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THE EFFECTS OF INTERACTION BETWEEN OF *STAPHYLOCOCCUS AUREUS* AND *SALMONELLA TYPHIMURIUM* ANTIGENS ON THE IMMUNE RESPONSE IN RABBITS

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Abstract

The research including preparation an antigens of killed whole cell sonicated antigen of *Salmonella typhimurium* (KWCSAg-ST) and killed whole cell sonicated antigen of *Staphylococcus aureus* (KWCSAg-STAPH.A), then evaluated their efficacy by using delayed type hypersensitivity (DTH)-skin test, and enzyme linked immuno-sorbent assay (ELISA). Twenty four rabbits randomly divided into three groups (eight animals for each group). The first group were immunized with KWCSAg-ST (1mg/ml) subcutaneously. The second group was immunized with KWCSAg-ST (1mg/ml) and KWCSAg-STAPH.A (1mg/ml) subcutaneously. The third group was injected by PBS (pH7.2) as control group. The highest diameter of the erythema was 22.00±1.67mm for 1st group as compared with 2nd group 16.00±0.58mm. There was an increase in the diameter of induration in 1st group at 48 hours. The highest diameter as recorded 15.33±1.52mm of 1st group compared with a significant difference ($P<0.05$). The immune response was estimated by using ELISA, The value of IgG concentration in 3rd group showed the elevation to 110.57±7.48µg at days 28 post immunization then decrease gradually to reach 92.29±4.22µg at days 56 post immunization with significant difference ($P<0.05$). IgG concentration in 1st and 2nd groups was 107.21±4.61µg, and 108.83±5.97µg, at 28 post immunized respectively, with significant different ($P<0.05$), then decrease gradually to reach 108.83±10.63µg and 100.62±5.53µg at days 56 post immunized respectively with significant difference ($P<0.05$). While The IL-4 concentration in 3rd groups elevated to 25.11±1.88pg at days 28 post immunization then decrease gradually to reach 23.74±2.93pg at days 56 post immunization with significant difference ($P<0.05$). IL-4 concentration in 1st and 2nd groups was 23.94±0.83pg and 25.56±1.90pg and at day 28 post immunized respectively, and elevated gradually to reach 27.28±3.59pg and 26.89±2.37pg at day 56 post immunized respectively with significant difference ($P<0.05$).

In conclusion there was an interaction of the two antigens to stimulate and progress the cellular and humoral immune response, likewise give immunological reaction between bacterial antigens of *Salmonella typhimurium* and *Staphylococcus aureus* which act as adjuvant to give good potent synergistic effect on immunological response.

Key words: Interleukine 4 (IL-4), Immunoglobuline G (IgG), Antigen, *Salmonella typhimurium*, *Staphylococcus aureus*, ELISA

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INTRODUCTION:

Salmonella is a Gram-negative, rod-shaped, and non-spore forming bacterium. Salmonellosis, an infection caused by *Salmonella*[1], *Salmonella Typhimurium* is one of the leading serovars responsible for human and animal diseases[2], It has led to serious economic losses and healthy problems worldwide, including particularly gastroenteritis and diarrheal diseases related to ileal injury and intestinal flora disorder[3], *Salmonella typhimurium* is a highly transmissible pathogen in rabbits that causes significant losses[4], Microbial infections are controlled by host inflammatory responses that are initiated by innate immune receptors after recognition of conserved microbial products. As inflammation can also lead to disease, tissues that are exposed to microbial products such as the intestinal epithelium[5], Intestinal epithelial cells serve as not only a barrier to bacteria colonizing the gut but rather as an integral and essential component of the innate mucosal immune system of the host through its secretion of inflammatory cytokines, chemokines (IL-8) and anti-microbial peptides (human β-defensins) to defend against the invasion of *Salmonella*[6], The host immune response to *S. Typhimurium* involves a complex interplay between innate and adaptive immunity[7], The early immune response to *Salmonella* in Peyer's patches (PPs) and mesenteric lymph nodes (MLNs) involves the recruitment of neutrophils and inflammatory monocytes, and these responses are important for delaying the spread of bacteria to systemic tissues[8], In addition, *S. Typhimurium* induces a T helper 1 (TH1)–biased adaptive response, and neutralization of the key TH1 cytokine interferon-y (IFN-Y) causes increased bacterial burden in multiple organs, At the same time, regulatory T cells can abrogate the proliferation of CD4+ TH1 cells. In addition, antibody production by B cells is required for a protective response against *S. Typhimurium*[7].

Staphylococcus aureus is a widespread commensal bacterium and pathogen, Polymorphonuclear leukocytes (neutrophils) are the primary cellular host defense against *S. aureus* infections and a major component of *S. aureus* abscesses[9], Protein A is a *S. aureus* surface protein that binds immunoglobulins in the incorrect orientation, which represents an immune evasion mechanism because it inhibits antibody-mediated phagocytosis[10], The B cell-mediated immune response against *S. aureus* involves the production of antibodies directed against specific antigens of components of *S. aureus*[9], T helper (Th) cell subsets (CD4+ T cells), especially Th1, Th2, and Th17 cells, which have been implicated in the pathogenesis of *S. aureus* skin infections. Th1 cells produce IFN-γ and promote cell-mediated immune responses, Th2 produce IL-4 and IL-13 and promote antibody-mediated immune responses, and Th17 cells produce IL-17 (i.e., IL-17A and IL-17F) and IL-21 and IL-22 and IL-26 and promote neutrophil recruitment and abscess formation[11].

Due to the synergistic effect of *Salmonella typhimurium* and *Staphylococcus aureus* to stimulate an immune response more efficient than *Salmonella typhimurium* Ag only.

2. Materials and Methods:

2.1. *Salmonella typhimurium* and *Staphylococcus aureus* isolate:

The both isolates were obtained from the department of microbiology/ University of Baghdad.

2.2 Antigens preparation:

Killed whole-cell sonicated antigen of *Salmonella typhimurium* (KWCSAg-ST) was prepared according to **Motive (1992)** procedure[12].

Killed whole-cell sonicated antigen of *Staphylococcus aureus* (KWCSAg-STAPH.A) was prepared according to **Motive (1992)** procedure with some modification[12].

2.3. Laboratory animal immunization

Twenty four rabbits were used, randomly divided into three equal groups (eight animals for each group). The animals were housed in clean, disinfected cages with a well-balanced diet and carefully controlled environment.

2.4. Experimental design:

The first group was immunized by killed whole cell sonicated Ag of *Salmonella typhimurium* (KWCSAg-ST) (1mg/1ml) subcutaneously. The second group was immunized with mix killed whole cell sonicated Ag of *Salmonella typhimurium* (KWCSAg-ST) (1mg/1ml) and killed whole cell sonicated Ag of *Stapylococcus aureus* (KWCSAg-STAPH.A) (1mg/1ml) subcutaneously. The third group (control group) injected by 1ml of PBS (pH7.2) subcutaneously.

Booster dose was given at 14th day for the 1st and 2nd groups of KWCSAg-ST. At the 21st day the skin test was conducted to examine the DTH and the cellular immunity of the test animals. At the 28th, 42nd, and, 56th days blood was collected from each group and serum was obtained for the tests.

2.5. Delayed Type Hypersensitivity (DTH) Skin test

This test was done as below. At the site of flank region after clipping and shaving carefully the region was divided into four injection sites with different dilution of whole cell *Salmonella typhimurium* sonicated antigen; crude concentrated Ag, 1:2, 1:4 and PBS (pH7.2) as control by intradermal of all immunized groups. The erythema and induration of the skin of the injected sites were measured after 24, 48, 72 hours post injection by using standard vernia[12].

2.6 ELISA Kits:

The kits were Sandwich enzyme-linked immunosorbent assay of IgG and IL-4 in Rabbit serum, as per the instructions in the manual (Shanghai YL Biotec- China).

2.7. Statistical analysis

The data of the study has been analyzed using SPSS software (Statistical Package for the Social Sciences), to test the significancy of effect of the antigens used in the study, and compare the means within the same group and between different groups of the study, using one-way and two-way ANOVA.

3. Result

3.1 Delayed Type Hypersensitivity (DTH) - Skin test

The delayed type hypersensitivity test (DTH) was used to evaluate the cellular immune response, which comprises of erythema and induration that had developed after 21 days following immunization.

Results revealed that the differences among groups were significant ($P<0.05$) for the three periods. The mean of erythema, Fig. (3-1), and induration, Fig. (3-2) in the 2nd group was significantly

(P<0.05) higher than of other groups in all periods, On the other hand, the differences among periods within each group were not significant for all groups.

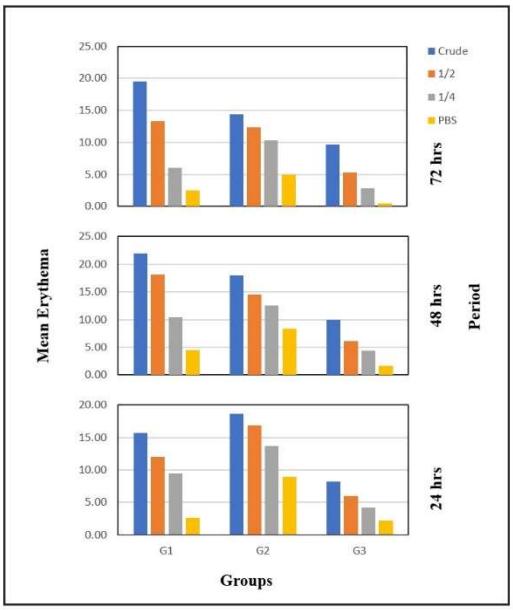

Figure (3-1) Mean of Erythema in rabbits immunized by KWCST-Ag

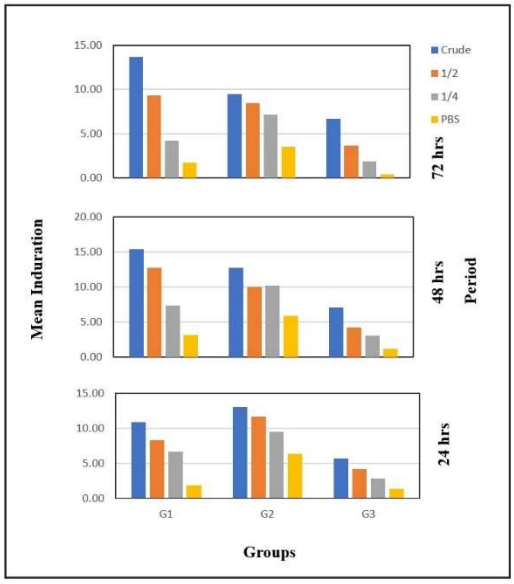

Figure (3-2) Mean of induration in rabbits immunized by KWCST-Ag

3.2 Enzyme-linked immunosorbent assay (ELISA).

ELISA test was used to determine the concentration of Immunoglobulin G and Interleukin-4. The antibody concentration is used to evaluate the humoral immune response, and the ability of the immune system to produce the important antibodies of the immune response.

The serum was drawn at days 28, 42, 56 following immunization.

3.2.1. Immunoglobulin G (IgG) test

The IgG concentration in 3rd group elevated to 110.57±7.48µg respectively at days 28 post immunization then decrease gradually to reach 92.29±4.22µg at days 56 post immunization with significant difference (P<0.05). IgG concentration in 1st and 2nd groups was 107.21±4.61µg and 108.83±5.97µg at 28 post immunized respectively, with significant different (P<0.05), then decrease gradually to reach 108.83±10.63µg and 100.62±5.53µg at days 56 post immunized respectively with significant difference (P<0.05) shown in Fig (3-3).
Results revealed that the differences among groups were significant (P<0.05) for the three periods. The mean of IgG concentration in 2nd group was significantly (P<0.05) higher than of other groups in all periods, Fig. (3-3).

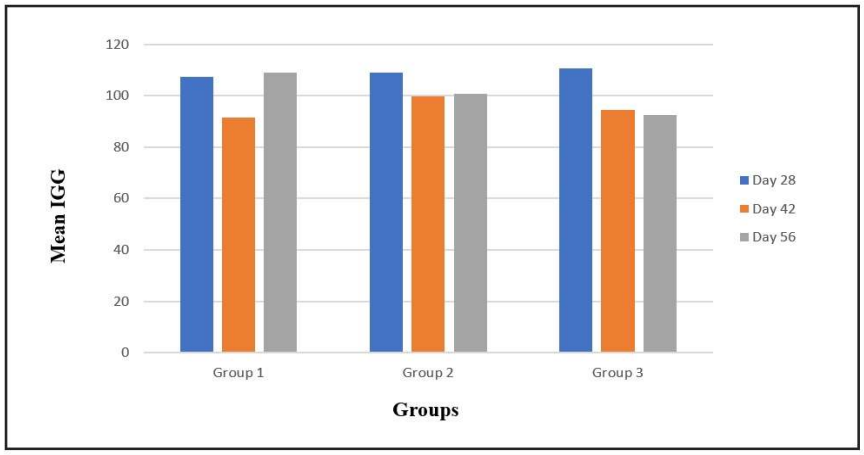

Figure (3-3) Mean of IgG concentration in rabbits immunized by KWCST-Ag

3.2.2. Interleukin 4 (IL-4) test

The IL-4 concentration in 3rd group elevated to 25.11±1.88pg respectively at days 28 post immunization then decrease gradually to reach 23.74±2.93pg at days 56 post immunization with significant difference (P<0.05). IL-4 concentration in 1st and 2nd groups was 23.94±0.83pg and 25.56±1.90pg at day 28 post immunized respectively, and elevated gradually to reach 27.28±3.59pg and 26.89±2.37pg at day 56 post immunized respectively with significant difference (P<0.05) shown in Fig (3-4).
Results revealed that the differences among groups were significant (P<0.05) for the three periods. The mean of IL-4 concentration in 2nd group was significantly (P<0.05) higher than of other groups in all periods, Fig (3-4).

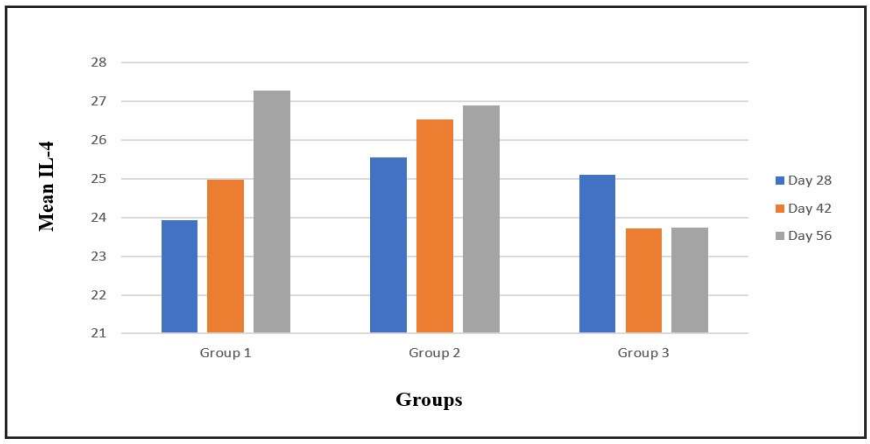

Figure (3-4) Mean of IL-4 concentration in rabbits immunized by KWCST-Ag

4. Discussion

4.1. Delayed type hypersensitivity (DTH) – skin test:

The results of delayed type hypersensitivity showed high mean of erythema at 24 hr. and induration at 48 hr. in the 2nd group due to immunized by two antigens of crude killed whole cell sonicated antigen- *Salmonella typhimurium* (1mg/ml) and crude killed whole cell sonicated antigen- *Staphylococcus aureus* (1mg/ml), DTH test indicated there was significant effect of the antigen injected on flank region. this result is in agreement with Strindelius (2004) who used DTH as a measure of cellular immunity in mice immunized with different types of Salmonella antigens the mice showed a significant increase in all immunized groups[13]. Rasheed (2011) recorded that the elevation in erythema at 24hrs and induration in 48hrs in group immunized with sonicated *S. typhimurium* and *S.enteritidis*[14].

4.2. Enzyme-linked immunosorbent assay (ELISA):

4.2.1. Immunoglobulin-G (IgG): In the present study, the IgG concentration at 28 day higher than other days with significant differences ($p<0.05$) in rabbits immunized by killed whole cell sonicated antigen-*Salmonella typhimurium* (1mg/ml) and killed whole cell sonicated antigen-*Staphylococcus aureus* (1mg/ml). This are in line with Kang who described that Immunoglobulin-G antibody response were stimulated to both the heterologous antigen rPspA and *Salmonella* lipopolysaccharide an OMPs, after a single oral immunization in Balb/C mice[15]. Another study observed the synergic reaction between *Salmonella typhimurium* and *Cryptococcus neoformans* antigen, with similar results given for IL-2, IL-6, and IL-8 (AL-Samarraae and AL –Maadhidi, 2018). Showing that the use of an adjuvant can enhance the killed antigen greatly[16].

4.2.2. Concentration of IL-4: The present study showed higher concentration of IL-4 at 28 day than other days with significant differences ($P<0.05$), in the 2nd group which agreed with Mohammed, that reported significant increase of IL-4 concentration in immunized group with killed whole cell sonicated antigen-*Salmonella typhimurium* (1mg/ml) and B. mellitensis (Rev-1) antigen $2x10^9$ cfu/ml compared with control group[17]. There is an agreement with Galdiero (1998)

who mentioned that killed Salmonella vaccines or purified bacterial components injected to mice was gives rise to an IL-4-dominated[18].

5. Conclusions

1- The interaction of both sonicated *Salmonella typhimurium* and *Staphylococcus aureus* antigens enhanced the skin immune response.

2- Stimulate the production cytokine such as IL-4 have been an indication for the cellular resistance to facultative intracellular *Salmonella typhimurium* infect.

3- Elevation of the levels of specific IgG-class antibody in serum can be regarded as serological evidence of *Salmonella typhimurium* immunization.

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