Immunological Effects Caused by Klebsiella pneumoniae Infections in White Rats and Select of Inhibitory for Rosmarinus Officinalis Extract's

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Abstract

This study was conducted in the laboratories of the Department of Biology, College of Education for Pure Sciences, Tikrit University, and the study aimed to isolate and diagnose Klebsiella pneumoniae,and study its immunopathology after infection in white male mice, 65 clinical samples were collected from sputum. Twelve isolates belonging to Klebsiella pneumoniae were diagnosed, and they constituted 35.2% of the total 34 gram-negative isolates. 25 laboratory animals were used, distributed into 5 groups, 5 animals per group. The first group represented the control group, while the second group was injected with a bacterial suspension in a volume of 1.5 ml and was not treated with any substance, while the third group was injected with a bacterial suspension with a volume of 1.5 ml and was treated with aqueous extract of rosemary leaves, and the fourth group was injected with a bacterial suspension at a volume of 1.5 ml and was treated with alcoholic extract rosemary leaves, As for the fifth group, it was injected with the bacterial suspension in a volume of 1.5 ml and was treated with the antibiotic Imipenem. The results of the study showed a decrease in the rate of IL-1α, which amounted to 59,714 pg / ml compared to the control group, and an increase in the rate of IL-17 was recorded, which amounted to 469.153 pg / ml The results confirmed the presence of a cellular and humoral immune response caused by infection with the bacteria Klebsiella pneumoniae.

Keywords. Klebsiella pneumoniae, Rosemary officinali, IL-17, IL-1α.

1. Introduction

The world today is facing a big problem, which is the undeclared war between germs and antibiotics. The most important of these bacteria is *Klebsiella* spp. This genus belongs to the enterobacteriaceae family, facultative anaerobes, immobile, gram-negative, and the usual environment for these bacteria is the intestinal tract of humans and animals. But it may move to another place, causing a wide range of infections, such as wound infection, urinary tract infection and respiratory infection [1]. *Klebsiella* spp. represent one of the types of opportunistic pathogens, and this bacteria accompanies AIDS patients and patients with chronic obstructive pulmonary disease, as well as patients with immunocompromised patients and are admitted to hospital [2], Klebsiella spp. infection is mainly caused by *Klebsiella pneumoniae*, and is the most pathogenically important species of the genus. *Klebsiella pneumoniae* is an important hospital pathogen, often causing many clinical manifestations including pneumonia, urinary tract infection and meningitis [3]. In Iraq, Yaseen [4] discovered that both immune and cellular infection play an important role in controlling bacterial infection [4]. Rosemary is a tree herb belonging to the family Lamiaceae, and this plant grows in the Mediterranean basin, and spreads in many countries of the world because of its various uses, including as a spice for cooking and food preservation because it is an antioxidant, as it has been used as a medicinal herb for centuries because of its status for many diseases, anti-inflammatory [5],The plants of the oral family, including rosemary, are used to a large extent to treat respiratory diseases and acute lung inflammation, as the leaves of these plants are used effectively to resist infection more than the stem and others [6], The surfaces of the upper respiratory tract (including the nose, nasopharynx, oral passages, trachea, and pharynx) are colonized by normal flora bacteria. Diphtheroid aerial wedge, which can be cultured from the surfaces of the nose [7]. Interleukin-17, which can contribute to inflammation and autoimmunity [8] Recent studies indicate that IL-17 plays a role in the pathogenesis of respiratory distress syndrome [9] It was found that IL-17 is increased in the lung of mice infected with *P.*aeruginosa [10]. Interleukin IL-1α plays a key role in initiating and coordinating the immune response to most pathological inflammatory conditions including infections and infectious diseases [11] Previous studies have demonstrated that IL-1α is an inducer of epithelial cytokine receptors and enhances the proliferation and distribution of cytokines [12]. The study aims to know the effect of treatment with the bacteria Klebsiella pneumoniae on some biomarkers of white rats and to study the inhibitory effect of water and alcoholic extracts of Rosmmarinus plant in vivo.

2. Materials and Methods

2.1. Isolation and purification

65 samples were collected from Shirqat General Hospital from October 22 to December 22, 2021, and they were isolated from the respiratory system. The samples were cultured on blood agar medium, macConkey medium, and nutrient agar medium. The isolates were diagnosed, and biochemical tests were performed on Fox Proscore, Indole, Motility, Methyl Red, Catalase and Oxidase. Relying on a Berkee workbook [13], and what was done according to [14].

2.2. Prearation of aqueous and alcoholic

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The researcher's method was adopted [15], modified from the main method of the researcher [16],in the preparation of alcoholic extracts.

2.3. Experience design

The rats were divided into five groups, and each group included five rats: The first group, which is the control group, was given the standard diet of these rats with drinking water during 30 days. The second group, which is the only infected group: This group was given the standard diet with drinking water for 30 days. The third group was treated with aqueous extract of rosemary (100 mg/kg) orally for 5 days after infection, in addition to the ration with drinking water. The fourth group was treated with the alcoholic extract of rosemary (100 mg/kg) orally for 5 days after infection in addition to the ration with drinking water. The fifth group was treated with the antibiotic Imipenem (100 mg/kg) orally for 5 days after the injury in addition to the ration with drinking water.

2.4. Measurment of inteleukin 17 levels in albino rats

Interleukin 17 is measured using a ready-made analysis kit from the Chinese company Sunlong, and the method of work recommended by the company was followed.

2.4.1. Procedure

Dilution of the standard solution we leave an empty pit treated with control. In the pits of the sample add 40 µL of dilution solution and 10 µL of serum sample. Incubate at 37 Cº for 30 minutes after closing it with the membrane of the sealing plate. Dilute the concentrated washing solution with distilled water (30 times for 96 tests). Remove the shutter plate gently and repeat the washing process 5 times. We add 50 µL of HRP reagent to each hole except for the empty control hole. Incubation as described in step 3. Washing as described in step 5. Add 50 µL of chromogen solution A and 50 µL of chromogen solution B to all pits, mix with gentle stirring and incubate at 37 Cº for 15 minutes. We add 50 µL of stop solution to all pits. The pits to finish the reaction, and the color in the pits should change to yellow. At 450 nm using a Microtiter plate reader, the absorbance value of the empty control pit is set as zero, the test should be performed within 15 minutes after adding the stop solution.

Calculation of Results

The known concentrations of standard solutions of interleukin-17 and its corresponding OD reading are plotted on the x-axis and y-axis, respectively. The concentration of interleukin-17 in the sample is determined by plotting the sample O.D. On the y-axis, the dilution concentration is calculated by multiplying the dilution factor as in the fig. 1.

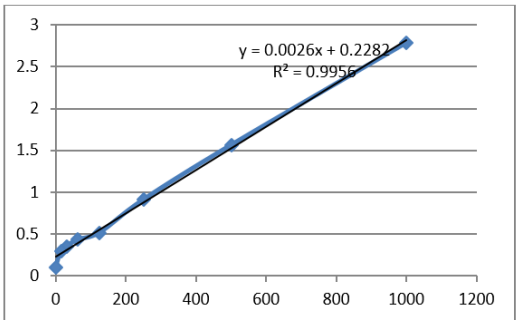

Figure 1. Interleukin 17 standard curve

2.5 Measurment of inteleukin 1alpha levels in albino rats

Interleukin 1α is measured using a ready-made analysis kit from the Chinese company Sunlong, and the method of work recommended by the company was followed.

2.5.1. Procedure

Standard solution dilution ten pits are set for the standard solution. In holes 1 and 2 add 100 µL standard solution and 50 µL standard dilution solution and mix well. In holes 3 and 4 add 100 µL solution from holes 1 and 2, respectively. Then 50 µL standard dilution solution is added and mixed well. Then 50 µL is removed from holes 3 and 4. In holes 5 and 6, add 50 µL of the solution from holes 3 and 4, respectively. Then add 50 µL of the standard dilution solution and mix well. In holes 7 and 8 add 50 µL solution from holes 5 and 6, respectively. Then 50 µL standard dilution solution is added and mixed well. In holes 9 and 10, add 50 µL of solution from holes 7 and 8, respectively. Then 50 µL standard dilution solution is added and mixed well. A 50 µL solution is discarded from holes 9 and 10. After dilution, the total volume in all pits is 50 µL and the concentration is 150 pg/ml, 100 pg/ml, 50 pg/ml, 25 pg/ml, 12.5 pg/ml, respectively. We leave an empty hole treated as a control. In the sample pits add 40 µL of dilution solution and 10 µL of serum sample. Incubate at 37 °C for 30 minutes after closing it with the membrane of the sealing plate. Dilute the concentrated washing solution with distilled water (30 times for 96 tests). Remove the membrane of the sealing plate. Gently and repeat the washing process 5 times. Add 50 µL of HRP reagent to each hole except the empty control hole. Incubation as described in step 3. Washing as described in step 5. Add 50 µL of chromogen solution A and 50 µL of chromogen solution B to all pits, mix, stirring gently and incubating at 37 Cº for 15 minutes. We add 50 µL of stopping solution to all the pits to finish the reaction, and the color in the pits should change to yellow. At 450 nm using a microtiter plate reader, the absorbance value of the control pit is set to zero, the test must be done within 15 minutes after adding the stopping solution.

Calculation of Results

The known concentrations of standard solutions of interleukin-1α and its corresponding OD reading are plotted on the x-axis and y-axis, respectively. The concentration of interleukin-1α in the sample is determined by plotting the sample O.D. On the y-axis, the dilution concentration is calculated by multiplying the

dilution factor as in the fig. 2.

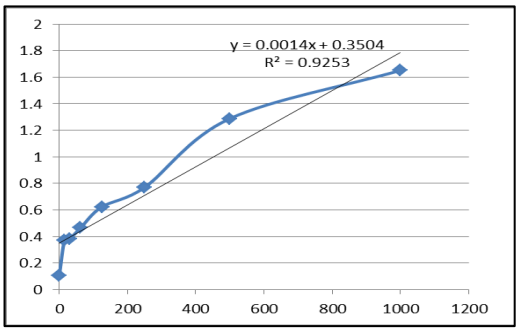

Figure 2. Interleukin 1α standard curve.

2.6 Statistical Analysis

Significant differences were extracted using ANOVA test, differences were confirmed by hard error, and significant differences were determined using Duncan's multinomial test [17].

3. Results and Discussion

3.1. DIAGNOSTIC RESULTS

The results of the biochemical examination that were adopted for the diagnosis of *Klebsiella pneumoniae* bacteria and were identical to the international diagnostic characteristics of this bacteria on MacConkey medium, according to the description [18].

3.2. MEASUREMENT OF THE LEVEL OF INTERLEUKIN-17 IN THE BLOOD SERUM

The results of the statistical analysis showed a significant increase in the infected group compared to the control group, and a significant decrease was recorded in each of the groups treated with antibiotic, treated with aqueous extract and the group treated with alcoholic extract compared with the control group, while the infected group had a significant increase as shown in the following fig. 3.

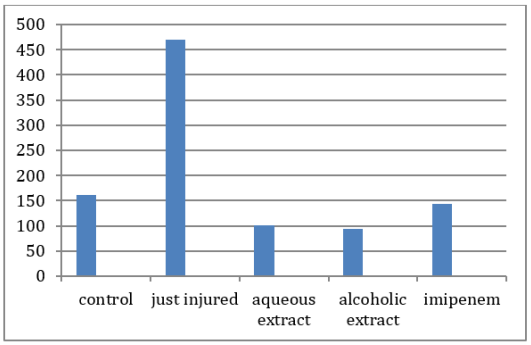

FIGURE 3. effect of the antibiotic imipenem, aqueous extract and alcoholic extract on the level of interleukin-17 in the blood serum

Interleukin-17 stimulates antimicrobial proteins and thus is a key mechanism for the elimination of pneumococcal bacteria [19]. Several studies indicate that IL-17 plays a key role in the host defenses inside the lungs, and it works to stimulate and migrate neutrophils to the site of inflammation [20] and people with pneumonia suffer from an increase in the level of IL-17, which works to generate an immune response and decreases again with treatment [21]. The results of the current study agree with Yasushi and his group [22] that infection with pneumococcus bacteria increases the level of interleukin 17, generates an immune response, and stimulates T cells and neutrophils to eliminate pathogens. Also, the results of the current study did not agree with what was found by Noorhan [23] in the level of interleukin 17 decrease in mice infected with *E.Coli.* The results of the study did not agree with what was found by Chen et al. [24] When measuring the level of interleukin-17 in the case of *Klebsiella pneumoniae*, the level of interleukin decreased in the epithelial layer of the lung, which plays an important role in creating chemical gradients necessary for mucosal immunity against pathogens of bacterial lungs.

3.3. Measurement of the level of interleukin 1α in the blood serum

The results of the statistical analysis showed a significant decrease for the affected group compared with the control group, and showed a significant increase in all treated groups compared to the control group, as shown in the following fig. 4

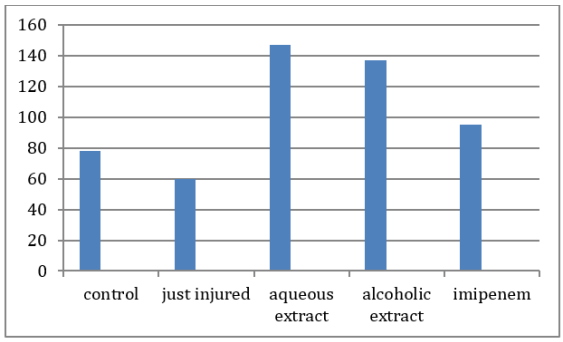

Figure4. Effect of the antibiotic imipenem, aqueous extract and alcoholic extract on the level of interleukin-1α in the blood serum.

IL-1α is a potent proinflammatory cytokine and is an important mediator of pathogenic bacterial pneumonia, whose elevation is evidence of bacterial infection [25] Superantigen also activates host cells to produce large amounts of bacterial inflammation-inhibiting cytokines especially IL-1α [26] Increased IL-1α in case of infection can lead to tissue damage and organ weakening [27],and [28] indicated that the gram-negative *Klebsiella pneumoniae* contains a LPS layer that stimulates the secretion of IL-1α in high concentrations, which stimulates natural killer cells, B cells, and neutrophils. The results of the current study did not agree with the findings [20]. When rats are infected with *Klebsiella pneumoniae*, an increase in IL-1α levels occurs. The results of the current study did not agree with [29] Increased levels of IL-1α when infected with *Pseudomonas aeruginosa* as a result of bacterial invasion. The results of the current study agreed with what was found [30] *Klebsiella pneumoniae* infection and treated with doses of azithromycin reduced the levels of IL-1α, and the data indicate that the dose and duration of treatment with antibiotics and plant extracts may not be sufficient to eliminate the infection with *Klebsiella Pneumoniae*.

4. Conclusions

The aqueous and alcoholic extracts of rosemary leaves

have the ability to inhibit the growth of isolates by 100%. Injecting laboratory animals with *Klebsiella pneumoniae* stimulated the cellular and humoral immune response. The alcoholic extract of rosemary leaves provided high protection among the treatments compared to the control group. Conducting histological studies of the important organs of laboratory animals, such as the lung, liver, heart and kidney, in order to evaluate histologically through what is caused by infection with *Klebsiella pneumoniae*.

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