morones-Ramirez *et al.,* Showed the mechanism of silver increased the cell death because silver can disrupted the bacterial cellular processes as metabolism; iron homeostasis and disulphide bond formation , therefore these changes can be caused increased in permeability membrane that can potentiate the activity of a broad range of dye (methylene blue) against Gram-negative bacteria in different metabolic states, as well as same mechanism may be playing when using nanoparticle (AgNPs) as an adjuvant with methylene blue (Gurunathan *et al.*, 2014). The effect of the diode laser on the bacteria was illustrated by the figures 4 and 5. Figure 4 showed high increased activity of AgNPs and dye (methylene blue) against both Staphylococcus *aureus and Pseudomonas aeruginosa* were observed with laser light 650 nm (500 mw) in 15 second. Interestingly, the maximum increased activity of both material (AgNPs and dye) when increased the time from 15 second to 2 minutes (figure 5). In present study showed laser light was able to inhibited both bacterial growth (*Staphylococcus aureus* and *Pseudomonas aeruginosa*) at low fluences in the all range time , In a parallel study, the Sousa *et al.,* (2015) found similar results by laser light was capable to inhibiter growth of both *Staphylococcus aureus* and *Pseudomonas aeruginosa* bacteria at the low fluences over time(no time-dependent effect), On the other hand, our study had different results from Guffey and Wilborn (2006) who showed no significant result by using laser at lower or higher fluences, Nevertheless, this wavelength is close to the ultraviolet light spectrum, whose antibacterial effects are well known (Maclean *et al.*, 2008) as well as Guffey *et al*., (2013) reported that highest inhibition (59.49%) after the 4th day of irradiation, but bacteria may be become more resistant to the irradiation as a result of treatment;, proliferating; more over time. As well as Bumah *et al.*, (2013) appearance in his study the low inhibited at higher bacterial concentrations and demonstrating that bacterial density is relevant, for a successful; treatment. comparable results by Guffey and Wilborn, (2006), who observed inhibited P. *aeruginosa* by lasers of 405, 470nm, 160 mW and 150 mW, because gram-positive (*S. aureus*) bacteria have thicker cell walls, whilst gram-negative (*P. aeruginosa*) bacteria have thinner cell walls (Morita et al., 2013). These structural divergences may be; factors determining the; penetration of the laser irradiation as biological effects (Sousa et al., 2015). the best result when combination of antibiotic treatment with laser for two-minute, bacteria which began to wreak and increased the sensitivity to antibiotics but Al-Hakak (2017) showed that the best his result was at one minute.

V. Conclusion:

Silver nanoparticles (AgNPs) showed stronger antimicrobial activity against *Pseudomonas aeruginosa* than *Staphylococcus aureus*, therefore can be considered as the potentially useful in the biological applications.

Silver nanoparticles (AgNPs) synergistic with dye (methylene blue) showed highest antibacterial activity. but both of them more activity with laser light at 650 nm.

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REFERENCES

Miclescu, A. andL. Wiklund, Methylene blue, an old drug with new indications", Jurnalul Român de Anestezie Terapie intensive, 17(1), 35-41, (2010).

Ali, C.I., (2005) Immunomodulatory effect of low power laser light on phagocytic function and virulence factors of *Pseudomonas aeruginosa* clinical isolates, Ph.D. thesis, Tikrit university Pp 1 – 7, 25 – 29 (2005).

Al-Hakak, Z.M., A pilot study for the treatment of microbial skin infections caused by bacteria pseudomonas Aeruginosa bacteria that resistance to antibiotic in human and animal by (ND: Yag laser) technology. Pak. J. Biotechnol. 14(3):417-422 (2017).

Anthony K.J.P., Murugan M. and S. Gurunathan, Biosynthesis of silver nanoparticles from the culture supernatant of Bacillus marisflavi and their potential antibacterial activity. J. Ind. Eng. Chem. 9: 1505–1510 (2014).

Bumah V.V., Masson–Meyers D.S., Cashin S.E., and C.S. Enwemeka, Wavelength and bacterial density influence the bactericidal effect of blue light on methicillin-resistant *Staphylococcus aureus* (MRSA). Photomed Laser Surg. 31: 547 –553 (2013).

Chaloupka K., Malam Y. and A.M. Seifalian, Nano silver as a new generation of nanoproduct in biomedical applications. Trends Biotechnol 28(11): 580-8 (2010a).

Christensen F.M., Johnston H.J., Stone V., Aitken R. J., Hankin S., Peters S. and K. Aschberger, Nano silver feasibility and challenges for human health risk assessment based on open literature. Nanotoxicology 4(3): 284-295 (2010).

Dar M.A., Ingle A. and M. Rai, Enhanced antimicrobial activity of silver nanoparticles synthesized by Cryphonectria sp. evaluated singly and in combination with dye (methyl blue). Nanomed: Nanotechnol. Biol. Med. 9: 105–110 (2013).

Fayaz A.M., Balaji K., Girilal M., Yadav R., Kalaichelvan P.T. and R. Venketesan, Biogenic synthesis of silver nanoparticles and their synergistic effect with dye (methyl blue): a study against gram-positive and gram-negative bacteria. Nanomed: Nanotechnol Biol Med. 9: 103–109 (2010).

Guffey J.S., Payne W., Jones T. and K. Martin, Evidence of resistance development by Staph*hylococcus aureus*to an *in vitro*, multiple stage application of 405 nm light from a supraluminous diode array. Photomed. Laser Surg. 31: 179–182 (2013).

Guffey J.S. and J. Wilborn, In vitro bactericidal effects of 405nm and 470nm blue light. Photomed Laser Surg. 24: 684–688 (2006)

Gurunathan, S., Han, J.W., Kwon, D.N. and J. Jin-Hoi Kim, Enhanced antibacterial and antibiofilm activities of silver nanoparticles against Gram-negative and Gram-positive bacteria. Nanoscale Res Lett. 9(1): 373 (2014).

Hwang I.S., Hwang J.H., Choi H., Kim K.J. and D. G. Lee, Synergistic effects between silver nanoparticles and dye (methyl blue) and the mechanisms involved. J. Med. Microbiol. 9: 1719–1726 (2012).

Inphonlek S., Pimpha N. and P. Sunintaboon, Synthesis of poly (methyl methacrylate) core/ chitosan-mixed-polyethyleneimine shell nanoparticles and their antibacterial property. Colloids Surf B: Biointerfaces 9: 219–226 (2010).

Kim J.S., Kuk E., Yu K.N., Kim J.H., Park S.J., Lee H.J., Kim S.H., Park Y.K., Park Y.H., Hwang C.Y., Kim Y.K., Lee Y.S., Jeong D.H. and M. H. Cho, Antimicrobial effects of silver nanoparticles. Nanomed. Nanotechnol. Biol. Med. 9: 95–101 (2007).

Maclean M., MacGregor S.J., Anderson J.G. and G. Woolsey, High-intensity narrow-spectrum light inactivation and wavelength sensitivity of *Staphylococcus aureus*. FEMS Microbiol. Lett. 285: 227–232 (2008).

Morita S., Tagai C., Shiraishi T., Miyaji K. and S. Iwamuro, Differential mode of antimicrobial actions of arginine-rich and lysine-rich histones against gram-positive *Staphylococcus aureus*. Peptides 48: 75–82 (2013).

Nel A., Xia T., Madler L. and N. Li, Toxic potential of materials at the nanolevel. Science 9: 622–627 (2006).

Sabnis, R.W., Hand Book of Biological Dyes and Stains, Synthesis and Industrial Applications, John Wiley & Sons, Inc, Hoboken, New Jersey (2010).

Rai M.K., Deshmukh S.D., Ingle A.P. and A.K. Gade, Silver nanoparticles: the powerful nanoweapon against multidrug-resistant bacteria. J Appl. Microbiol. 112(5): 841-852 (2012).

Ramgopal, M., Sai sushma, Attitalla I.H. and A.M. Alhasin, *Res. J. Microbiol*. 6: 432-438 (2011).

Sondi I. and B. Salopek-Sondi, Silver nanoparticles as antimicrobial agent: a case study on E-coli as a model for Gram-negative bacteria. J Colloid Interface Sci. 9: 177–182 (2004).

Sousa, N.T., Santos, M.F., Gomes, R.C., Brandino, H.E. Martinez, R. and R.R. Guirro, Blue Laser

Inhibits Bacterial Growth of *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Photomed. Laser Surg. 33(5): 278–282 (2015).

Sunitha, Abdeen, Rimal Isaac, R.S. Sweetly, G., Sornalekshmi, S. Arsula, R. And P.K. Praseetha, Evaluation of Antimicrobial Activity of Biosynthesized Iron and Silver Nanoparticles Using the Fungi Fusarium Oxysporum and Actinomycetes sp. on Human Pathogens. Nano Biomed. Eng. 5(1): 39-45 (2013).

Usacheva, M.N., Teichert, M.C. and M.A. Biel, The Role of the Methylene Blue and Toluidine Blue Monomers and Dimers in the Photoinactivation of Bacteria. *Journal of Photochemistry and Photobiology B-Biology* 71: 87-98 (2003).

Vladimirov,Yu A., Osipov, A.N. and G.I. Klebanov, Photobiological principles of therapeutic applications of laser radiation. Biochem. (Moscow) 69(1): 81 – 90 (2000).

Wainwright, M., Safe Photoantimicrobials for Skin and Soft-Tissue Infections. *Int. J. Antimicrob. Agents* 36: 14-18 (2010)