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The preventive role of lactoferrin on some immune variables in rats experimentally infected with E. coli leading to amelioration the infection

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ABSTRACT

Male albino rats were used in this study and were obtained and bred in the animal house of the College of Veterinary Medicine, Tikrit University. A total of thirty two animals were used in the experiment and divided into two groups, each containing 16 animals. The first group received 100 mg/kg of lactoferrin orally. The second group (the control group) was given only water. Then, the animals were given 0.5 ml of E.coli bacterial suspension containing 4×106 CFU/ml . This dose was determined by the LD50 test.Blood was drawn from the eye socket on days (3, 6, 12, 24) from the start of the experiment and placed in special tubes to conduct the tests required in the research, which are, IgG, CD8, IL-1β, TNF-α,The tests were performed using ELISA technology. The highest CD8 value on day 24 for animals in the control group was given only E.coli. Interleukin 1 β showed the highest value on days 3 and 6 for the lactoferrin group given bacteria. IgG showed the highest value on day 24 for the lactoferrin group given bacteria. Finally, TNF- α showed the highest value on day 3 for the lactoferrin group given bacteria. Lactoferrin influences immune variables and bacterial resistance.

Introduction

Lactoferrin is an iron-binding glycoprotein of the transferrin family with a molecular weight of 80 kDa (Hao et al., 2019) consisting of 703 amino acids. It is found mainly in milk, in various mammalian external fluids and secretions such as tears, saliva, cervical mucus, and others (Rascón-Cruz et al., 2021). Lactoferrin is a protein with multiple physiological functions. Studies have shown that lactoferrin performs 20 different functions in mammals, such as regulating iron absorption in the intestine, antioxidant, anticancer, antimicrobial, and anti-inflammatory (Artym and Zimecki, 2021). One of the most important functions of lactoferrin is its antibacterial effect, which is due to its ability to extract iron from the bacterial medium and reduce its availability. In addition to the direct association that occurs between lactoferrin and the components of the cell wall surface, which affects the stability of this wall and thus the death of the bacterial cell (Sienkiewicz et al., 2022). The positive charge carried by lactoferrin enables it to bind with the negatively charged molecules of some components of the cell wall of Gram-positive bacteria, including lipoteichoic acid, while its binding with Gram-negative bacteria is with lipopolysaccharide LPS (Nurul Aini et al., 2019). Therefore, in this research, the effect of lactoferrin on animals experimentally infected with E.coli bacteria was studied.

Material and methods

Thirty two male Albino rats were used in this experiment, divided into two groups, with 16 animals in each group. The first group, the control group, was given only water, and the second group was given lactoferrin orally at a rate of 100 mg/kg daily throughout the experimental period. In the second day, the animals were given 0.5 ml of E.coli bacterial suspension containing 4×106 CFU/ml, this dose was determined using the LD50 test (Randhawa, 2009). Blood was drawn from the eye socket on days (3, 6, 12, 24) from the start of the experiment and placed in special tubes to conduct the tests required in the research, which were IgG, CD8, IL-1β, TNF-α, the tests were performed using ELISA technology.

A sandwich enzyme-linked immunosorbent assay (ELISA) kit (Manufacturer Name) was used. The antibody specific for the variant to be tested is pre-coated on a microplate, samples are added to the wells, and any molecules of the desired variant bound to the antibody are immobilized after the suspended matter was removed. The biotin-conjugated antibody specific to the desired variant was then added to the wells. After washing, peroxidase (HRP) solution was added to the wells. After washing, any unbound reagent is removed. Then, the substrate solution is added to each well. The color develops in proportion to the number of variables to be examined. The reaction is stopped, and the optical density of the samples (OD) is measured and compared with the standard curve.

Statistical Analysis

The ready-made statistical program SAS (2005) was used to analyze the data. A complete randomized design (CRD) was used to analyze the data. Individual differences between treatments were tested using the Duncan (1995) multiple range test, based on the following mathematical model:

$Yij = \mu + Ti + eij$

Ti = type effect Yij = observation value of the studied trait

eij = Random error that is normally distributed with mean of zero and variance of δ2e for each characteristic

Results and Discussion:

Table (1) and Figure (1) showed the level of Tumor Necrosis Factor Alpha (TNF-α) cytokine. where the control group showed a slight increase on day (3) at a rate of (72.75 pg/ml) today (6) at a rate of (77.25 pg/ml).After this period, the level of cytokine began to decrease on day (12) at a rate of (65.5 pg/ml) and on day (24) at a rate of (62.25 pg/ml). As for the group that was given lactoferrin accompanied by E.coli bacteria, it showed the highest level on day (3) at a rate of (86.75 pg/ml) comparedo the control group, and then this level began to decrease on day (6) and day (12) at a rate of (73.5 pg/ml), which continued to decrease to a lower level at a rate of (60.5 pg/ml) on day (24). It is clear that giving lactoferrin accompanied by E.coli bacteria orally stimulated a strong immune response of TNF-α cells, and this was noticeable initially on day (3) compared to the control group, but this response began to decline to a level lower than the level of control cells on day (24). This decrease in the level of TNF-α cells over time is an indication of the process of eliminating inflammation and restoring tissue integrity (Lima et al., 2022). Some studies have indicated that lactoferrin may enhance the release of TNF from immune cells, such as macrophages, in response to bacterial infection. Lactoferrin can act as a stimulator of a group of cytokines, including TNF, contributing to the inflammatory response necessary to combat infections such as E. coli (Drago-Serrano et al., 2017)

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Table (1): the effect of lactoferrin and E.coli bacteria on the level some of immunological markers of experimentally infected rats through oral dosing.

CD8 pg/ml	IL-1β	IgG ng.ml	TNF α pg.ml	Treatment & Time
155.93 ± 15.53 f	230.33 ± 21.19 a	5352.25 ± 95.79 d	72.75 ± 1.75 bc	**C+ve 3**
232.11 ± 11.71cd	169.25 ± 8.79 b	5590.75 ± 81.53 d	77.25 ± 1.93 b	**C+ve 6**
267.02 ± 14.18 cb	134.55 ± 3.13 d	6033.75 ± 61.34 c	65.50 ± 1.75 cd	**C+ve 12**
361.47 ± 16.93 a	116.50 ± 3.93 d	6231.25 ± 42.31 c	62.25 ± 1.25 d	**C+ve 24**
294.52 ± 13.25 b	205.55 ± 3.19 a	6035.00 ± 70.44 c	86.75 ± 2.83 a	**L+ *E.coli* 3**
247.89 ± 13.97 c	211.12 ± 9.05 a	6589.75 ± 68.43 b	73.50 ± 2.50 bc	**L+ *E.coli* 6**
210.25 ± 12.56 d	157.32 ± 11.59 bc	6786.00 ± 80.06 b	73.50 ± 5.10 bc	**L+ *E.coli* 12**
179.27 ± 18.58 d	153.15 ± 6.28 bc	7198.50 ± 90.19 a	60.50 ± 2.21 d	**L+ *E.coli* 24**
*	*	*	*	**Moral level**

C+ve = control positive , L= lactoferrin , (*) used for p-values
Different letters within a column indicate significant differences at a significance level of p<0.05.

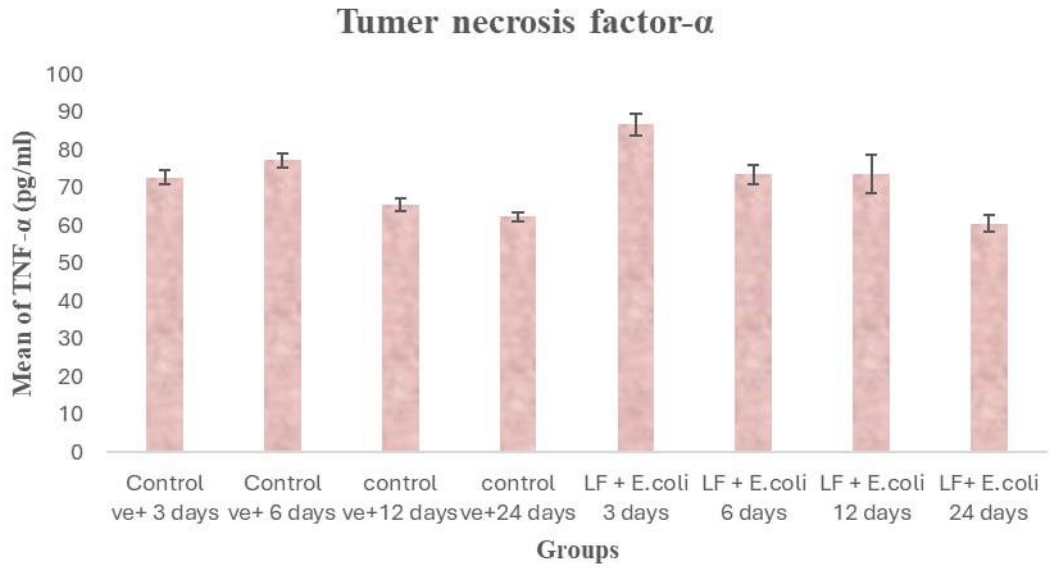

Figure (1): the level of Tumer necrosis factor in rat groups after Administration of lactoferrin and E.coli orally at different periods.

From the above data, it is clear that the level of TNFα cytokine was at its highest level when lactoferrin and E. coli bacteria were given to rats at the same time at the beginning of the dosing period on day (3). Studies have confirmed that infection with E. coli bacteria, especially when injected intravenously, can cause a significant and noticeable increase in the levels of TNFα cytokine (Cazanga et al., 2023). This increase usually quickly reaches its peak after exposure to E. coli bacteria or exposure to lipopolysaccharide (Wang et al., 2022)

From Table (1) and Figure No. (2), it is clear that the level of CD8 (cluster of differentiation 8) in the control group showed a constant increase at a rate of (155.93 pg/ml) on day (3), which continued to increase on day (6) at a rate of (232.11 pg/ml). The level of cytokine continued to rise, but at a lower rate on day (12) compared to the rise on day (6), which ranged at a rate of (267.02 pg/ml). As for the level of cells on day (24), it showed an increase over the level of cells at a rate of (361.47 pg/ml), which is an increase over the level of cells on day (12). The increase in

the level of cells in the control group showed an unstable pattern in the level of increase in this group.

However, in the group that was given lactoferrin and E. coli bacteria orally, it showed a steady decrease in the level of (CD8) cells over time. The level of cells on day (3) was at its highest level at a rate of (294.52 pg/ml), which began to decline at a rate of (247.89 pg/ml) on day (6). The level of (CD8) cells continued to decline but at a lower rate on day (12) and day (24) at a rate of (210.25 pg/ml) and (179.27 pg/ml), respectively. The level of cells in the control group showed an increase in the level of the adaptive immune response over time. Furthermore, the early increase in CD8 cell levels, followed by a steady decline over time, indicates that the administration of lactoferrin in conjunction with E. coli bacteria altered the dynamics of the immune response, indicating an enhanced adaptive immune response (Ohradanova-Repic et al., 2023).

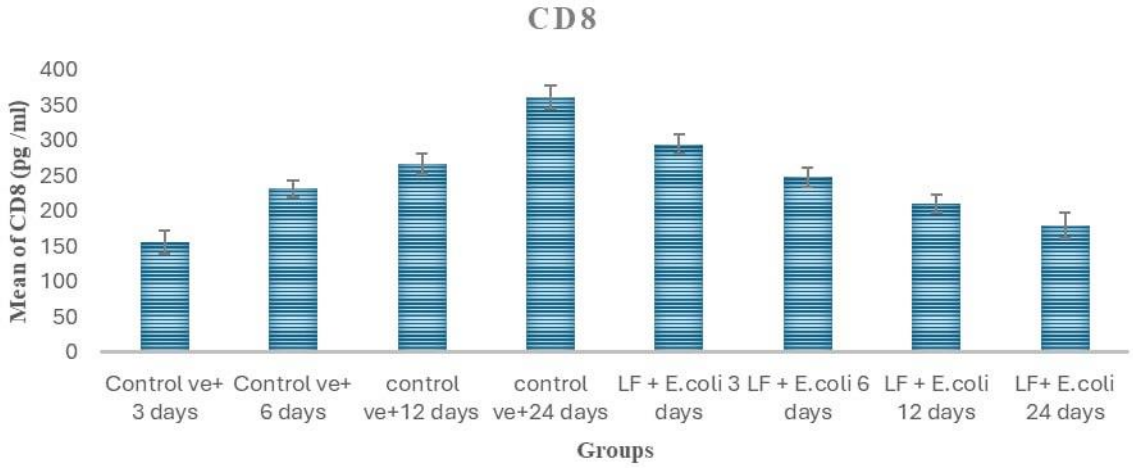

Figure (2): the level of CD8 in rats after Administration of lactoferrin and E.coli bacteria orally at different periods.

The data showed that the highest level of CD8 cells was on day (3) after the rats were inoculated with E.coli bacteria and lactoferrin compared to the rest of the groups except for the positive control group that was dosed with E.coli bacteria only. Studies have shown that increasing the dose of E. coli bacteria accelerates the transfer of CD8+ T cells, indicating their important role in stimulating the early immune response. The study also reported a significant decrease in the CD4+/CD8+ cell concentration ratio due to the increased concentration of CD8+ T cells during the first hours of infection (Mehrzad et al., 2008). In another study, it was confirmed that lactoferrin has an important role in stimulating the differentiation of lymphocytes (CD4- CD8-) into T helper cells (CD4+), which indicates its important role in the maturation of T cells rather than directly affecting the levels of T cells (CD8+ T) (Elass et al., 2002).

It is shown from Table (1) and Figure (3) that the level of immune cytokine (IL-1β) for the control group was a steady decrease throughout the study period, as it showed the highest level on day (3) at a rate of (230.33), and on day (6) it showed a noticeable decrease compared to day (3) at a rate of (169.25). As for day (12), it decreased compared to day (6) at a rate of (134.55). As for day (24), it recorded a decrease compared to day (12) at a rate of (134.55).

As for the group that was given lactoferrin and E.coli bacteria orally, it showed a complex pattern. It is clear from Table (1) and Figure (3) that the level of cells (IL-1β) on day (3) was at a rate of (205.55) and then increased on day (6) compared to day (3) at a rate of (211.12). After this period, the level of cells showed a significant decrease on day (12) compared to day (6) at a rate of (157.32), followed by a decrease on day (24) compared to day (12) at a rate of (153.15). From these readings, we conclude that the level of immune cells (IL-1β) showed a steady decrease in the level of the pro-inflammatory cytokine, which indicates the elimination of inflammation naturally. As for the group that was given lactoferrin and E.coli bacteria orally, it showed a conservative pattern in terms of level with a slight increase on the sixth day (6) before a decrease on the day (24), which was higher compared to the control group. This pattern suggests that lactoferrin associated with E. coli bacteria may prolong a type of immune response, especially the production of IL-1β, which enhances immunity, as shown in the figure (3).

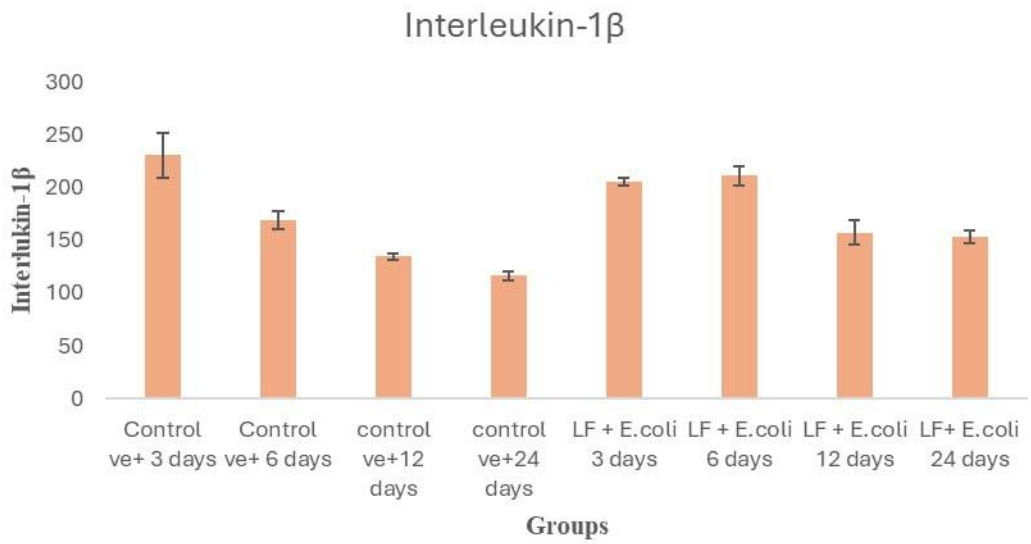

Figure (3): the level of IL-1β in rats after Administration of lactoferrin and E.coli bacteria orally at different periods.

In the levels of (IL-1β), it was found that the highest level was in the group that was given E.coli bacteria accompanied by lactoferrin on day (6) compared to the other groups except for the control group, which showed a slightly higher level. Studies have confirmed that infection with E.coli bacteria, especially strains that produce Shiga-toxin, stimulates neutrophils to produce high levels of (IL-1β) (Sabbione et al.,2023). Studies have confirmed that lactoferrin plays a complex role in modulating the immune response when the body is infected with inflammation. It can also lead to reduced levels of other types of interleukins, such as IL-10, IL-1β, and IL-8, further underscoring its importance in the immune response (Lutaty et al., 2020; Pehlevan et al., 2020; Widjaja et al., 2023). Therefore, lactoferrin and E. coli may work antagonistically to modulate cytokine levels.

It is clear from Table (1) and Figure (4) that the level of immunoglobulin G (IgG) parameter in the control group showed a steady increase in the level over time. The highest level of cells was shown on day (3) at a rate of 5352.25 ng/ml. The level increased on day (6) over day (3) at a rate of (5590.75 ng/ml). The increase continued day (12) over day (6) at a rate of (6033.75 ng/ml). In addition, on day (24), the level of (IgG) cells continued to increase compared to day (12) at a rate of (6231.25 ng/ml).

The group that administrated lactoferrin and E. coli orally showed a steady increase throughout the study period. Table (1) and Figure (4) show clear significant differences between groups, the level of (IgG) parameter for this group on day (3) was (6035 ng/ml), while on day (6) it showed an increase over day (3) at a rate of (6589.75 ng/ml). As for day (12), the level of cells increased over day (6) at a rate of (6786 ng/ml), and this increase continued to reach a level of (7198.5 ng/ml) on day (24), which represents an increase over day (12). The highest percentage of IgG was on day 24 for the group dosed with lactoferrin and bacteria, indicating that lactoferrin stimulates the body's immune response to produce antibodies against bacteria.

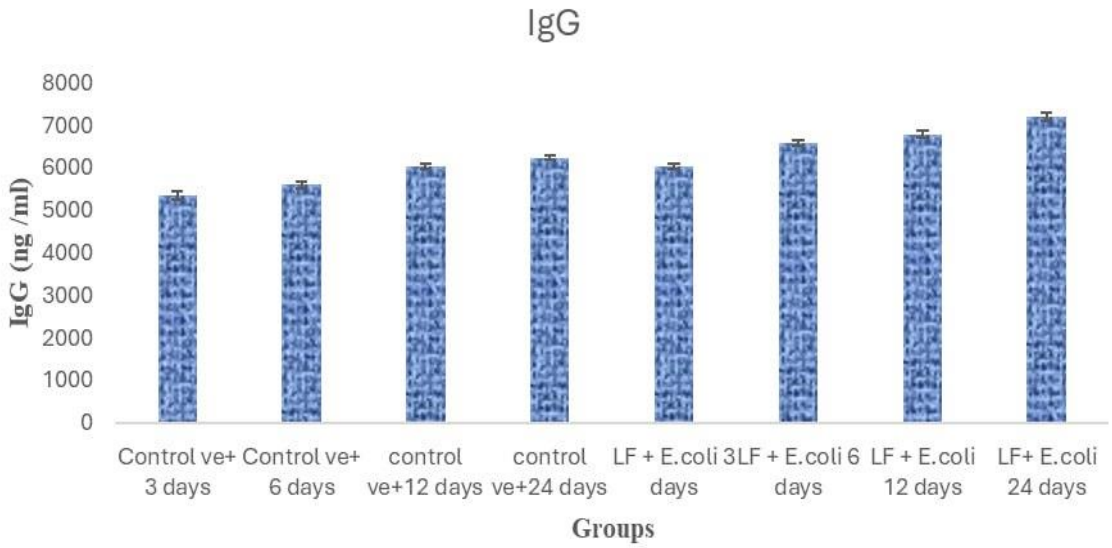

Figure (4): the level of IgG in rats after Administration of lactoferrin and E.coli bacteria orally at different periods.

From the above data, it is clear that oral administration of lactoferrin and E.coli to rats enhanced the humoral immune response as measured by the level of IgG , and that this group not only showed the highest level of increase in IgG level on day (3) but also maintained this level of increase throughout the study period. These readings indicate that the combination of lactoferrin and E.coli in oral administration enhanced the production of antibodies compared with the control group (Avalos-Gómez et al., 2022).

As for the IgG immunoglobulin cells, the data showed that the highest levels were observed after giving lactoferrin and E.coli bacteria on day (24). This is because lactoferrin helps stimulate the immune system to produce more antibodies (IgG) in response to combat E. coli bacteria (Liu et al., 2021).

Conclusion

Lactoferrin enhances the immune response in rats against E. coli by increasing the levels of CD8 cells and IgG antibodies and regulates inflammatory cytokines such as IL-1 and TNF-α.

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الدور الوقائي للاكتوفرين على بعض المتغيرات المناعية في الجرذان المصابة تجريبياً ببكتيريا الإشريكة القولونية E.coli والمؤدية الى التخفيف من حدة الاصابة

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الملخص

تم استخدام ذكور الفئران البيضاء في هذه الدراسة وتم الحصول عليها وتربيتها في بيت الحيوانات بكلية الطب البيطري جامعة تكريت. تم استخدام ما مجموعه اثنين وثلاثين حيوانًا في التجربة وقسمت إلى مجموعتين تحتوي كل منهما على 16 حيوانًا. تلقت المجموعة الأولى 100 ملغم / كغم من اللاكتوفيرين عن طريق الفم. أعطيت المجموعة الثانية (مجموعة الضبط) الماء فقط. بعد ذلك، أعطيت الحيوانات 0.5 مل من معلق بكتيريا الإشريكية القولونية الذي يحتوي على 4 × 106 وحدة تشكيل مستعمرة / مل. تم تحديد هذه الجرعة عن طريق اختبار LD50. تم سحب الدم من تجويف العين في الأيام (3، 6، 12، 24) من بداية التجربة ووضعها في أنابيب خاصة لإجراء الاختبارات المطلوبة في البحث وهي IgG و CD8 و IL-1β و TNF-α، أجريت الاختبارات باستخدام تقنية ELISA. أعطيت أعلى قيمة CD8 في اليوم 24 للحيوانات في المجموعة الضابطة فقط E.coli. أظهر إنترلوكين 1 بيتا أعلى قيمة في اليومين الثالث والسادس لمجموعة اللاكتوفيرين المُعطاة للبكتيريا. كما أظهر IgG أعلى قيمة في اليوم الرابع والعشرين لمجموعة اللاكتوفيرين المُعطاة للبكتيريا. وأخيرًا، أظهر TNF-α أعلى قيمة في اليوم الثالث لمجموعة اللاكتوفيرين المُعطاة للبكتيريا. يؤثر اللاكتوفيرين على المتغيرات المناعية ومقاومة البكتيريا.

الكلمات المفتاحية: اللاكتوفرّين، الإشريكية القولونية، الخلايا التائية السامة، الغلوبيولين المناعي G، الإنترلوكين1 .