PREPARATION OF VACCINE FOR DIABETIC FOOT PATHOGENIC BACTERIA USING LOW LEVEL DIODE LASER

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ABSTRACT

Since the invention of laser in 1960, lasers have been developed and approved in many fields. Lasers can now be regarded as practical tools with unique properties that have been utilized effectively in several applications in fields of medical and biological sciences.

six bacterial isolates were isolated from human samples of diabetic foot infections, which used for preparation of vaccines. The experiment was conducted on fifteen adult male rabbits, they were divided into three groups with 5 rabbits each.

After irradiation of the bacterial suspensions with the diode laser for different exposure times and different frequencies, and the wavelengths used were (660, 820, 915) nm, the growth of bacterial isolates decreased until killed of bacteria at (40) min.

The aim of the current study was to preparation of vaccines (live attenuated and killed) by irradiation of the bacteria by the low level diode laser. Wavelength (660) nm was more effective in killing the bacteria, and the variations were not significant between the live attenuated and the killed vaccine.

INTRODUCTION

LASER is an acronym for light amplification by stimulated emission of radiation; common usage today is to use the word as a noun (laser) rather than as an acronym (1). Laser is a device that converts electrical or chemical energy into light energy. In contrast to ordinary light that is emitted spontaneously by excited atoms or molecules, the light emitted by laser occurs when an atom or molecule retains excess energy until it is stimulated to emit it. The radiation emitted by lasers including both visible and invisible light is more generally termed as electromagnetic radiation (2).

Diabetic Foot Ulcers (DFUs) are a common and much feared complication of diabetes, with recent studies suggesting that the lifetime risk of developing a foot ulcer in diabetic patients may be as high as 25% (3). Infection is a frequent (40%-80%) and costly complication of these ulcers and represents a major cause of morbidity and mortality. It is estimated to be the most common cause of diabetes-related admission to hospital and remains one of the major pathways to lower-limb amputation (4).

A *vaccine* is a suspension that contains a part of a pathogen that induces the immune system to produce antibodies that combat the antigen (5). When the vaccine is injected into a body the chemicals in the vaccine cause tissue irritation. This results in blood flow to the injection site and with the blood comes white blood cells. The white

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blood cells become exposed to the antigen and begin a series of processes that cause antibodies to be produced to the antigen. The period of time from when the vaccine is injected until production of antibodies takes days or weeks (6).

The immunoglobulins are Proteins, present on the surface of B- lymphocytes , secreted in response to stimulation, that neutralize antigens by binding specifically to their surface (7).

The aim of the current study was to preparation of vaccines (live attenuated and killed) by irradiation of the bacteria by the low level diode laser.

MATERIALS AND METHODS

Bacterial species: six bacterial isolates (*Staphylococcus aureus, S. epidermidis, Pseudomonas aeruginosa, Escherichia coli*, *Proteus mirabilis,*and *Klebsiella pneumoniae*) were isolated from human samples which collected from patients lying in Al- Hussain Teaching Hospital, Al– Muthanna Province, suffering from diabetic foot infections, using sterilized cotton swabs. These samples were identified according to Bergeys manual using different morphological and biochemical tests (8).

Vaccine preparation: six isolates of bacteria were cultured on blood agar at 37˚C for 24hr. washing the surface of the plates with normal saline using glass rods.The bacterial suspension mixed with vortex for 3min. (5 ml) of the suspension from each culture was cold centrifugation at 6000 r.p.m for 10 min.The sediment of the bacteria washed three times by normal saline (pH=7.2) then mixed with vortex to be suspended once again in 5 ml of normal saline and compared with Macferland solution. The bacterial suspension irradiated with laser to obtain live attenuated and killed vaccines (9). Then bacterial suspension recultured on blood agar and incubated at 37℃ for 24hr. to determine the live attenuated and killed bacteria in which the growth not observed.

Vaccine program: Fifteen male rabbits, were divided into three groups and injected as following. **First group:** n =5 inoculated with live attenuated vaccine (A vaccine) consist of a mixture of (*S. aureus, S. epidermidis, Pseudomonas aeruginosa, Escherichia coli*, *Proteus mirabilis,*and *Klebsiella pneumoniae*) 1.8ml (0.3ml from each one) subcutaneously.**Second group:** n =5 inoculated with killed vaccine (K vaccine) consist of a mixture of bacterial isolates 1.8ml (0.3ml from each one) subcutaneously.**Third group:** n=5 (control group) injected with physiological normal saline.

Collection of Blood samples: 3 ml blood samples were collected from the marginal ear vein of the rabbits after one month of the vaccination, for the purpose of measuring the concentration of the immunoglobulins which present in their serum. by using Radial Immunodiffusion (RID) method. using specialized kits (LTA-Italy).

Statistical Analysis: all the results obtained were estimated statistically by using Minitab program 2 sample T – test .

RESULTS

Radial Immunodiffusion (RID) method used for determination immunoglobulins concentration (IgG, IgA, IgM, C_3, and C_4). which present in the serum of the rabbits which were inoculated with live attenuated and killed vaccine , there were an increasing in the level of IgG for all the animals groups . The variations between the live attenuated vaccine group and the control group were very high significant $P < 0.001$, but were high significant $P < 0.01$ between the killed vaccine group and the control one, and not significant between the attenuated vaccine group and the killed vaccine one, as observed in table (1), and fig. (1). The results of IgA concentrations for the three groups were highly significant $P < 0.01$, when comparing the attenuated with control group, while were significant $P < 0.05$ between the killed vaccine and control groups , and not significant variations between the attenuated and killed groups , as shown in table (1) , and fig. (2). The same results were obtained for IgM, C_3 and C_4 concentrations showed significant variations $P < 0.05$, for the both groups , attenuated and killed when compared with the control group , but not significant between the attenuated and killed groups, as shown in table (1), fig. (3-5).

DISCUSSION

The concentrations of IgG showed very high significant variations , these results agreed with (10), who found that IgG responses were significantly greater in the mice groups immunized with both subunits, 10 μg of antigen (*S.aureus*) mixed with 3 μg of cholera toxin. While the results of IgA concentrations were highly significant when comparing the attenuated vaccine with control groups, these results nearly agreed with (11), who measured the level of IgA specific for cholera toxin subunit B in serum before and after immunization. There were significant increase in IgA ($P < 0{\cdot}001$) , in both the control and the patient groups.

The concentrations of IgM for the three groups showed significant variations, these results not agreed with (12), who found after *E.coli* endotoxin infusion the Ig concentrations increased rapidly with significantly higher (IgM $p < 0.001$, and IgA $p < 0.05$) , and also not agreed with the results of (13), who reported that immunization with a formalin-killed *E.coli* strain gives significant results in serum IgG and IgA response directed against the immunizing strain that is comparable to that observed after immunization with a live strain. The main role of secretory IgA is to inhibit bacterial attachment and neutralize viruses in mucosal tissue. In addition IgA, but not IgG, are translocated across epithelial tissue and can neutralize viruses intracellularly. This indicates that IgA is the first line of defence in the mucosal compartment. Secretory IgA is generally considered to be a non inflammatory antibody because it does not trigger inflammatory processes when it binds to antigens (14) .

The results of complement C_3 and C_4 concentrations for the three groups showed significant variations, $P < 0.05$, for the both groups , attenuated, and killed when compared with the control group, these results not agreed with those got by (15),

who recalled that the variations were not significant in colonization density between non-bacteremic and bacteremic complement- depleted mice or between bacteremic complement - depleted and control mice. Complement is central to innate humoral immunity, interacting with a host of soluble and membrane proteins. In addition to the anti-bacterial activity of the complement cascade (16).

Table (1): Immunoglobulins concentration rate (mean) of the live attenuated vaccine , killed vaccine and control groups .

Immunoglobulins	Groups		
	Attenuated	Killed	Control
IgG	2750	2335	1656
IgA	906	820.9	551
IgM	363.0	313.8	223.2
C_3	300.0	299.9	234.5
C_4	100.44	97.00	75.4

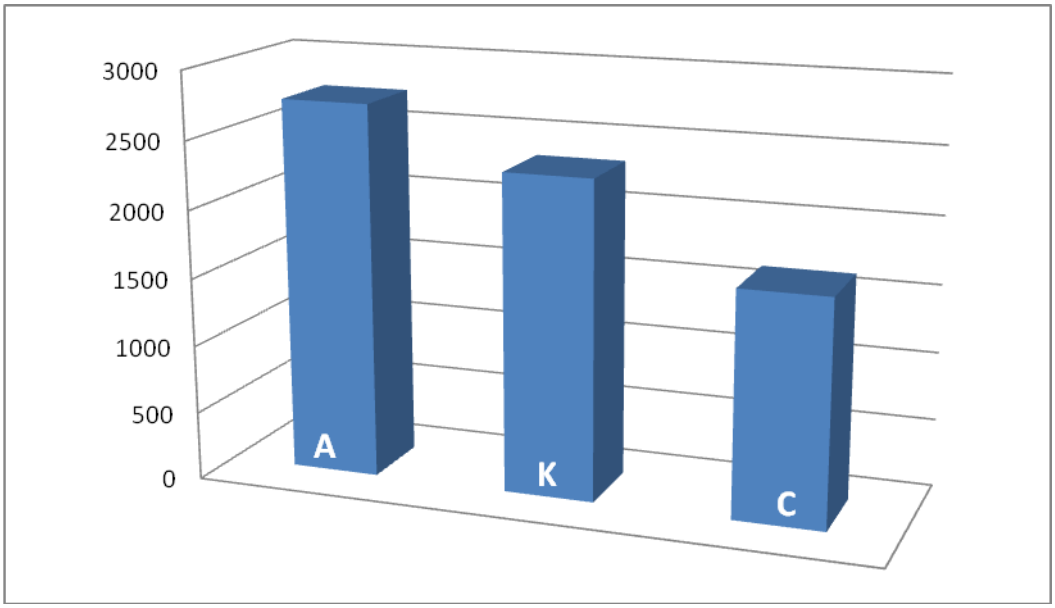

Fig. (1): shows the mean of IgG concentrations for the three groups (Attenuated vaccine, Killed vaccine and Control) .

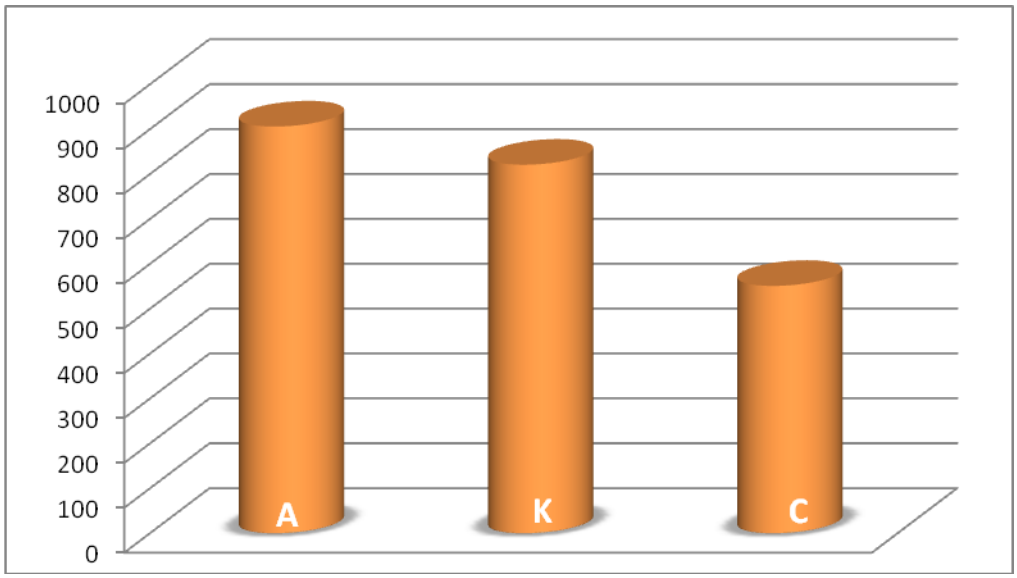

Fig. (2): shows the mean of IgA concentrations for the three groups (Attenuated vaccine, Killed vaccine and Control).

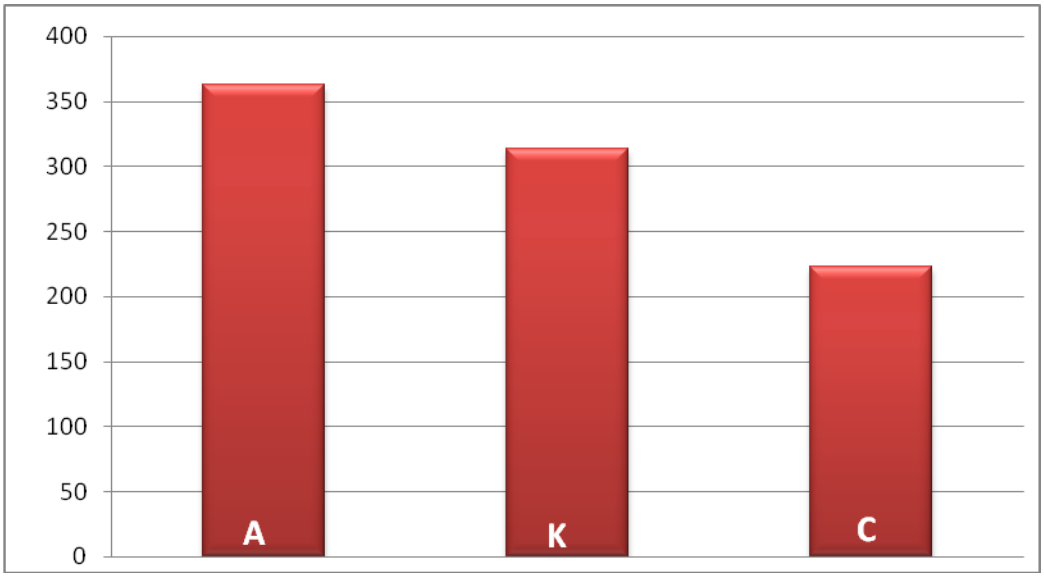

Fig. (3): shows the mean of IgM concentrations for the three groups (Attenuated vaccine, Killed vaccine and Control) .

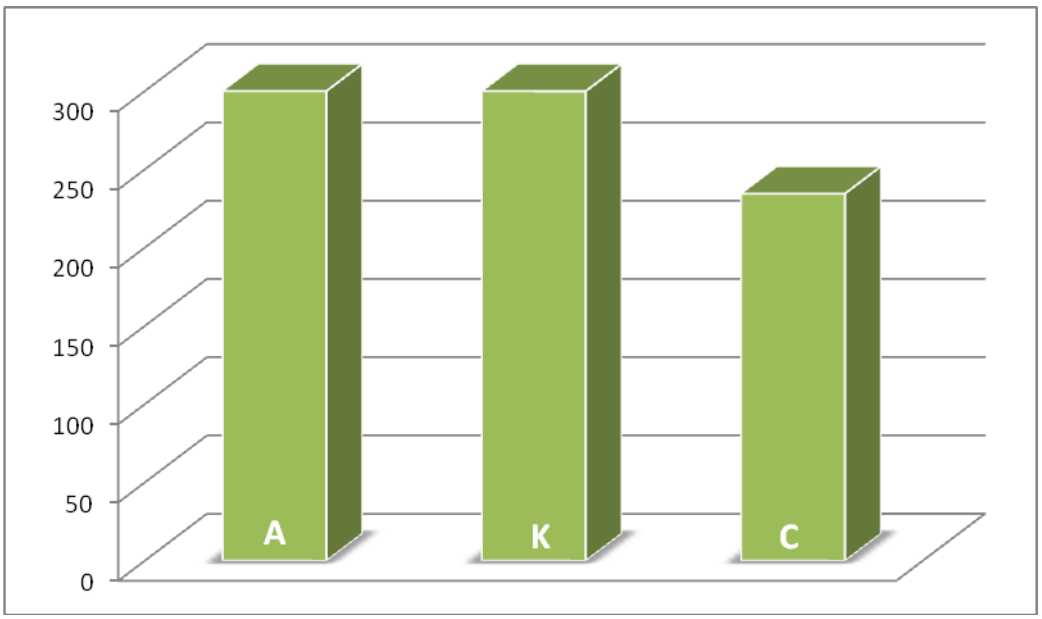

Fig. (4): shows the mean of C_3 concentrations for the three groups (Attenuated vaccine, Killed vaccine and Control) .

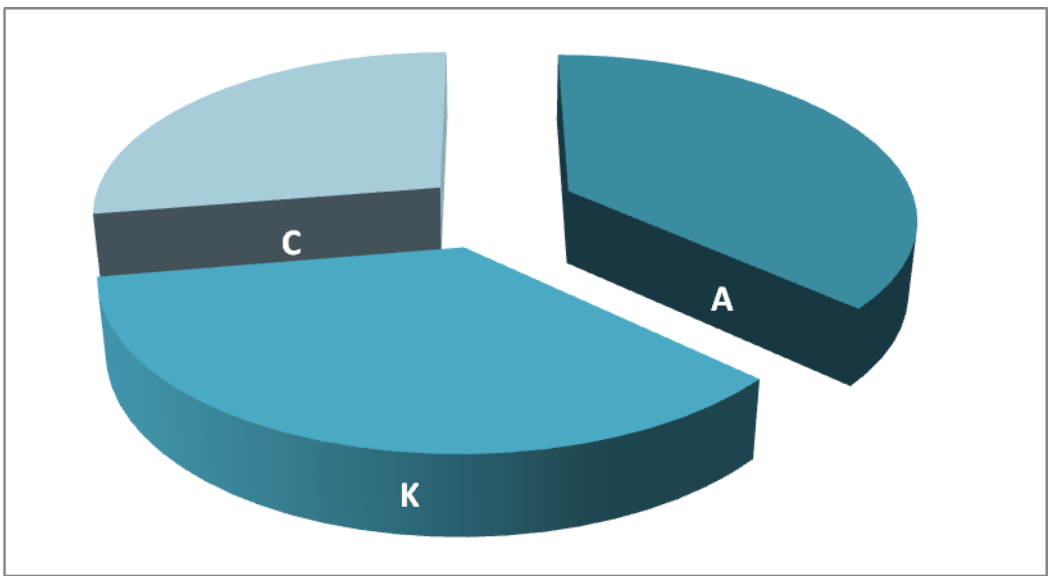

Fig. (5): shows the mean of C_4 concentrations for the three groups (Attenuated vaccine, Killed vaccine and Control) .

تحضير لقاح مضاد للبكتريا المرضية المسببة لإلتهاب القدم السكري بإستخدام التشعيع بالليزر واطئ الطاقة

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الخلاصة

منذ أختراع الليزر في عام 1960 ، بدء يتطور ويطبق في العديد من المجالات العلمية ، يمكن أن يعتبرالليزر أداة علمية تملك خصائص مميزة تستعمل في حقول العلوم الطبية والحيوية . هدفت الدراسة الحالية إلى تحضير نوعين من اللقاحات (الحي المضعف واللقاح المقتول) بواسطة تشعيع البكتريا بالليزر الدايود واطئ الطاقة.

تم استخدام ست عزلات بكتيرية والتي عزلت من حالات القدم السكري من المرضى الراقدين في مستشفى الحسين التعليمي في محافظة المثنى وشخصت هذهِ العينات بالإعتماد على الأختبارات المورفولوجية والبايوكيميائية حسب طرق التشخيص ل Bergeys manual. واجريت التجربة على 15 ارنب من الذكور البالغة والتي قسمت الى ثلاث مجموعات (5) ارانب لكل مجموعة . بعد تشعيع البكتريا بالليزر لأوقات مختلفة مع ترددات مختلفة و كانت الأطوال الموجية المستخدمة (660، 820 ، 915) نانومتر .

بدء النموالجرثومي ينخفض وصولاً الى قتل البكتريا عند (40) دقيقة . حيث كان الطول الموجي 660 نانومتر الأكثر فعالية في قتل البكتريا ، ولم تلاحظ أي فروق معنوية بين اللقاح الحي المضعف واللقاح المقتول .

كان الطول الموجي 660 نانومتر الأكثر فعالية في عمليات تضعيف وقتل البكتريا ، ولم تسجل فروقات ملحوظة بين اللقاح المضعف والمقتول من حيث المعالم الدموية .

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