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Modulation of Immune system by Oral Administration Pathogenic *Escherichia coli* Isolated from Cow Mastitis

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Abstract

The immune system has an effective role in the defense mechanism against different microbial agents. This study aimed to identify the effect of experimentally oral administration of pathogenic *Escherichia coli* isolated from cow mastitis on modulation of immune system in rats. 15 Wister albino rats divided in to three groups,5 each , control group, G1 orally infected group by 10^5cfu/ml *E.coli* and G2 orally infected group by 10^6cfu/ml *E.coli*, after 15 days of infection ,blood samples collected and serum separated for identification the level of CD4, CD8 and TNF-alpha, by using ELISA test. Our result revealed that there was no significant difference in CD4 and CD8 between control group and oral group G1, while there was a significant increase in CD4 and CD8 concentration between control group and oral group G2, and a significance increase in TNF-α level in G1 and G2 groups when compared with control group. Conclusion: Oral administration of pathogenic *Escherichia coli* had a great modulatory effect on the immune system and the dose 10^6 cfu/ml *E.coli* was more potent than10^5 cfu/ml *E.coli* dose in stimulation and modulation of immune response.

Key words: *E coli*, immune system, CD4, CD8, TNF-α

Introduction

The immune system has a major role in the defense mechanism against different microbial agents. It can recognize the self from non self cells. It also classified to specific and innate immunity (1) The immune system has interconnected reactions which protect the body from foreign material invasion. It fights pathogens through innate immunity and acquired immunity (2) Immunoglobulin's, helper T-cells, polymorphonuclear, cytotoxic T-cells, and dendritic cells are the main cells of the immune system (1).

When bacterial infection occurs, CD4 T cells stimulated by the expression an antigen found on MHC class II but CD8 T cells stimulated by the expression of the antigen present on MHC class I. CD4 cells activation will stimulate production of IFN-gamma, which stimulate the macrophages ,which increased the secretion of nitrous oxide which lead to death of bacteria. More over CD8 cells have an effective role in the

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protection through cytotoxicity, by destruction of macrophages which infected (3). CD8 T Cells also are important for many intracellular bacterial infections (4). When infection with *Escherichia coli* occurred, CD8 cells appeared firstly in while CD4 appeared at the later hours of infection (5).Tumor necrosis factor alpha (TNF-α) was firstly known to induce necrosis for tumor cells, but recently it has additional role on autoimmune disease (6). It is a cytokine protein containing 157 amino acids which has a major role in regulation of inflammatory response and modulation of immune system was occur through infection with various pathogen like *Candida albicans* caused modulation in the effect on the level of IL-3, GM-CSF and IL-25 (7, 8). This study aimed to identify the modulatory effect of experimentally oral administration of pathogenic *Escherichia coli* isolated from cow mastitis on immune system in rats.

Material and methods

Animals used in this study

In this study, 15 Wister male albino rats aged (8-9) weeks and their weight between (132-188 gm). The rats were obtained from animal breading center, in Iraq. The study regarded the ethical role of Vet. Medicine, Diyala University. The rats were caged in the animal house of the supplier for 19 day at standard humidity (50–60%), temperature (22–30°C). They had free excess of food (standard pellets) and drinking water (ad libitium) during all experiments.

Preparation of bacterial strains:

Escherichia coli species which isolated on MaCconkey and EMB agar from field cow mastitis cases then confirmed by VIETK II system (Biomerieux) and were adjusted at concentration of 10^5 and 10^6 cfu/ml at microbiology lab. of Vet. Medicine, Diyala University.

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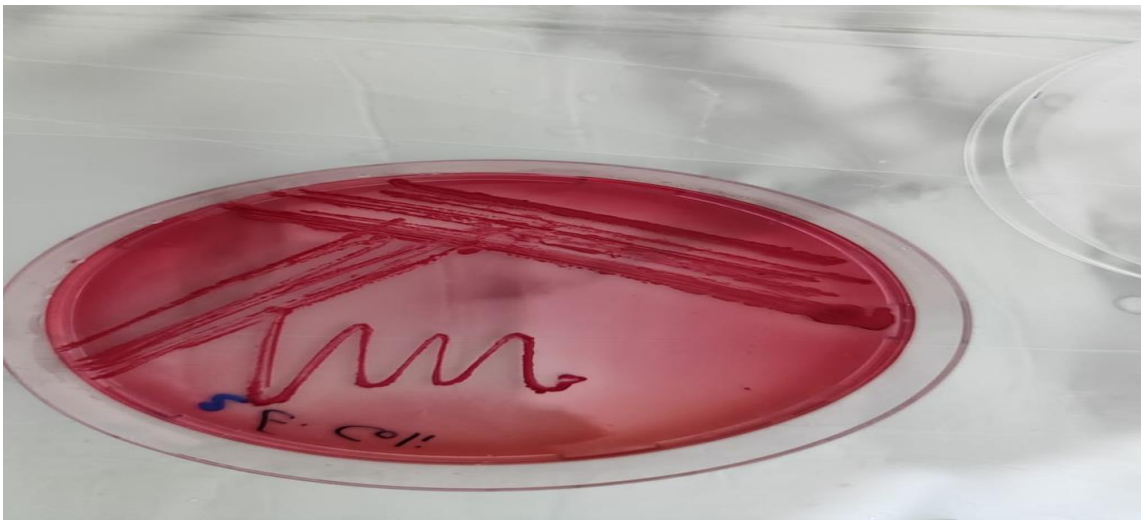

Figure1: E.coli showed rounded, mucoid bright pink colonies (lactose fermenter) on the surface of MacConkey' s agar medium

Experimental design

Sixteen Wister albino male rats were divided into three main groups: **Control group:** not infected with *E. coli* , **G1:** infected orally with 100 µl suspension of *E.coli* (10^5 cfu/ml), while **G2:** infected orally with *E.coli* (10^6 cfu/ml). After 15 day of infection, rats' blood sample was collected in tubes, and left for 30 minutes to coagulate at 25 °C. Followed by spun at 2000 xg for 15 minutes at 4^o C using a centrifuge (Hermle), the serum was store at -80^o C until it was used. ELISA Kits (from BT LAB Company) was used for detection of CD4, CD8 and TNF-α with patch number E0044Ra, E0045Ra and E0764Ra respectively, the method was done according to manufacture instructions.

Figure 2: sample blood collection

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Statistical Analysis

The data was analysed by using IBM SPSS statistics 20 for analysis of data, and ANOVA tests. The data were presented as (mean ± SD). Variance significance was at (P value ≤ 0.05).

Results

The results showed the effect of *E. coli* infection on the level of CD4 in the serum of infected rats, as presented in Figure (1), there was no significance between control group which was (0.15 ± 0.04) U/mL and oral group G1 which was (1.07± 0.35) U/mL, while there was a significant increase in CD4 concentration in G2, which was (2.26± 1.29) U/mL.

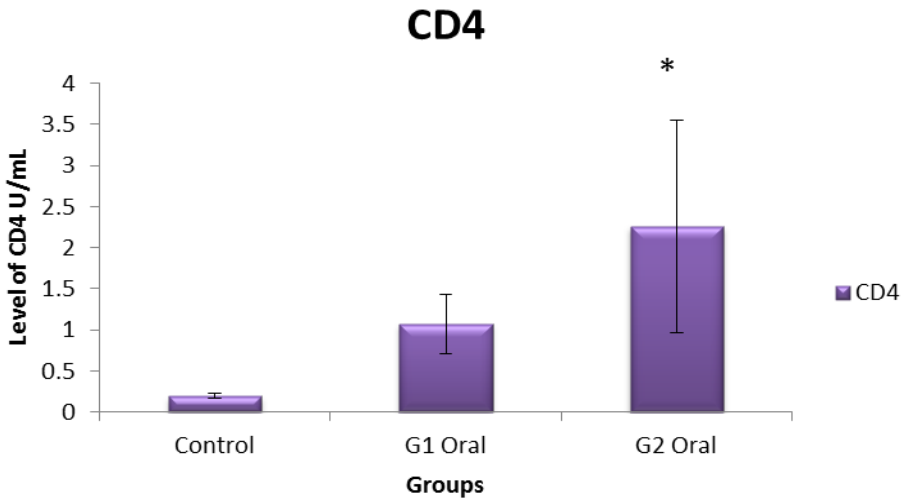

Figure 3: The effect of induced *E. coli* infection on the level CD4 U/mL in the rats among study groups: G1(10^5 cfu/ml) and G2 (10^6 cfu/ml). *Significant P ≤0.05

As shown in Figure (2) there was no significance in CD8 level between control group which was (64.47 ± 11.97) ng/mL and G1 which was (74.31± 6.60) ng/mL, while there was a significance increase in CD8 level between control group and G2 which was (99.04± 14.97) ng/mL.

As shown in Figure (3) there was a significance increase in TNF-a level at G1 and G2 compared with control group , which were (67.72 ± 22.01, 90.11± 14.14 and 113.10± 28.71) ng / L respectively.

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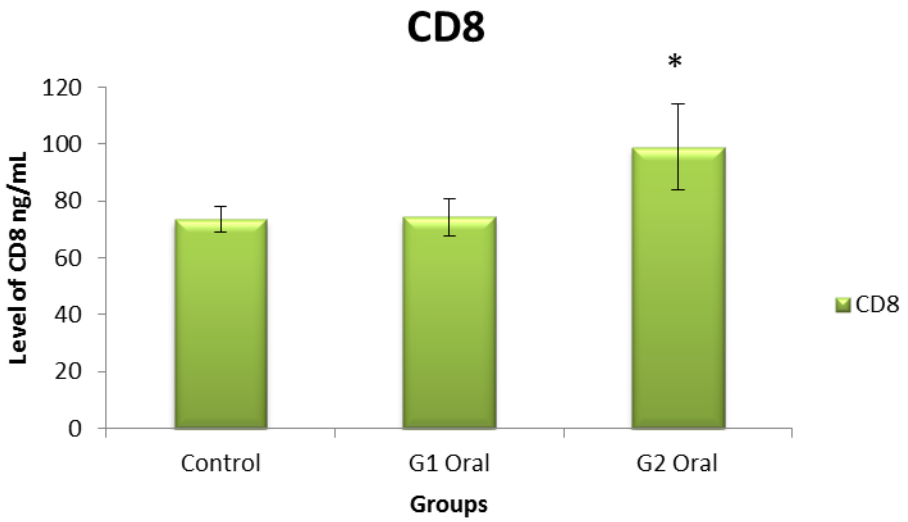

Figure 4: The effect of induced E. coli infection on the level of CD8 in the rats among study groups: G1 (10^5 cfu/ml) and G2 (10^6 cfu/ml). *Significant P ≤0.05

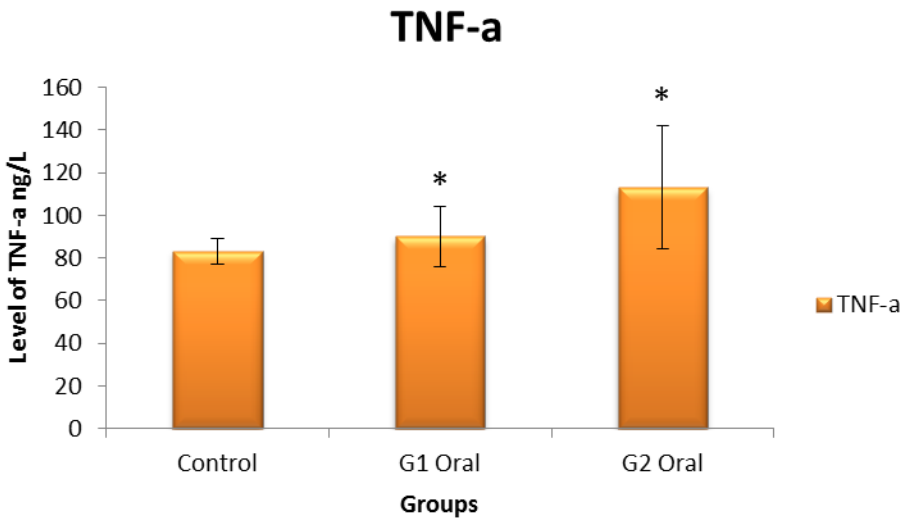

Figure 3: The effect of induced *E. coli* infection on the level of TNF-α ng/ L in the rats among study groups: G1 (10^5 cfu/ml) and G2 (10^6 cfu/ml). *Significant P ≤0.05

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Discussion

The immune system has a major role in protection of the body and defense against different microorganisms infections. The cellular immune response can inhibit replication of pathogen and get rid of infected cells (9). For studying of immunomodulatory effect of oral infection of *E.coli* in rats, the level of CD4, CD8 and TNF- α were investigated. The results revealed that there was no significant difference between control group and G1, while there was a significant increase in CD4 concentration between control group and G2 ,these promoted that stimulation for immune response occur at certain concentration of the bacteria. CD8 cells play an important role in the protection mechanism as they have cytotoxic effect not only on infected cells but also on infected macrophages (3, 10). The finding of this study showed that there was no significance in CD8 level between control group and G1, while there was a significance increase in CD8 level between control group and G2, which proved that oral infection with 10^5 cfu/mL *E.coli* was less potent in stimulation of immune response than infection with 10^6 cfu/mL *E.coli*, this result the same with notice in pervious study found that infection of *E. coli* activated CD4 and CD8 T cells macrophages produce TNF-α in , response to many Gram-negative bacterial infection. As pathogen activates the NF-*κ*B which is essential for stimulation of inflammatory cytokines as TNF-α and interleukins which have a potent effect in fighting infection (11, 12). In the present study there was a significance increase in TNF-α level between control group, G1 and G2. With the higher concentration in oral infected G2 group , these result agreed with (13) who found that the level of inflammatory factor TNF-α increased after infection in mice, also our result go on parallel with (14) who found that there was a significance increase in TNF-a level between control group and infected group which were (64.66 ± 1.36 and 195.22 ± 10.21), respectively. and (15) who reported that TNF-α. and IL-10 were significantly increased in serum of *E.coli* infected groups**.**

Conclusion: The results of this study was concluded that oral administration of pathogenic *Escherichia coli* can modulate the immune system and the dose 10^6 cfu/ml *E.coli* was more potent than10^5 cfu/ml *E.coli* dose in stimulation of immune response

Conflict of interest: No conflict of interest

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