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Preparation of Vaccine against Diabetic Foot Pathogenic Bacteria Using Low Level Diode Laser

A thesis

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Abstract

The objective of this study is to prepare two types of vaccine (Live attenuated and killed vaccines) against pathogenic bacteria of diabetic foot infection in humans, using laser irradiation.

Samples collected from forty patients suffered from diabetic foot infection, using sterile cotton tinge (Swab), the samples diagnosed depending on a number of morphological examinations, biochemical tests and culturing on selective media, as well as to use of the API system. The bacterial isolates obtained including the followings:

Staphylococcus aureus (24.59 %) , *Klebsiella pneumoniae* (22.95 %), *Escherichia coli* (21.31 %), *S. epidermidis* (9.84 %) , *Pseudomonas aeruginosa* (9.84 %) , *Proteus mirabilis* (8.20 %) ,and other species (3.28 %) .

The sensitivity of the bacterial isolates before irradiation to a number of antibiotics were examined , they all gave high resistance to the antibiotics, except two types (Amikacin & Ciprofloxacin) , which the bacterial isolates were sensitive to.

The bacterial isolates irradiated with laser using wavelengths (660, 820, and 915nm) to increase their sensitivity reaching to a step of attenuating or killing the bacteria with increasing exposure times. Then the attenuated and killed bacteria from each isolate used to prepare a mixed vaccine .

Laser effect on the bacterial isolates showed a significant decrease in the viability of the bacteria of all species when the dose was increased , where occur killing the bacteria after 20 min and more of laser dose .

The efficiency of the vaccine was tested by using laboratory animals, fifteen rabbits were used in the current study , they were divided into three groups with five rabbits each, one group for live attenuated vaccine inoculation, and the other one for the

killed vaccine while the third group used as a control group. One month after the completion of the vaccination, the concentrations of the immunoglobulins (IgG, IgA, IgM C3, and C4) in the rabbit serum measured using Radial Immunodiffusion (RID) method.

The results showed very high significant differences $P < 0.001$ for the level of IgG between the live attenuated vaccine group when compared with the control one, high significant differences $P < 0.01$ for the level of killed vaccine group compared with the control one .

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 group and the control one, it was also significant for the level of IgM, C3, and C4, when compared both the live attenuated and killed vaccine groups with the control one respectively.

There were no significant differences between the live attenuated and the killed vaccine groups of all measured concentrations.

Subsequently the animals inoculated again with a live dose of the bacterial isolates and the levels of (IgM, IgA, IgG, C3, and C4) were measured, the same results as in the vaccinations readings were obtained.

Finally the animals were inoculated with the challenge dose of all the isolated live bacteria. The animals of the control group died, while the immunized animals remained healthy revealing the efficacy of the vaccine and the vaccination program.