

In Vitro Study of the Phototherapy Activity of Low-Power Laser Against Embryonic Development and Nit Shell Degradation of Human Head Lice, *Pediculus Humanus Capitis*

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Abstract

Head lice eggs, called nits, cling to the hair base. Nit removal has become important in head lice treatment because main insecticides are not 100% nictidal. The aim of this study is to provide a new method for controlling and treating head lice by inhibiting the development of embryo in their nits (eggs) and nit shell degradation through the use of He-Ne and Nd:YAG laser beams. For this purpose, seventy five nits (ova) of *P. humanus capitis* were collected from children's hair then all nits were divided on three clean Petri dish, two Petri dish were exposed to He-Ne and Nd:YAG laser then put in incubator at $23\pm 2C^{\circ}$ for about a week then followed up the nit hatching rate on daily basis. Results indicate that both types of laser having a powerful role in the inhibition of the head lice nits. The number of hatched nit is equal to 4/25 nits (16%) among Nd:YAG laser expositied nits while the number of hatched nit is equal to 0/25nits (0%) among Gas laser, He-Ne Laser expositied nits in comparing to the control sample, number of hatching egg is equal to 14/25 nits (56%). Chi square association (P. value =0.037) confirmed that the He-Ne laser beam slightly having a stronger effect on the degradation of the head lice nit shell while it is strongly depending on the period of nit exposure.

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1. Introduction

The early stages of the development of laser devices were observed in research laboratories. Still, in recent times, they have obtained clinical acceptance and become product lines for both consumers and researcher [1,2] *Pediculus humanus capitis*, commonly known as a head lice are ectoparasite that affect human head for thousands of years and various pediculicides have been utilized for the treatment of pediculosis since the early 20th century [3]. As a response to the failure of major insecticides to be 100% nictidal, nit removal has become an important part of head lice therapy. Nit refers to the egg of the human head lice which attaches to the base of the hair in

an affected person. It is covered by a thick shell wall and prevents the entry of any type of antiparasite. The head louse, scientifically known as *Pediculus humanus var. capitis* (Anoplura: Pediculidae), is a blood-sucking parasite that is responsible for causing pediculosis. It is one of the most prevalent ectoparasites worldwide [4]. Head lice is a major health issue among children aged 3 to 13 [5]. The main clinical symptom of this parasite is intense itching of the scalp. Additionally, head lice biting can lead to secondary infections caused by various microorganisms [6]. The novelty and unique aspects of this study specifically present low-power laser therapy, using He-Ne and Nd:YAG

lasers, as a novel, non-chemical method to impede embryonic development in head lice nits and decompose nit shells—filling a notable void in existing treatments. This work specifically illustrates a statistically significant impact of He-Ne lasers compared to Nd:YAG lasers under particular settings, offering a comparative analysis that has not been thoroughly investigated in previous studies. Moreover, it targets the increasing resistance to chemical pediculicides by providing a strategy that reduces treatment duration, lowers expenses, and decreases side effects, distinguishing it apart from current procedures that rely exclusively on chemical treatments. The aim of this study is to put a new strategy in order to control and treat the head lice through inhibition of the nit embryonic development using He-Ne and Nd:YAG laser.

2. Materials and Methods

The names and model of equipment used in the study were summarized in table 1). Nits of *Pediculus humanus capitis* were obtained from the hair of children, with a specific focus on dark brown nits. A total of seventy-five nits were collected and distributed evenly into three sterile Petri plates, with each dish having 25 nits. The first and second Petri dishes were utilized as experimental samples, each subjected to a different laser type, whereas the third Petri dish

Table 1. The names and model of equipment used in the present study.

No	Name of equipment	Model
1	Incubator	WP25AB
2	Microscope	Novel/XSZ-N107T
3	Biological safety cabinet and enclosure	Model:CJ-600N
4	Camera	HUAWEI NOVA 7i/ JNY-LX1, 2019
5	Nd:YAG laser	GS-530-500
6	He-Ne laser	AL650T100-TO18

3. Results and Discussion

Results appear that both types of lasers having a powerful role in the inhibition of the head lice nits, number of hatched nit is equal to 4/25 nits (16%) among Nd-YAG exposed nits while number of hatched nit is equal to 0/25nits (0%) among Gas laser, He-Ne Laser exposed nits in comparing to

was designated as the control group. The initial experimental group exposed nits to Nd:YAG laser operating at a power of 500 mW and radiating light at a wavelength of 530 nm for a duration of 3 minutes each day. After being exposed to laser, the nits were placed in an incubator at a temperature of 23°C ± 2°C for around one week. The second experimental group was subjected nits to a helium-neon (He-Ne) laser with a power of 100 mW and a wavelength of 650 nm for a duration of 3 minutes each day. The nits were then placed in an incubator under identical conditions. A similar technique was utilized away wherein both laser types were subjected to a protracted exposure period of 6 minutes. The control group nits were cultured in the absence of laser exposure, while being subjected to the same circumstances. In order to track the progress of embryonic development, every day a review of a single nit from each group was conducted using a microscope with a 40x magnification. This procedure followed the methods described by [7]. Photographs capturing the various phases of embryonic growth were captured on a daily basis using a HUAWEI NOVA 7i (JNY-LX1, 2019) camera and then compared to the control group. The control group nits were affixed to slides and secured with tape, but they were not exposed to laser treatment. These nits were used as a reference point for comparison.

the control sample, number of hatching eggs is equal to 14/25 nits (56%). Chi-square association (P. value =0.037) confirmed that the He-Ne laser beam slightly having a stronger effect on the degradation of the head lice nit shell after nit exposure to laser beam for 3 minutes (Table 2). It is also confirmed that there is a strong relationship between Exposure time to laser beam and percentage of nit hatching. In the present study, results confirm that an increase in time for nit exposure to laser beam leads to increase the efficiency of the laser to penetrate nit shell and inhibit embryonic development 0/25nits especially in case of Nd-YAG using. Therefore, statistically there is no significant relationship between the efficiency of (Nd-YAG) and (He-Ne) Laser when exposure time for Nd-YAG is over as twice as (6 minutes) compared to the exposure time for He-Ne laser (Table, 3; Figure 1).

After nit exposure to laser beam, solid state laser (Nd-YAG), the following steps of sing nit embryonic development had been recorded, at the first day, small spots named, embryonic cell were appeared then the next day, the slow aggregation

of all embryonic cells in the center of the nit had been recorded followed by the degradation of embryonic cells and nit shell at the third day, in contrast the gas laser (He-Ne Laser) lead to nit shell degradation at the second day (Figure 2 and Figure 3). It is very important to mention that the normal embryonic development required seven days and the following embryonic development

had been recorded without the effect of laser beam includes, appearing of the embryonic cells, then aggregation of all cells in the center of the nit followed by appearing of the eye spot and legs at the fourth and fifth day. Two days later (sixth and seventh day) fully embryonic development had been completed and hatching occurred in order to emerge first nymph (Figure 4).

Table 2. Chi-square association compare the efficiency of He-Ne laser Nd-YAG laser as antilice (Exposure period is 3 minutes/day).

Tests	Positive hatching	Negative hatching	Total
He-Ne laser	0 2.0	25 0.2	25 2.2
Nd-Yag laser	4 2.0	21 0.2	25 2.2
Total	4 4.0	46 0.3	50 4.3
chi2(1) = 4.3478 Pr = 0.037			

Table 3. Chi-square association compare the efficiency of He-Ne laser Nd-YAG laser as antilice (Exposure period is 6minutes/day)

Tests	Positive hatching	Negative hatching	Total
He-Ne laser	0 0.5	25 0.0	25 0.5
Nd-Yag laser	1 0.5	24 0.0	25 0.5
Total	1 1.0	49 0.0	50 1.0
chi2(1) = 1.0204 Pr = 0.312			

Figures 1A and 1B illustrate the effect of laser exposure duration (3 minutes and 6 minutes daily) on nit hatching rates. The data indicate an independent time-dependent relationship, where continuous laser exposure significantly reduces hatching rates for both He-Ne and Nd:YAG lasers. The He-Ne laser consistently attained a 0% hatching rate, even with decreased exposure times, demonstrating its higher effectiveness in inhibiting embryonic development compared to the Nd:YAG laser, which showed a 16% hatching rate under the same conditions. This result shows the efficacy of the He-Ne laser as an effective technique for nit control. Figures 2 and 3 present whole microscopic evidence of the embryonic changes caused by laser exposure. Figure 2 represents the impact of Nd:YAG laser on the nit during a three-day period, demonstrating initial embryonic cell clumping, subsequent degradation, and the eventual disintegration of the nit

structure. Conversely, Figure 3 illustrates the He-Ne laser's expedited impact, with observable degradation occurring as early as the second day. The visual observations corroborate the quantitative data, further illustrating the He-Ne laser's superior capacity to penetrate the nit shell and impede its development compared to the Nd:YAG laser. Figure 4 acts as a control reference, illustrating the typical advancement of nit embryonic development in the absence of laser treatment. The images illustrate the sequential development of embryonic cells, the emergence of eye spots and legs, and the subsequent hatching of live nymphs over a span of seven days. Comparing them with Figures 2 and 3 shows the disruptive impacts of both laser types, which not only impede embryonic growth but also increase nit shell degeneration. The integration of statistical results, visual evidence, and time observations collectively provide a thorough

understanding of the efficacy and mechanisms of laser-based treatment for head lice nits. This discourse underscores the study's contributions to

investigating innovative, non-chemical methods for pediculicidal treatment.

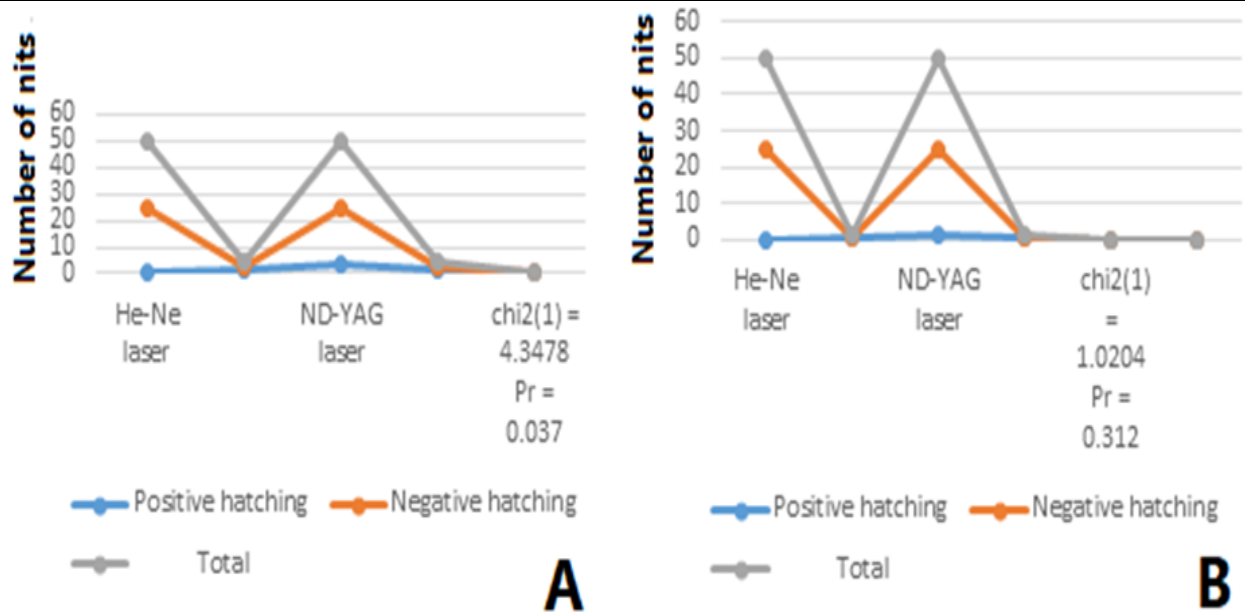


Figure 1. Line charts show effect of laser time exposure and number of nit hatching, three minutes' exposure per day (A) and six minutes of exposure per day (B).

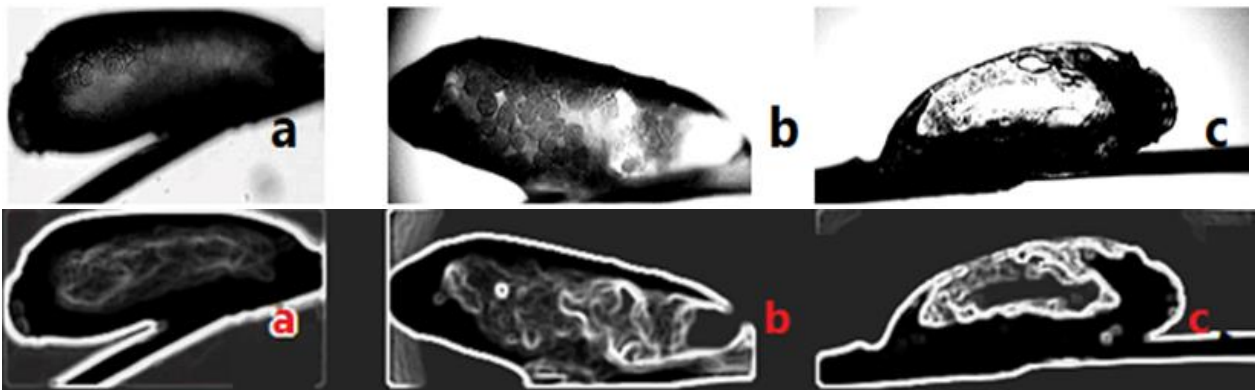


Figure 2. Nit embryonic changing under the effect of Nd:YAG laser (400X) (a) First day, small spots named, embryonic cell were appeared. (b) Second day, the slow aggregation of all embryonic cells in the center of the nit. (C) Third day, degradation, shrinking and death of embryonic cells.

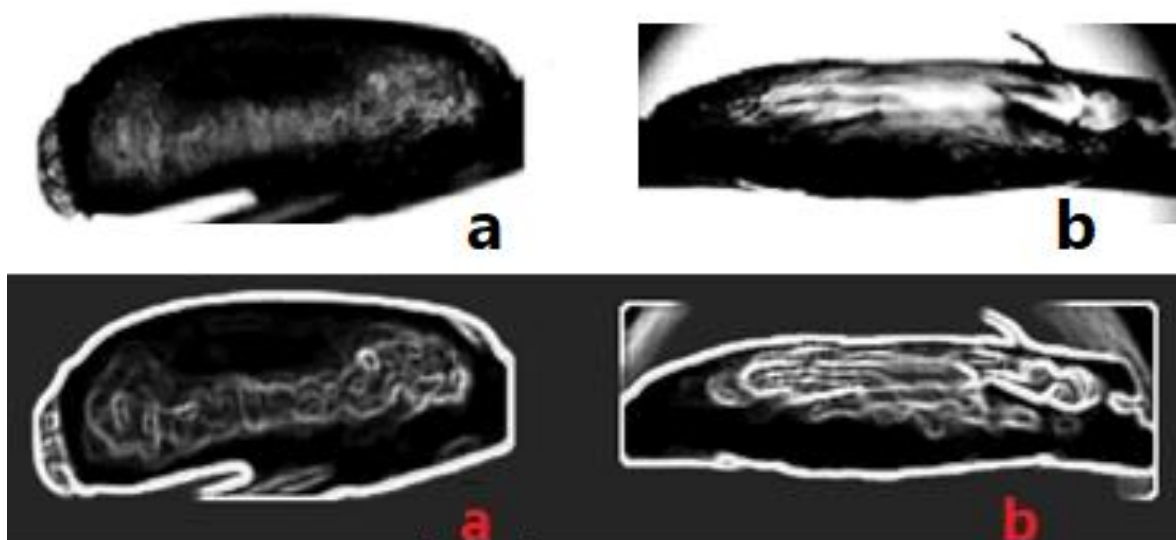


Figure 3. Nit embryonic changing under the effect of He-Ne laser. (400X). a) First day, small spots named, embryonic cell were appeared. b) Secondary degradation of embryonic cells.

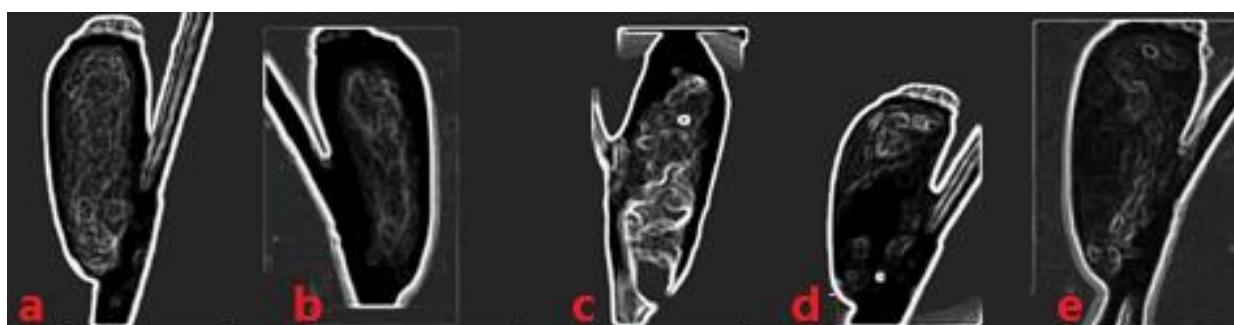


Figure 4. Nit embryonic changing in the absence of laser exposure, control sample (400X). a) Nit with yolk matter at early stage after laying. b) First day, appearing of the embryonic cells. c) Second and third day, aggregation of all cells in the center of the nit. d) Fourth day, development of eye spot. e) Fifth and sixth day, development of legs and live nymph ready to exist.

In biological systems, the laser effect depends on the wavelength, power, and duration of exposure. It is well-known that laser energy can cause damage to proteins and lipids [8] by inducing changes in temperature, which in turn leads to the modulation of these chemical substances [9]. It had been recorded that the head lice nit reissuance is return to presence of several chemical composition of the nit sheath includes protein, lipid (fatty acid) and chitin especially several amino acids like Tyrosine and Phenylalanine which helps in the formation of chemical complex and the later are relatively resistance to degradation by different anti nit as mentioned by [10]. Nowadays, various chemical substances are used as antilice example, 1,2-octanediol, dimeticone, herbal and essential oils,

isopropyl myristate and malathion while the resistance of this parasite is increased [11]. Recent data confirmed that the increasing of insecticide resistance in head lice in the United States is due to knockdown resistance (kdr)-type mutation [12]. Several advantages of the new mode of head lice eradication had been measured includes, limited treatment time, reduce the cost and get rid of side effects. 1.

4. Conclusions

This study concludes that low-power lasers, particularly He-Ne and Nd:YAG lasers, demonstrate significant possibility in inhibiting head lice nits by interrupting embryonic development and disintegrating nit shells. The findings indicate that the He-Ne laser completely

inhibited nit hatching (0%), but the Nd:YAG laser produced a 16% hatching rate under comparable conditions, indicating the He-Ne laser's higher efficacy. The study also shows that the efficacy of both lasers is significantly influenced by the duration of exposure, with extended exposure times resulting in stronger effects.

These data confirm the safety of laser therapy as an innovative, non-chemical substitute for conventional pediculicides. This method targets significant issues, including the growing resistance of head lice to chemical treatments and their related negative effects, providing a safe, economical, and effective alternative. This study provides the basis for subsequent research aimed at improving laser parameters and investigating wider applications of laser technology in parasitology and allied disciplines, facilitating progress in non-invasive and eco-friendly treatments.

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References

- [1] Thiel, H; "Low power laser therapy—an introduction and a review of some biological effects". *J Can Chiropr Assoc*, 30 (3): 133-138, 1986.
- [2] Janocha, A.; Ziemba, P.; Jerzak, A.; Jakubowska, K ; "Clinical use of lasers and energy-based devices in selected skin diseases". *Educ. Health Sport J*, 74:5172651726. 2024.
- [3] Heukelbach, J.; Speare, R.; Canyon, D; "Natural products and their application to the control of head lice: an evidence-based review". In *Chemistry of Natural Products*, 2nd ed; Goutam Brahmachari; Recent Trends and Developments: India, 277-302, 2006.
- [4] Neuberg, M.; Banfić, I.; Cikač, T.; Ribić, R.; Zember, S.; Meštrović, T; "Knowledge, attitudes, psychosocial perspectives and applied epidemiology in the control of head lice (pediculosis capitis) in croatian preschool children: a qualitative study on childcare professionals and health coordinators". *Children*, 9(1):1-16.2022.
- [5] Leal Cardoso, J. H.; Noronha C. De Souza,A.; Militão De Souza, F.; Sa Preire, S.; Pinçon, C; "Treatment of Head Louse Infestation with a Novel Mixture Made of Semi-Crystalline Polymers and Plant Extracts: Blind, Randomized, Controlled, Superiority Trial". *Cosmetics J*. 7 (1): 25.2020.
- [6] Madke, B.; Khopkar, U; "Pediculosis capitis: An update. *Indian J Dermatol Venereol and Leprol*", 78 (4): 429-438.2012.
- [7] Sonnberg, S.; Oliveira, F.; De Melo, I. A.; De Melo Soares, M.; Becher, H.; Heukelbach, J.; "Ex vivo development of eggs from head lice (*Pediculus humanus capitis*)", *Open Dermatol J*, 4(1): 82-89.2010.
- [8] Fischer, F.; Aulmann, M.; Maier-Borst, W.; Lorenz, W. J; "Blood Cell Damage afterin VitroIrradiation of Fresh Whole Blood with 630 nm Laser Light". *Blood Cells, Mol and Dis*, 24(3): 385-395.1998.
- [9] Avci, P.; Nyame, T. T.; Gupta, G. K.; Sadasivam, M.; Hamblin, M. R "Low-level laser therapy for fat layer reduction: a comprehensive review". *Lasers Surg Med*, 45(6): 349- 357.2013.
- [10] Burkhart, C. N.; Stankiewicz, B. A.; Pchalek, I.; Kruge, M. A.; Burkhart, C. G; "Molecular composition of the louse sheath".*Parasitol J*, 85 (3): 559-561.1999.
- [11] Burgess, I. F; "Head lice: resistance and treatment options".*Pharmaceut J*, 297(7893):1-9. 2016.
- [12] Gellatly, K. J.; Krim, S.; Palenchar, D. J.; Shepherd, K.; Yoon, K. S.; Rhodes, C. J.; Lee, S. H. *et al*: "Marshall Clark, J;" Expansion of the knockdown resistance frequency map for human head lice (Phthiraptera: Pediculidae) in the United States using quantitative sequencing". *Med Entomol J*, 53(3),653-659.2016.