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Research article

Photodynamic therapy for hair removal

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ABSTRACT

Background: Unwanted hair is one of the most common medical problems affecting women of reproductive age inducing a lot of psychological stress and threatening their femininity and self-esteem. Old methods of removing unwanted hair include shaving, waxing, chemical depilation, and electrolysis, all of which have temporary results. However laser-assisted hair removal is the most efficient method of long-term hair removal currently available. It is desirable to develop a reduced cost photodynamic therapy (PDT) system whose properties should include high efficiency and low side-effects.

Method: Mice skin tissues were used in this study and divided into six groups such as controls, free methylene blue (MB) incubation, liposome methylene blue (MB) incubation, laser without methylene blue (MB), free methylene blue (MB) for 3 and 4 h and laser, liposome methylene blue (MB) for 3 h and laser. Methylene blue (MB) was applied to wax epilated areas. The areas were irradiated with CW He-Ne laser system that emits orange-red light with wavelength 632.8 nm and 10 mW at energy density of 5 J/cm^2 for 10 min. The UV-visible absorption spectrum was collected by Cary spectrophotometer.

Results: Methylene blue (MB) is selectively absorbed by actively growing hair follicles due to its cationic property. Methylene blue (MB) untreated sections showed that hair follicle and sebaceous gland are intact and there is no change due to the laser exposure. Free methylene blue (MB) sections incubated for 3 h showed that He:Ne laser induced destruction in hair follicles, leaving an intact epidermis. Treated section with free methylene blue (MB) for 4 h showed degeneration and necrosis in hair follicle, leaving an intact epidermis. Liposomal methylene blue (MB) sections incubated for 3 h showed He:Ne laser induced destruction in hair follicles with intradermal leucocytic infiltration.

Conclusion: Low power CW He:Ne laser and methylene blue (MB) offered a successful PDT system in selectively damaging hair follicles, leaving an intact epidermis. The current PDT system provides better outcome than hair destruction through laser heat transfer procedures and laser-mediated hair removal, due to complete destruction of hair follicles.

Keywords: Photodynamic therapy (PDT), hair removal, Methylene blue (MB) photosensitizer and He:Ne laser, UV-visible spectroscopy

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1. INTRODUCTION

Hair reduction and hair removal are two of the most common procedures performed by dermatologists all over the world, either for cosmetic reasons[1] or for therapeutics reasons as in cases of hirsutism, which is an important medical problem affecting about 8% of women in the reproductive age inducing a lot of psychological stress since it is an embarrassing condition that threatens both a woman's perception of her femininity and her self-esteem.[2,3]

Old methods of removing unwanted hair include shaving, waxing, chemical depilation, and electrolysis, all of which have temporary results. However laser-assisted hair removal is the most efficient method of long-term hair removal currently available.[4] There has been an explosive increase in the use of lasers for hair removal since the first lasers were approved in 1996. Several hair removal systems have been shown to be effective in this setting: the ruby laser (694 nm), the alexandrite laser (755 nm), the diode laser (800 nm), an intense pulsed light source (590 to 1200 nm), and the neodymium:yttrium-aluminum-garnet (Nd:YAG) laser (1064 nm), with or without the application of carbon suspension. The parameters used with each laser system are effective in removing hair without damaging the surrounding skin is based on selective photothermolysis—the theory that at a particular wavelength, pulse duration, and fluence, thermal injury is confined to a target that contains a light-absorbing molecule called a chromophore.[5] The target chromophore in laser hair removal is melanin in the hair shaft, whose removal is accomplished through follicular unit destruction.[6] Thus individuals with red or light-colored hair and Fitzpatrick phototype II skin have decreased efficacy of laser treatment than those with dark-colored hair and the same phototype.[7] Concurrently, the abundance of melanin in the epidermis of patients with dark skin color has always been regarded as hazardous due to the increased incidence of side effects in this patient population, including hyperpigmentation.[8]

Multiple lasers and intense pulsed light sources have been shown to provide long-term hair removal, where, some treatments with alexandrite and diode lasers lead to temporary short-term 50% hair reduction up to six months after treatment.[9] However, the persistence of follicular elements after two and three treatments indicates that there is no evidence of permanent follicle death and that laser either inhibits or inhibits or alters normal hair cycle.[10]

Since complete damage of the hair follicle without damaging the surrounding skin is desirable, in the present study we present a technique in which selective damage of the hair follicle leaving an intact epidermis, by using topical photosensitiser and laser radiation can be expected to be a long lasting effect. Photodynamic therapy for hair removal has been suggested by several authors (https://www.bcidaho.com/providers/medical_policies/med/mp_20,144.asp),[11] however no experimental attempt has been carried out.

The clinical applications of the PDT sometimes are costly. Therefore, it is desirable to develop reduced cost PDT system whose properties should include high efficiency and low side-effects. To fulfil these requirements, some organic dyes have been proposed as good PDT candidates. Methylene blue (MB) structure is shown in Figure 1,[12] a well-known dye with high light absorption at 665 nm, is effective

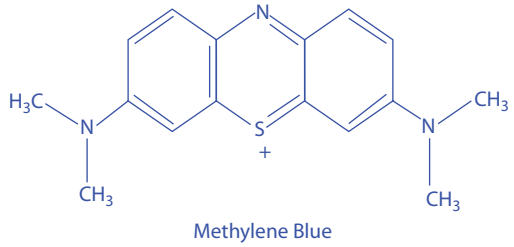

Figure 1. Structure of Methylene Blue (MB).

in PDT, showing ability to generate $^1O_2$ and photodynamic activity for clinical applications against several diseases.[13,14] Even without light, Methylene blue (MB) has natural antifungal and antibacterial activity,[15,16] whose toxic potential can be increased by light activation.

The aim of the present study is to contribute to PDT development by researching to find an adequate PDT system including He-Ne laser source and its effect on the photodynamic activity of Methylene blue (MB) in biological experiments. The investigation of the effectiveness of PDT with of Methylene blue (MB) and a He-Ne device was carried out on mice skin.

2. METHODOLOGY

2.1 Photosensitizer and light

Methylene blue (MB) (C_{16} H_{18} $ClN_3S.3H_2O$), which has MW of 373.90 g mol^{-1} (Figure 1),[12] and all other reagents and solvents used were of analytical grade. The methylene blue (MB) stock solution of 2.65×10^{-2} mol l^{-1} was prepared in deionized water and then diluted by the appropriate volume to obtain the test solutions. Continuous wave (CW) He-Ne (CVI Melles Griot Co., Japan) light system that contains 4 units in series that emitted orange-red light with wavelength 632.8 nm at energy density of 5 J/cm^2 was used. He-Ne laser source was controlled using a handheld laser power meter. The UV-visible absorption spectrum was obtained by a Cary 50 spectrophotometer (Agilent Technologies, South San Francisco, CA, USA). The Methylene blue (MB) treated and untreated skin samples were exposed to 10 mW CW He-Ne laser (wavelength 632.8 nm) at energy density of 5 J/cm^2 for 10 min and the distance between the laser source and the skin tissue sample is 5 cm.

2.2 Preparation of Methylene blue loaded liposomes

Multilamellar vesicles containing methylene blue (MB) were prepared by traditional hydration method, 3-sn-phosphatidylcholine from soybean, and Cholesterol at weights (100 mg Soy PC: 60 mg CHOL) respectively were dissolved in 10 ml chloroform.[17] The thin lipid film was obtained by removing the organic solvent under a vacuum condition using a rotary evaporator of 240 min^{-1} speed under reduced pressure and nitrogen gas to prevent the oxidation of the phospholipids. 100 mg Methylene blue (MB) in 20 ml phosphate buffer of pH 7 was added to the dried thin film in the flask that kept rotated in water bath at 50°C for 2 h. Liposomal suspension was placed in ultrasonic water bath for 30 min. The un-entrapped methylene blue was removed by size exclusion gel chromatography using sephadex G-75. The gel column was washed three times with phosphate buffer of pH 7 for the elution. Methylene blue (MB) in the prepared liposomes, 0.5 ml of liposomes suspension was dissolved in 0.5 ml detergent (10% Triton X100).

The diameter of the liposomes was determined using dynamic light scattering system (LPA PARIII, Otsuka Electronics, Japan) at 25°C scattering angle of 90° and 12,000 count per second. Liposome dispersions were diluted with pH 7.4 PBS and zeta potential values were measured at 25°C using Zetasizer 2000 (Malvern Instruments, Malvern, U.K.). Liposomal encapsulation efficiency was measured by gel filtration method using Sephadex column (Sephadex G-25 column, Amersham Pharmacia, Sweden).

2.3 Preparation of control hydrogels

To compare the effects of using liposomes on the skin permeation of methylene Blue, liposome- free control hydrogels were prepared separately. Because of low water solubility of methylene blue (MB), it was solubilized with Transcutol® and dispersed the mixture into poloxamer gel matrix, in which the amount of methylene blue (MB) added was 0.2%.

2.4 Preparation of Liposomal Hydrogels

The liposomal hydrogel (lipogel) was prepared by dispersing liposomal solution into pre-swollen 25% poloxamer gel matrix at 4°C. The final concentration of methylene blue (MB) in lipogels was the same as the control hydrogel.

2.5 Passive Skin Permeation Study

The mice dorsal skin was removed carefully leaving the fat tissue and rinsed with PBS. Prepared skin samples were stored at 70°C until skin permeation experiments. One gram of hydrogel samples was loaded onto the donor compartment of Franz diffusion cells with a permeation area of 2.00 cm^2 and PBS containing 30% ethanol was used as a receptor solution (11 ml) maintained at 37°C.

2.6 Skin permeation study under electrically assisted condition

The experimental condition of electrically assisted skin permeation studies was basically the same as the passive diffusion studies except for the application of electric current supplying system. To mimic electric current supplying conditions from skin massager, we prepared power supply and applied a fixed cathode current of 0.4 mA/cm^2. The reason for being chosen a current of 0.4 mA/cm^2 was because current densities below 0.5 mA/cm^2 were generally considered to be well tolerated in humans.

In this study, the electric current was applied for 30 min in a pulsatile manner with on and off every 30 s to simulate skin massage condition.

2.7 Animals

Mice (male and female) 20 gm were used to evaluate the penetration of liposomal MB and free gel methylene blue (MB) into pilosebaceous unit, and its ability to destruct pilosebaceous unit loaded with of methylene blue (MB), the experiments were performed in accordance with the animal use committee guide lines, this the protocol was approved by our animal use and ethic committee, the mice were anaesthetized with chloroform and ether 1:1 after shaving the remaining hair was removed using cold wax strip the skin was cleansed with ethanol the formulation was applied onto the entire back of each animal. The animal divided into six groups such as controls (3 animals), Free methylene blue (MB) (6 animals), liposome methylene blue (MB) (6 animals), laser without methylene blue (MB) treatment (6 animals), free methylene blue (MB) and laser (6 animals) and liposome methylene blue (MB) exposed cells (6 animals).

3. RESULTS

Figure 2 shows the UV-visible absorption spectrum of methylene blue (MB) that allows for a wide range of electromagnetic radiation options within the visible spectrum with maximum absorption band centred at 665 nm. The spectrum showed full absorption compatibility with He-Ne laser emitted wavelength at 632.8 nm. The full compatibility between the absorption spectrum of the photosensitizer and the source of laser is one of the main factors that determine the PDT effect. Low power continuous wave (CW) He-Ne laser light sources with wavelength 632.8 nm used to enhance the cosmetic effect of PDT procedure. The combination between short contact methylene blue (MB) and low power continuous wave (CW) He-Ne laser may make PDT more practical and attractive to laser cosmetics.

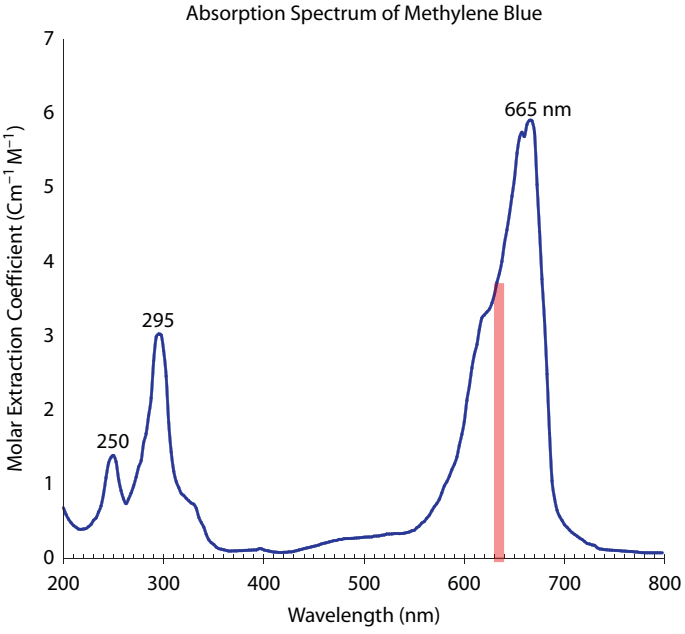

Figure 2. UV-visible absorption spectrum of Methylene blue (MB). The spectrum is showing absorption maxima at 665, 295 and 250 nm. Overlying wavelength (632.8 nm) of continuous wave (CW) He-Ne laser source devices.

Figure 3 shows a microscopic cross-section of mice skin before undergoing to photodynamic therapy (PDT) procedure. The Figure shows that the structure of the skin contains numerous amounts of intact hair follicle and sebaceous glands. The cross-section of mice skin after treatment with free methylene blue (MB) for 3 h is shown in Figure 4. The methylene blue (MB) photosensitizer is selectively absorbed by actively growing hair follicles in the skin and the leukocyte infiltration and hyperplasia were also observed. It is known that, the absorption of the Methylene blue will depend on the activation and

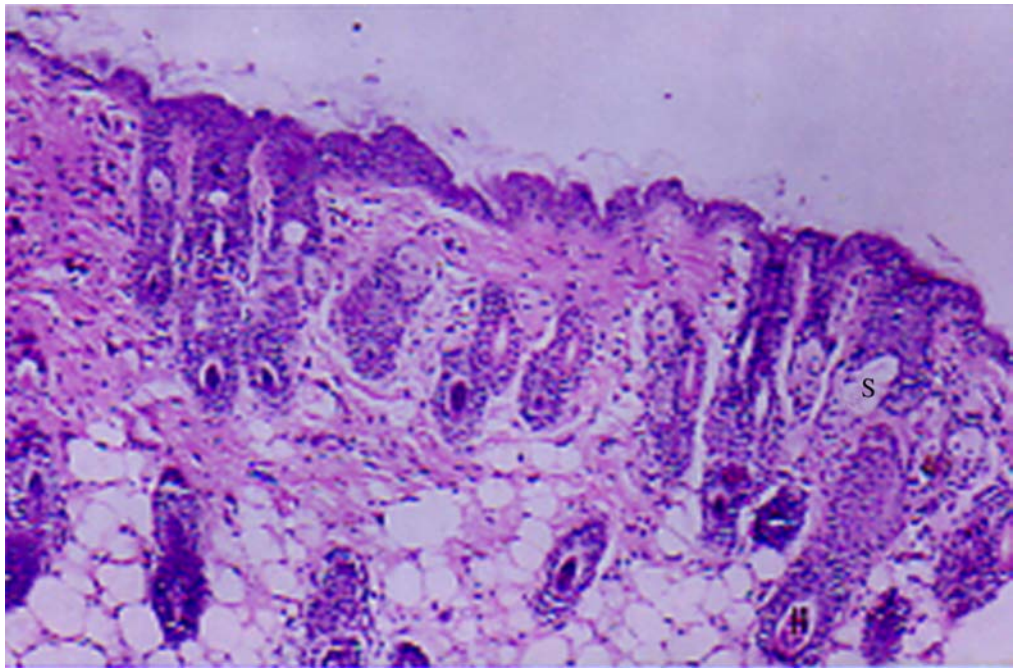

Figure 3. A control cross-section of mice skin, showing normal hair follicles (H) and sebaceous glands (S).

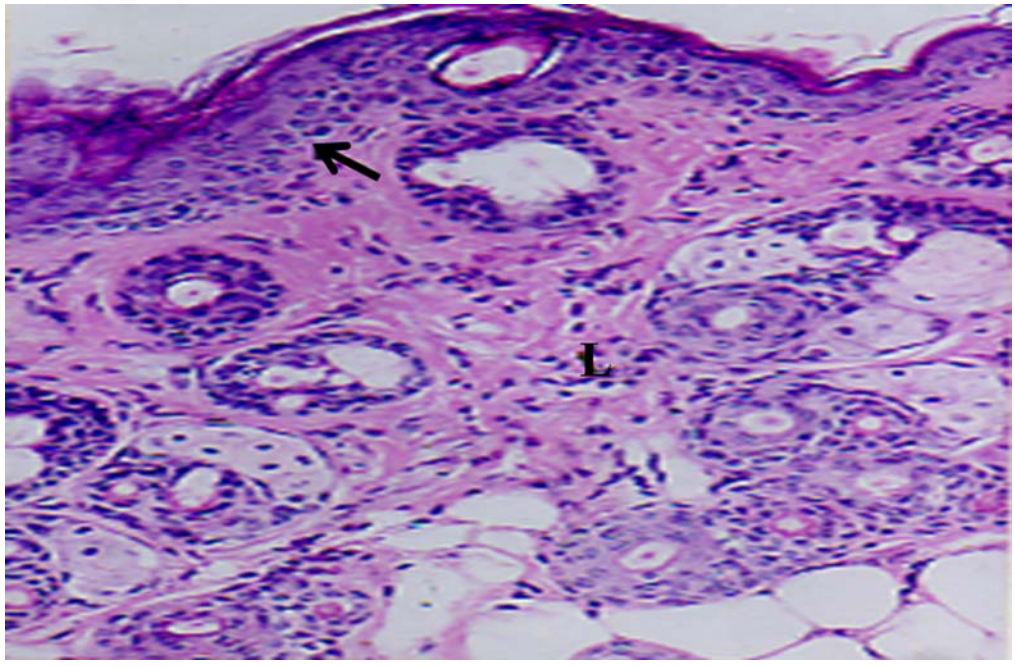

Figure 4. Free MB mice skin sections, incubated for 3 h. Arrow is referring to the epidermal hyperplastic epithelium, and L shows intradermal leukocytic infiltration.

differentiation of the absorbing cells and methylene blue (MB) is selectively absorbed by the mitochondria in actively growing cells due to its cationic property.[18]

Figure 5 shows methylene blue (MB) free skin cross section after irradiated by 10 mW He-Ne laser (wavelength 632.8 nm) at energy density of 5 J/cm^2 for 10 min. The result shows that the hair follicle and sebaceous gland are intact and there is no change due to the laser exposure. This result is due to the fact that the modulation effect of laser irradiation is depending on the dose applied and on the absorbing photosensitiser.[19–22] 3 h methylene blue (MB) incubated skin cross section and irradiated

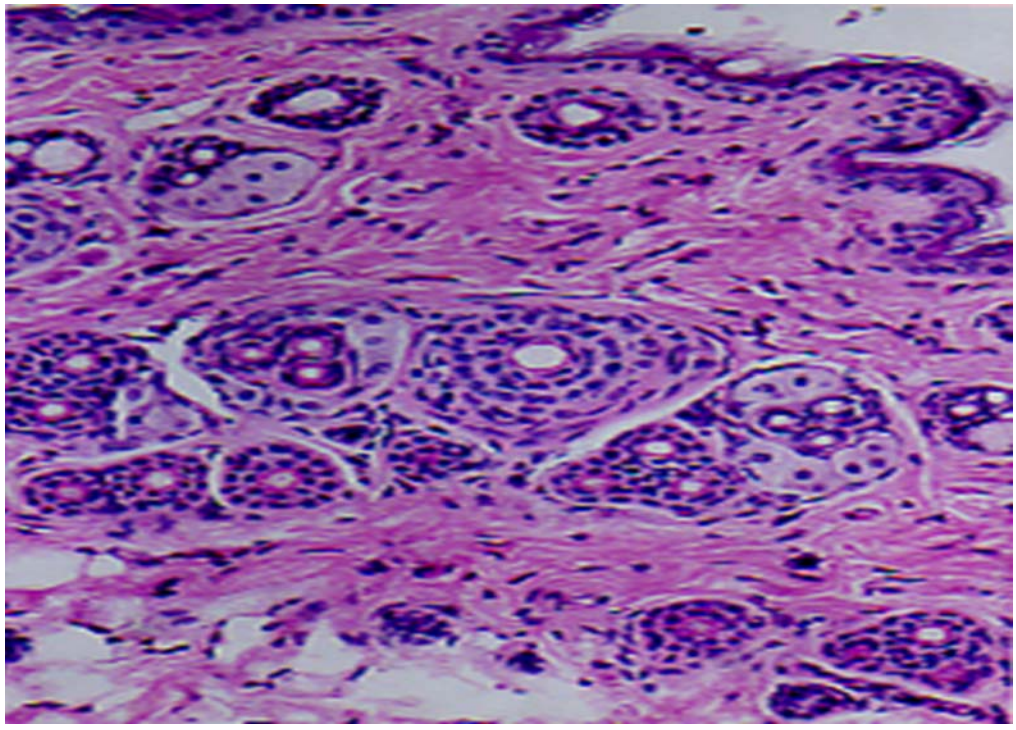

Figure 5. A cross-section of mice skin post exposure of 10 mW He-Ne laser (wavelength 632.8 nm) for 10 min. The slides are showing apparently normal hair follicle (H) and sebaceous gland(s).

by 10 mW He-Ne laser for 10 min is shown in Figure 6. The result shows that He:Ne laser induced destruction in hair follicles, leaving an intact epidermis.

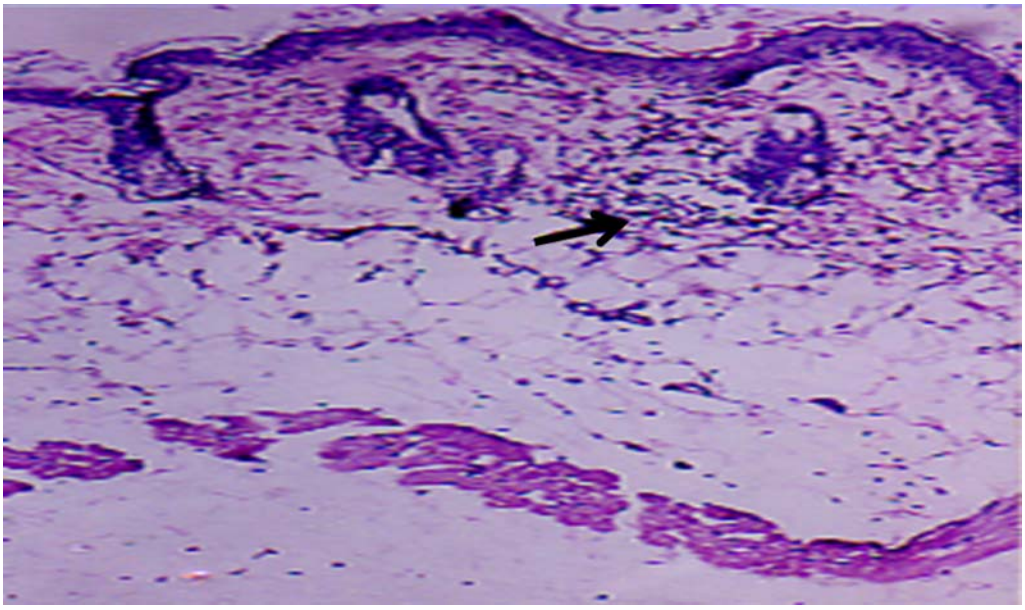

Figure 6. Free MB mice skin sections incubated for 3 h, post-exposure to 10 mW He-Ne laser for 10 min. The slide shows degenerative changes in the hair follicle and intact epidermis.

Figure 7 shows mice skin cross-section incubated in methylene blue (MB) for 4 h and irradiated by 10 mW He-Ne for 10 min. The result shows that degeneration and necrosis in hair follicle, leaving an intact epidermis. 3 h liposomal methylene blue (MB) incubated mice skin cross-section and irradiated by 10 mW He-Ne for 10 min is shown in Figure 8. The result shows that He:Ne laser induced destruction in hair follicles with intradermal leukocytic infiltration as pointed by the black arrows in Figure 8.

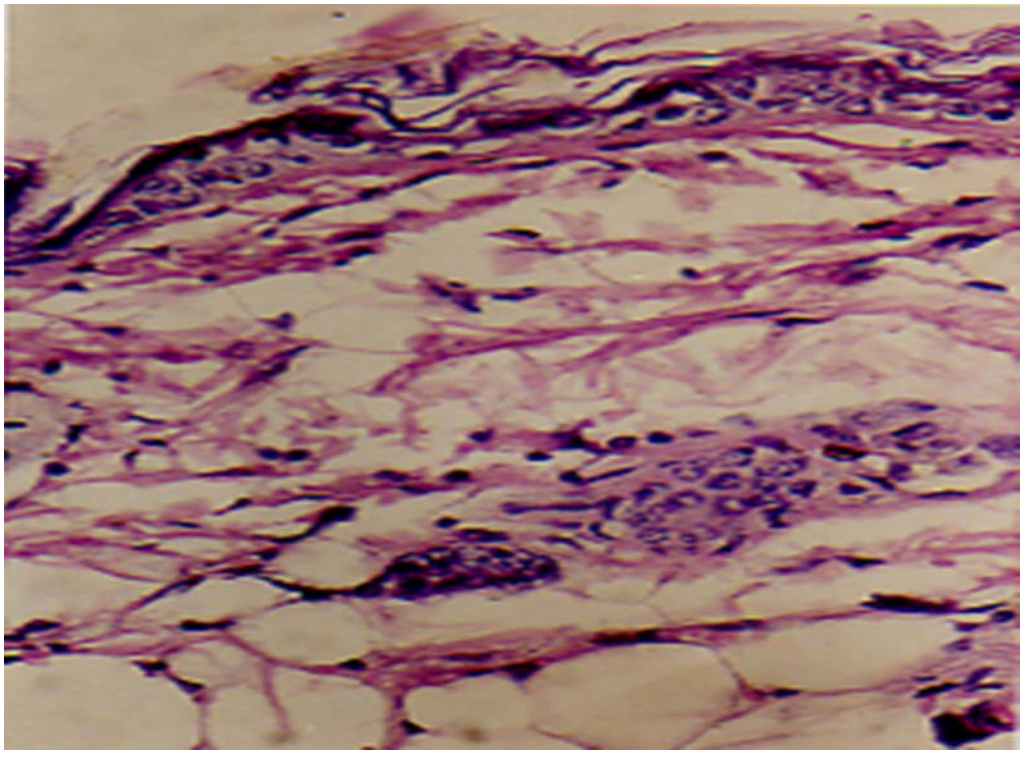

Figure 7. Free MB mice skin sections, incubated for 4 h, post-exposure to 10 mW He-Ne laser for 10 min. The slide shows degeneration and necrosis in the hair follicle, leaving an intact skin epidermis.

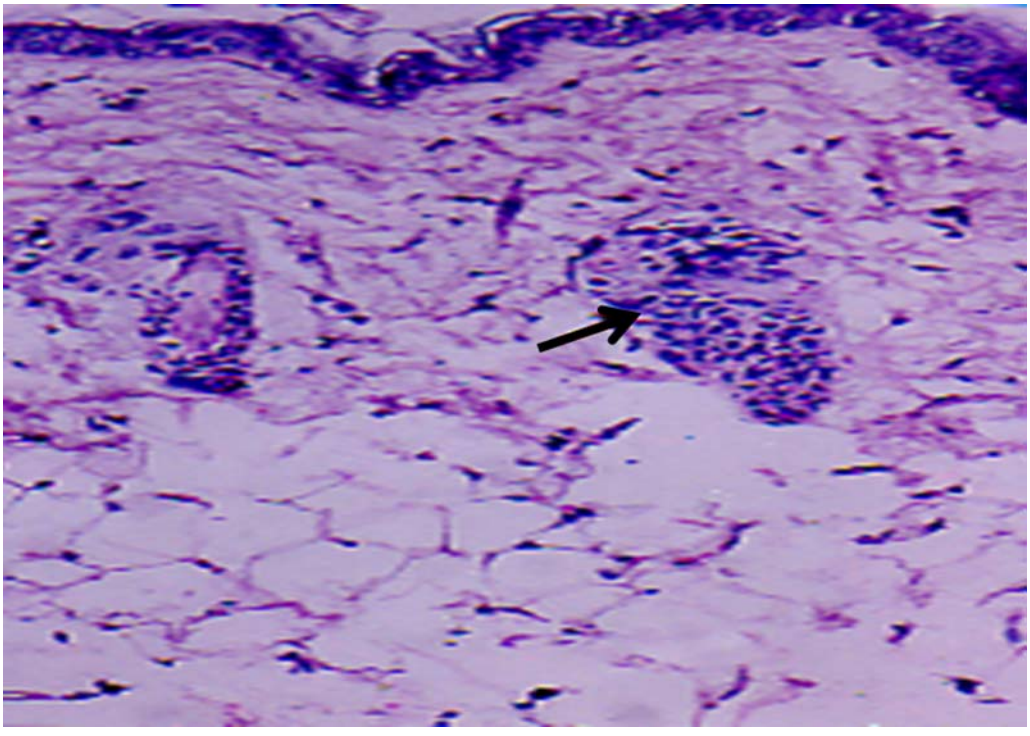

Figure 8. Liposomal MB mice skin sections incubated for 3 h, post-exposure to 10 mW He-Ne laser for 10 min. The slide shows a degenerative hair follicle with intradermal leukocytic infiltration (arrow).

The result in Figure 5 shows less incubation time of the treated skin with methylene blue (MB) can cause certain and limited PDT effect such as destruction in hair follicles. On the other hand, long incubation time can lead to extensive PDT effect such as degeneration and necrosis in hair follicle as shown in Figure 7. Liposomal methylene blue (MB) treated skin tissue samples shows destruction in hair follicles with intradermal leukocytic infiltration (Figure 8), which can refer to the severe effect of the PDT in comparison to the free methylene blue (MB) treatment for the same skin samples.

Figures 5 and 6 can be explained that PDT involved the relatively selective uptake of methylene blue (MB) photosensitizing drug and subsequent irradiation with light of a suitable wavelength to excite it. The excited methylene blue (MB) photosensitizer can react directly with a cellular substrate to form free radicals or may transfer its energy to a molecular substrate such as oxygen to produce highly reactive singlet oxygen, which causes irreversible oxidation of some essential cellular component. Both reactions will culminate in induction of cytotoxicity to irradiated area.[23,24] As a lipophilic substance, methylene blue (MB) could accumulate in the mitochondrial membranes, thereby altering membrane properties and/or the mitochondrial membrane potential.

4. DISCUSSION

The selective absorption of the methylene blue (MB) by the active growing cells such as hair follicles provides a unique characteristic for methylene blue (MB) to be a good candidate for PDT hair removal system. The low power continuous wave He-Ne laser is also good candidate of the PDT system due to the lack of thermal effect, pain, skin burning sensation and post inflammation.[25] The combination between low power He-Ne laser and methylene blue (MB) photosensitizer provide an efficient and save system for hair removal procedure. The methylene blue (MB) incubation time is one of the main factors that affect the PDT system results. The 3 h tissue incubated MB and He-Ne irradiated PDT system gave the optimum hair removal treatment conditions since it is free from post inflammatory hyperpigmentation, and epidermal damage. The tissue- Methylene blue incubation procedure is another factor that affecting the PDT results. The free methylene blue (MB) incubation system showed limited and less extensive results than the liposomal Methylene blue system. The current MB-(CW) He-Ne PDT system showed that is more practical and safe than the short-contact 5 ALA-pulsed – red light PDT system that showed time-consuming treatment, which involved pain, post inflammatory hyperpigmentation, and epidermal damage (https://www.bcidaho.com/providers/medical_policies/med/mp_20,144.asp).[11,25]

The present study incorporated methylene blue (MB) as photosensitizers because it is a well-known dye in medicine and has been discussed as an easily applicable drug for the topical treatment during photodynamic therapy (PDT).[26] Since 1996, there have been numerous advances in hair laser removal that utilize melanin as a chromophore.[6] However, this technique is not effective with light fair hair due to lack of sufficient chromophore[7] and causes permanent hyper-pigmentation in dark skin patients.[8]

The current PDT system in this present study showed incubation with methylene blue (MB) and irradiation with low power CW He:Ne laser induced destruction in hair follicles, leaving an intact epidermis. Photodynamic therapy procedure in completely destructing the hair follicle provides a far better outcome than destructing only hair shaft through laser heat transfer during photoepilation procedure[25] and Laser-mediated hair removal.[27] This is due to the complete destruction of hair follicle using the PDT procedure. The destruction of hair follicle using the current PDT procedure may take up to three years until the hair follicle regains its activity.[28] This is because a hair follicle is periodically regenerate by spontaneously undergoing repetitive cycles of growth (anagen), apoptosis-driven regression (catagen), and relative quiescence (telogen).[29]

Methylene blue (MB) photosensitizer that binds to mitochondria has been proven to induce apoptosis upon exposure to the appropriate wavelength via mitochondria-dependent apoptosis cascade.[30–32] Also, being a photosensitizers that binds to the plasma membrane or lysosomes, methylene blue (MB) will also kill cells, but less efficiently by a non-apoptotic mechanism.[33,34] Previous studies proved the efficiency of the PDT system in RIF-1 murine fibrosarcoma cells[35] and in bladder cancer cells.[36] The results showed that the PDT system induced changes in cellular size, granularity, protein content, RNA and DNA as analysed by flow cytometry.[36] Concurrently, squamous-cell carcinomas of the upper aero- digestive were destroyed *in vitro* through photosensitization with Methylene blue and subsequent radiation with red laser light.[37]

5. CONCLUSION

Dermatologists and cosmetic laser surgeons have become more interested in photodynamic therapy and the most significant practical goal is to develop long lasting effect and safe PDT system for hair removal. It is important to refer to the tremendous impact PDT would have if it ever became a practical and viable option. Low power CW He:Ne laser and methylene blue (MB) offered a very successful PDT system in the present study in selective destructing the hair follicle, leaving an intact epidermis. The current methylene blue (MB)-(CW) He-Ne PDT system results was confirmed using pathological examination and showed that this technique is a safe and appears to provide a long lasting action. Photodynamic therapy using the light activated anti-microbial agent, Methylene blue kills safely methicillin resistant staphylococcus aureus (MRSA) in superficial and deep excisional wounds.[38] Methylene blue (MB) has been already used as antimalarial agent for long time. Recent studies has shown the efficacy of Methylene Blue as an safe, effective and cheap antimalarial agent for Falciparum Malaria treatment.[39] The current PDT system provides better outcome than hair destruction through laser heat transfer procedures and laser-mediated hair removal, due to complete destruction of hair follicles.

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