INTERNATIONAL JOURNAL OF SCIENCE AND NATURE

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EFFECT OF DIODE LASER (805) NM ON THE PATHOGENICITY OF *PROTEUS VULGARIS*

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ABSTRACT
The effect of diode laser (805 nm) single exposure radiation on *Proteus vulgaris* has been studied. Pure isolated of *Proteus vulagris* was exposed to diode laser for one minute, then three groups of mice injected intraperitoneal (IP) with 0.25 ml of the following: PBS, unirradiated and irradiated bacteria. Biochemical tests, antibiotics sensitivity and histopathological study were examined before and after irradiation. The results showed slightly different between two groups.

KEYWORDS: diode laser, intraperitoneal, Biochemical tests.

INTRODUCTION
Proteus is a genus of *Enterobacteriaceae,* which occurs widely in humans, animals and in the environment, and it can recover easily from sewage, soil, garden vegetables and many other materials[1, 2]. They can cause many illnesses, such as kidney stones, bladder stones, peritonitis, septicemia, pyelitis infection. In addition, the diagnosis of pyelonephritis and urinary tract infection can found when the *Proteus* concentration in urine is greater than 10^5 cells/ml. Urease production by bacteria has been showing to increase the risk of pyelonephritis in experimental animals[3, 4]. *Proteus* species are highly resistant to antibiotics, so infections can be difficult to cure. Their plasmids are responsible for spreading antibiotics resistance genes in a microbial population [5]. Since laser invention, its offers hope for new treatment of bacterial infections, even those that are resistant to the current drug, now diode lasers involve many materials and forms, contributed in many fields[6]. This study design to study the effect of laser technique on *Proteus vulgaris* and its influence on the viability, antibiotic resistant and pathogenesis.

MATERIALS & METHODS
Identification of bacteria
Pure isolated bacteria of *Proteus vulagris* obtained from biotechnology department in a science college in University of Baghdad. Bacterial isolate recultured on MacConky and Blood agar. Different morphological and biochemical tests were used for identification and diagnosis bacterial isolate followed by using Api 20E system**.**

Irradiation Method
A loopful of the culture transferred from the BHI slant to a test tube containing BHI broth and incubated at 37°C for overnight. The suspension centrifuged at 3500 r.p.m for 10 minutes, supernatant removed and the precipitate was resuspended using physiological saline. The suspension mixed using the vortex to get a homogenous suspension, which compared with the McFarland solution (1.5*108 CFU/ml)[7]. One ml of the diluted bacteria suspension transferred to a sterile Eppendorf tube and exposed to laser light for one minute. Another tube also contains 1ml of the suspension did not expose to laser light used as a control, then irradiated and the non-irradiated suspension was placed on BHI agar and incubated at 37°C for overnight, then tested for biochemical characteristics, their sensitivity to antibiotics and alpha toxin production[8, 9].

Antibiotics sensitivity test
The sensitivity of *P. vulgaris* isolate had performed by Kirby-Bauer disc diffusion assay [7]. The following antibiotic discs were used during this study: Cefotaxime (CTX) (30µg), Norfloxacin (NOR) (10µg), Vancomycin (VA) (30µg), Erythromycin (E) (5 µg).

Laboratory animals
Ten BALB/c mice divided to three groups. The first group injected IP (four mice) with 0.25 ml of the unirradiated isolate. The second group (four mice) injected 0.25 ml of the irradiated isolate. The third group contain two mice used as a control and injected 0.25ml of PBS. After four days, all the animals sacrificed for histopathological study. Specimens of different organs take it and fixed in 10% formalin for 5-7 days, dehydrated in several concentrations of alcohol and clearing in xylene, embedding in paraffin and then blocking. Sectioning at 5µm onto glass slides and then deparaffinized, rehydrated and stained with Harris Haematoxylin & Eosin[10].

RESULTS & DISCUSSION
Some biochemical and antibiotics tests done before irradiation, then one ml of bacteria suspension was exposed to 2 w for one minute of the laser after that the irradiated bacteria was re-tested to the biochemical test and compare the result with unirradiated bacteria as showed in the table (1). The unirradiated bacteria and the irradiated bacteria cultured on Muller Hinton agar and

tested for their sensitivity to an antibiotic (Norfloxacin, Cefixime, Vancomycin, Erythromycin). The irradiated bacteria showed change in their sensitivity to cefixime from (20mm) to (15mm) than other antibiotics (Norfloxacin, Cefixime, Vancomycin, Erythromycin). Also, changes have seen in citrate utilization after irradiation this might be due effect of laser on bacteria DNA. Histopathological study showed little changes between irradiated and unirradiated group as shown in figure 1, 2, 3, 4 and 5, while no changes was seen in Brain, heart and Seminal prostatein both groups (irradiated and unirradiated bacteria).

Previous studies [11,12] found that effect of diode laser increase the susceptibility of bacteria to antibiotics, with increasing time and dose of laser exposure, this may be due to mutant occur to the bacteria plasmid contents decrease gradually after laser irradiation this can be seen for small and large plasmid [6]. Some studies on gram positive bacteria found that no effect to the laser light on the antibiotics sensitivity of gram positive bacteria [13]. While, others found *Staphylococcus aureus* lost its ability to produce β- hemolysine and α-toxin and increase susceptibility to antibiotics. While their ability to produce catalase, coagulase and mannitol fermentation was not affected by laser irradiation[8]. Seyedmousavi *et al*. [14], concluded that a direct laser-based approach without using a photosensitizing agent may be a promising novel treatment approach for superficial and mucocutaneous *Candida albicans* infections.

TABLE 1: Effect of laser on some biochemical characteristics and antibiotic sensitivity of *Pro. vulgaris*

Biochemical & Antibiotic Tests	Un irradiated bacteria	Irradiated bacteria
TSI agar	K/A H_2S	K/A H_2S
Citrate utilization	+	-
Urease test	+	+
Motility test	+	+
Norfloxacin	10mm	10mm
Cefxime	20mm	15mm
Vancomycin	R	R
Erythromycin	R	R

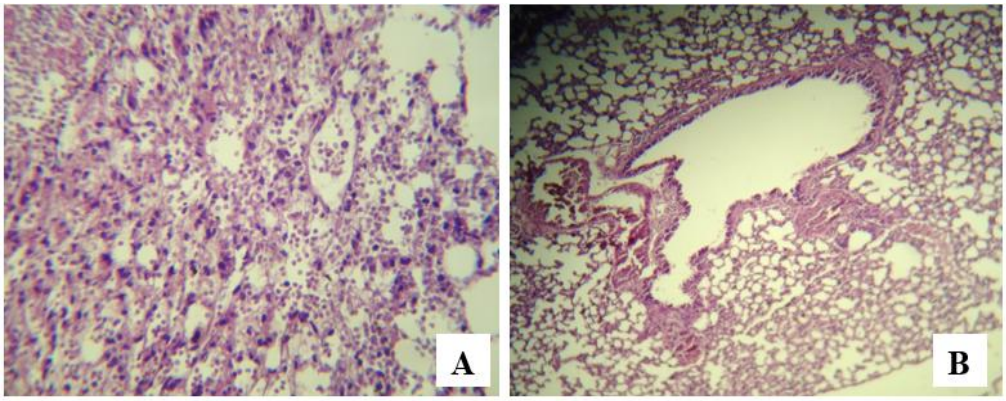

FIGURE 1: Histological section of mouse lung showing A: irradiated group with mild thicking of alveolar septa with hemorrhage exudates or congestion. B: unirradiated group showing congestion with mild thickening of alveolar septae (X200; H&E).

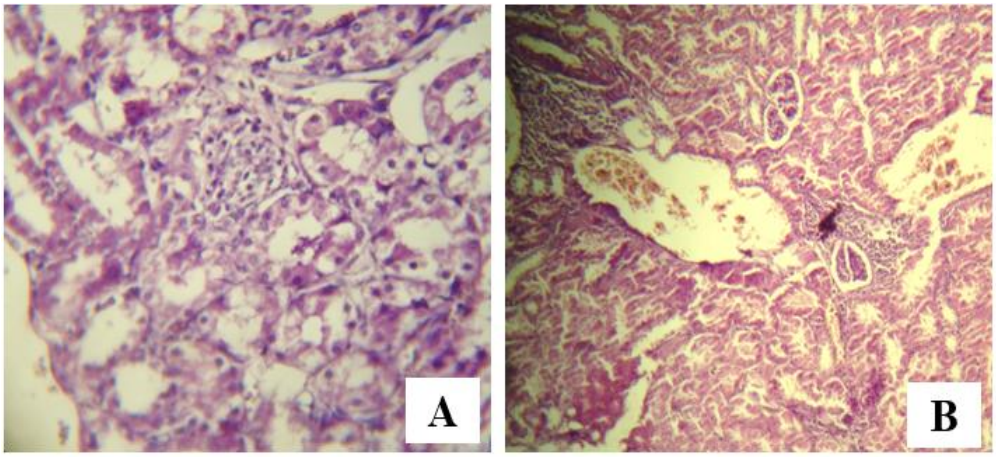

FIGURE 2: Histological section of mouse kidney showing A: irradiated group with certain degenerative, necrosis, renal tubules with mild inflammatory cell infiltration and congestion. B: unirradiated group with congestion, inflammatory cell infiltration between the renal tubules with degenerative changes of tubules (X200; H&E).

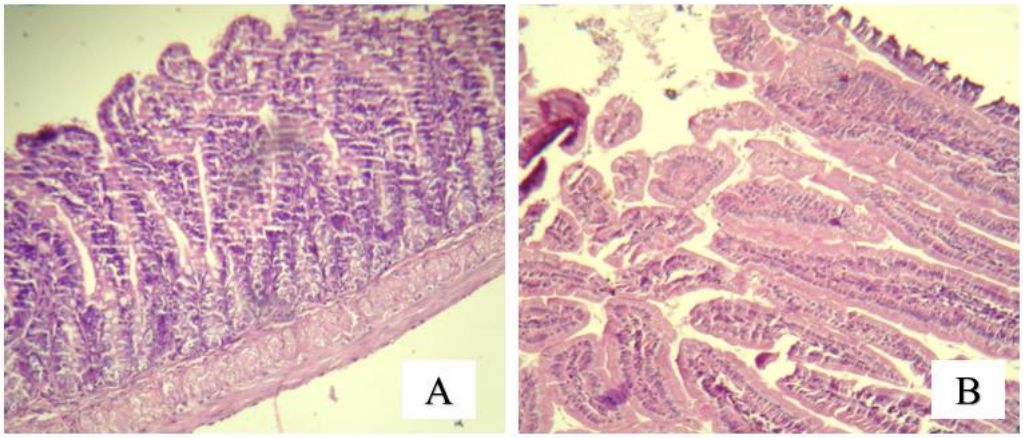

FIGURE 3: Histological section of mouse intestine showing A: irradiated group with normal intestinal villa, mild broading and mild inflammatory cell infiltration. B: unirradiated group with normal appearance of intestinal villa (X200; H&E).

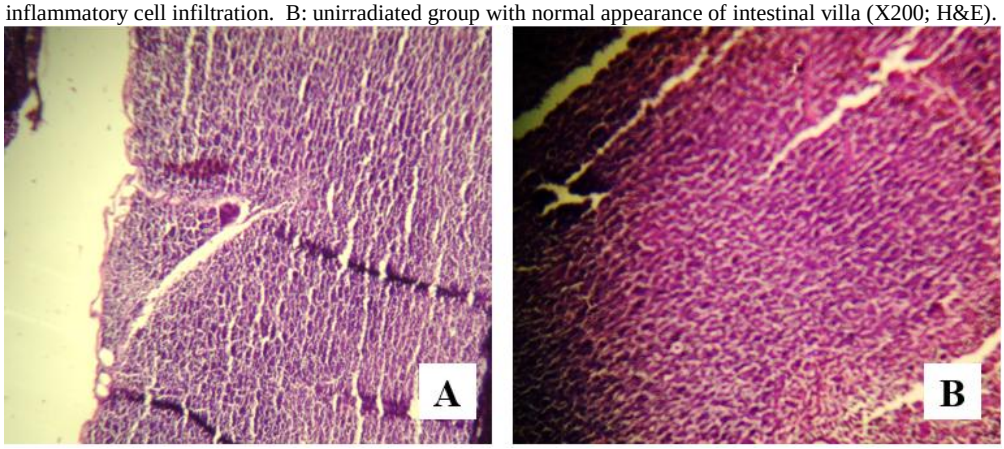

FIGURE 4: Histological section of mouse spleen showing: A (irradiated group) & B (unirradiated group) with widen of white pulp with reduction of red bulb (X200; H&E).

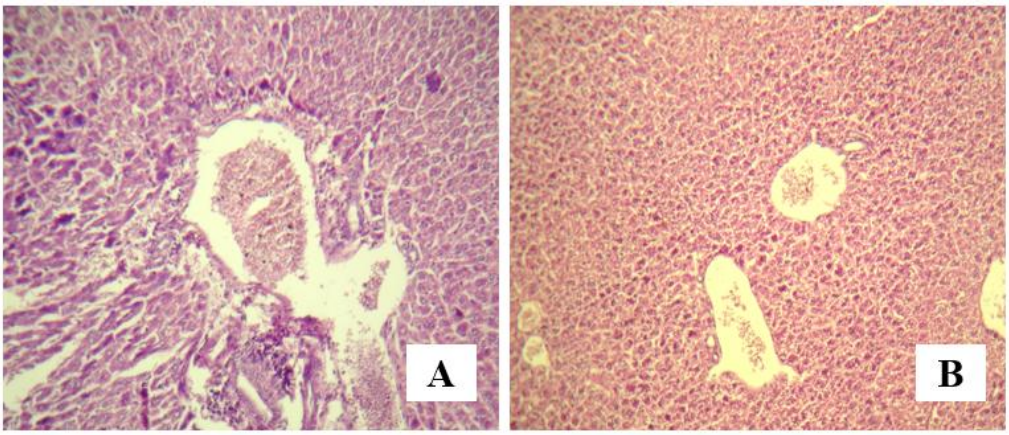

FIGURE 5: Histological section of mouse liver showing A: irradiated group with a focal degenerative change, mild inflammatory cell infiltration and congestion of blood vessels. B: unirradiated group with accumulation of glycoprotein (X200; H&E).

CONCLUSION

This study showed that little changes seen between irradiated and unirradiated group. This may be due to short time of irradiation cause little effect on bacterial genome, therefore more research required to study the effect of different wave length of laser and different time of exposure on virulence factors of pathogenic microorganism to find suitable treatment to disease

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