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The Effect Of Propolis On Level Of Interleukin 17 And Interleukin 37 In Experimentally Infected Mice With Acinetobacter Baumannii

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Abstract

The objective of the current study was to determine the impact of low and high different concentrations of propolis on enhancing the immune response orally in experimentally infected wounds with Acinetobacter baumannii. In this study, propolis was used at two different gradient concentrations (30, 40, 50) mg/kg and (100, 150, 200) mg/kg to enhance the immune response in mice with infected wounds through study its impact on levels of both Interleukin17 and Interleukin37. The results showed that concentrations of (30, 40, 50) mg/kg of propolis showed a higher effect than the concentration of (100, 150, 200) mg/kg in stimulating the production of IL-7, as its concentration was (125.68±6.26) pg/ml in the immunized infected and treated group, while it was reached (24.20±1.07) pg/ml in the group of the same treatment but immunized with the concentration of (100, 150, 200) mg/kg compared with an infected positive control group which reached (32.28±8.00) pg/cm^3 and (54.69±0.38) pg/ml in the negative control group, the level of cytokine IL-37 was reduced in the group immunized with a concentration of (30, 40, 50) mg/kg of propolis with infected and treated wounds as it reached (3.06±0.06) pg/ml compared with (2.44±0.02) pg/ml in those immunized with concentration (100, 150, 200) mg/kg, while reached (11.54±3.72 and15.66±7.76) pg/ml in the infected control positive mice untreated and treated respectively. The study showed an increase in the concentration of IL-17 in the mice of the uninfected group and infected untreated group immunized bee venom which was (156.6±0.40) pg/ml and (147.90±4.31) pg/ml for both groups respectively, while it showed a reduction in the level of IL-37 which reached (2.89±0.90) and (3.05±0,12) pg/ml for both groups respectively.

Keywords: Propolis, Interleukin 17. Interleukin 37, Acinetobacter baumannii

Introduction

Antibiotic resistance is one of the biggest threats to public health, and bacteria may change their response to antibiotics and show resistance to them making it more difficult to be treated and gain recovery, and this resistance may arise from the misuse and overuse of antibiotics, and bacteria have many mechanisms for antibiotic resistance including the production of some

enzymes, reducing membrane exudation, and the occurrence of mutations (Uddhav and sivagurunathan, 2016).

A. baumannii is a widespread opportunistic pathogen that causes nosocomial infections and it causes many pathological conditions such as sepsis, urinary tract infection, meningitis, pneumonia, burn injuries, and wounds and is found in intensive care units, as well as in soil and infects humans and animals, and it affects immunosuppressed people and may cause deaths (Yadav et al, 2020).

A. baumannii is characterized by being Gram-negative bacilli, spherical to bacillary shaped, aerobic, immotile, non-lactose fermenting, and characterized by its ability to resist dehydration. These bacteria have been associated with long-term hospital admissions, morbidity, and mortality (Raut et al., 2020).

These bacteria possess several virulence factors, including their ability to form biofilms, which facilitate their survival in the hospital environment, their ability to adhere to living and non-living surfaces, and enhancing their resistance to antibiotics (Leitao, 2020).

To control bacterial resistance to treat infections resulting from it, many attempts have been made to use many substances, including stimulating the immune system using propolis, as it is a biological product collected by bees from plants. It is a resinous substance and has biological effectiveness against many microorganisms, as it contains substances with antibacterial nature, such as flavonoids and some phenolic acids. It is also considered an antioxidant, anti-tumor, and immunomodulator (Przybylek and Karpinski, 2019).

Several studies have linked different types of propolis and its different components to anti-inflammatory activity. Flavonoids and their anti-inflammatory effects have been studied, which begins with a complex series of chemical signals after tissue injury, as it increases the response of host cells to repair its injured tissues. There are two phases of acute and chronic inflammation, where acute inflammation mediates from by activating immune system cells that migrate to the site of damage, it stimulates the release of cytokines and causes chronic inflammation when acute inflammation is not successfully treated (costa et al., 2012).

Material and Methods

1. Experimental design

Sixty-four adult male albino mice were used and divided randomly into 8 groups, wound, and infection with A. baumannii were induced in some groups, some groups were treated with 20 mg/ml of Rifampin antibiotic, as follow:

- **Immunization with the concentrations of 30, 40, 50 mg/kg of Propolis groups:**

G1: Mice group immunized gradientally with two doses from the concentrations of (30, 40, 50) mg/kg of Propolis, then wound, infection was induced and treated with an antibiotic.

G2: Mice group immunized gradientally with two doses from the concentrations of (30, 40, 50) mg/kg of Propolis, then wound, infection were induced and left without treatment.

- **Immunization with the concentrations of 100, 150, 200 mg/kg of Propolis groups:**

G3: Mice group immunized gradientally with two doses from the concentrations of (100, 150, 200) mg/kg of Propolis, then wound, infection was induced and treated with an antibiotic.

G4: Mice group immunized gradientally with two doses from the concentrations of (100, 150, 200) mg/kg of Propolis, then wound, infection was induced and left without treatment.

- **Control groups:**

G5: control positive group: wounded, infected, and untreated mice.

G6: control positive group: wounded, uninfected, and untreated mice.

G7: control positive group: wounded, infected, and treated mice.

G8: control negative group: without wounded mice (healthy).

2. Development of wound and infection

Mice were anesthetized with ketamine and then hair was removed from the dorsal side and sterilized with 70% ethanol, and then a 6-mm incision was made using a scalpel.

3. Measurement of IL-17 and IL-37

The levels of IL-17 and IL-37 in serum were measured by ELISA using a commercial mouse kit.

- Principle of the test: Microplate with pits coated with anti- antibodies were used for the examination (IL-17) and IL-37, which binds with the cytokines found in the blood serum to form the antigen-antibody complex. When adding the antibodies labeled with the enzyme, it binds with the first complex, which reacts with the substrate of the enzyme when added and leads to the production of a color whose intensity is directly proportional to the concentrations of IL-17 and IL-37.

4. Statistical analysis

Data were summarized as Mean±Standard Error using SPSS (V.23) followed by Duncan multiple variances to extract the differences between groups.

Results

As shown in Table (4.1), the results showed a significant increase in the concentration of the cytokine IL-17 in the experimentally infected positive control group, which was left without treatment, as it reached 108.42 ± 0.82picogram/ml compared with the negative control group

(healthy mice), which its level reached 54.49±0.38 pg/ml and the positive control group with non-infected experimental wounds, which amounted to 52.24±4.62 pg/ml.

The results also showed a significant decrease in the level of IL-17 in the group of mice with infected wounds treated with Rifampicin antibiotic, as its level reached 32.28±8 pg/ml compared to the positive control group, which indicates the effect of the antibiotic on the immune response.

The current study showed that IL-17 level in the group of mice immunized with the concentrations of (30, 40, 50) mg/kg of body weight of propolis and treated with the antibiotic Rifampicin showed a significant increase, as it reached 125.68±6.26 pg/ml compared to the group activated with concentrations (100, 150). , 200) mg/kg and the infected group treated with Rifampicin, whose level was 24.20±1.07 pg/ml, as well as to the negative and positive control groups with uninfected wounds 54.49±0.38 and 52.24±4.62 pg/ml for the two groups, respectively.

The two groups of mice immunized with concentrations of (30, 40, 50) and (100, 150, 200) mg/kg of body weight before infection showed a significant increase in their level, reaching 130.28±0.40 and 83.22±0.40 pg/ml in the two groups, respectively,

As for the cytokine IL-37, its levels showed a significant increase in the group of positive control mice with wounds infected with untreated bacteria A. baunannii, as its level was 11.54±3.72 pg/ml compared with the group of mice with uninfected wounds (3.55±0.09) pg/ml and the group of healthy mice (3.36±0.17) pg/ml, also showed a significant increase in the group of mice with infected wounds treated with the antibiotic Rifampicin (15.66±7.76) pg/ml, while its level was low in all experimental groups, and its level showed a non-significant increase in the group of experimental mice with infected experimental wounds with bacteria and activated with concentrations (30, 40, 50) mg/kg of body weight and untreated, reaching 5.11±1.58 pg/ml compared to its level in the group of mice activated with concentrations (100, 150, 200) mg/kg under the same treatment conditions, as its level was 2.18±0.40 pg/ml, and as shown in Table (4.1) its level did not show a significant change in the two groups of mice activated with concentrations (30, 40, 50) and (100, 150 , 200) mg/kg body weight compared to the negative control mice group (healthy) (3.36±0.17) pg/ml and to the two groups of activated mice with the same concentrations of propolis and treated with antibiotic, which amounted to 3.06±0.06 and 2.44±0.02 pg/ml for the two groups, respectively.

Table (4.1) levels of Interleukin 17 and Interleukin 37 in mice immunized with different concentrations of Propolis.

Concentrations and Groups		IL-17 pg/ml	Il-37 pg/ml
Immunization with 30, 40, 50 mg/kg	G1	125.68±6.26 de	3.06±0.06 a
	G2	79.77±19.26 bc	5.11±1.58 ab
	Before infection	130.28±0.40 ef	2.60±0.35 a

Immunization with 100, 150, 200 mg/kg	G3	24.20±1.07 a	2.44±0.02 a
	G4	54.54±0.40 ab	2.18±0.40 a
	Before infection	83.22±0.40 c	3.60±0.40 a
Control	G5	108.42±0.82 cde	11.54±3.72 be
	G6	54.49±0.38 ab	3.55±0.09 a
	G7	32.28±8 a	15.66±7.76 c
	G8	54.49±0.38 ab	3.36±0.17 a

Different letters in each column indicate a significant difference at P≤0.05.

G1: Mice group immunized gradientally with two doses from the concentrations of (30, 40, 50) mg/kg of Propolis, then wound, infection was induced and treated with an antibiotic. G2: Mice group immunized gradientally with two doses from the concentrations of (30, 40, 50) mg/kg of Propolis, then wound, infection were induced and left without treatment. G3: Mice group immunized gradientally with two doses from the concentrations of (100, 150, 200) mg/kg of Propolis, then wound, infection was induced and treated with an antibiotic. G4: Mice group immunized gradientally with two doses from the concentrations of (100, 150, 200) mg/kg of Propolis, then wound, infection was induced and left without treatment. G5: control positive group: wounded, infected, and untreated mice. G6: control positive group: wounded, uninfected, and untreated mice. G7: control positive group: wounded, infected, and treated mice.G8: control negative group: without wounded mice (healthy).

Dissuasion

An increase in the concentration of the cytokine IL-17 in the experimentally infected positive control group, which was left without treatment in this study indicating the role of injury in stimulating the production of this cytokine**.** This was demonstrated by the study of Curtis and Way in 2009, which showed the protective effect of Th-17 cells against pathogenic bacteria K.pneumoniae, Citrobacter rodentium, and the rest of the extracellular bacterial pathogens, and that the protection resulting from Th-17 may act on fungi, as it protects against infection with fungi such as candida albicans infection also protects against intracellular infection with Salmonella enterica and Listeria monocytogenes, as well as against bacteria that cause tuberculosis. (Das and Khader, 2017) explained that exposure to bacterial, fungal, and viral infection activates IL-17 from specific and non-specific immune cells, which include epithelial cells, macrophage cells, and myeloid cells, thus stimulating the production of antimicrobial peptides, chemoattractants, and some other types of cytokines, the attractant proteins attract neutrophils and other immune cells to the site of infection, and this cytokine increases inflammatory reactions.

The antibiotic Rifampicin possesses immunosuppressive and anti-inflammatory properties, the study of (Yuhas et al., 2011) showed that the use of this antibiotic was associated with a significant increase in nitric oxide production in cell cultures of hepatic epithelial cells HEPG2 and that the increase in nitric oxide production stimulated by this antibiotic may affect the

production of other immunomodulators, the studies of (Dajani et al., 1972), and (Paunescu, 1970) showed the immunosuppressive properties of Rifampicin, and these may be a reason of decreasing level of IL-17 in the group of mice with infected wounds treated with Rifampicin antibiotic in the current study.

A significant increase in IL-17 level in the group of mice immunized with the concentrations of (30, 40, 50) mg/kg of body weight of propolis and treated with the antibiotic Rifampicin indicating that the use of propolis stimulates the production of some cytokines, including this cytokines, which is a pre-inflammatory cytokine, and this cytokine is a stimulus for the production of granulocyte colony-stimulating factor (G-CSF) and it acts as a mediator of inflammation during the presence of infection, it also has an important role in the chronic infections that accompany some immune diseases accompanied by chronic inflammation, such as the case of rheumatoid arthritis (kuwabara et al., 2017). This cytokine is produced by a type of T helper cell called Th-17, which includes a subset of T helper cells that differ from Th1 and Th2 cells and can be produced by ϬϬT cells and natural killer cells in a small percentage. Its action is link between specific and nonspecific immune mechanisms through its effect in stimulating toxic T-cell (CTL) responses against cancer (Xu and Cao, 2010).

This cytokine has been classified among the proinflammatory cytokines for its ability to stimulate the expression of inflammatory mediators involved in the proliferation of immune cells. This cytokine plays an essential regulatory role in the immune system in injury and inflammatory diseases (Jin and Dong, 2013).

As for the effect of propolis and its role in immune modulation, its effect and some of its active components in non-specific immunity have been shown by modifying the activity of neutrophils and mononuclear cells, which are important components of non-specific immunity and basic blood macrophages (Edwards, 1994; Gao et al. ., 2014), and that the macrophages that arise from the circulating mononuclear cells in the blood are present in most tissues, which connects between specific and non-specific immunity and this activity is stimulated by immunomodulators, including the propolis (Wolska et al., 2019).

Some studies (Draganova-Filipova et al., 2008; Gao et al., 2014) have suggested that the efficacy of alkaloids is highly dependent on their concentration, as low concentrations stimulate cellular immune responses, while high concentrations have inhibitory effects on lymphocyte proliferation and that these compounds deactivate the association of DNA with nuclear signaling factors NFAT with NF-kB (Marques et al., 2004).

A study of (Miossec, 2017) showed that the level of IL-17 increased in chronic diseases, inflammation, and autoimmune diseases, and it is hoped that IL-17 levels will be used as a target for follow-up treatment of bacterial infections.

IL-37 is a member of the IL-1 family, and studies indicate that this cytokine has inhibitory effects on both specific and acquired immunity by inhibiting many inflammatory mechanisms and bacterial infections, and has anti-bacterial and antiviral effects, as it stops inappropriate immune

activation and reduces inflammation caused by pathogens. Thus, this cytokine protects host tissues from the effects of infection by suppressing the increased inflammatory reactions (Nold et al., 2010; Wang et al., 2018; Allam et al., 2020), and this cytokine has been shown to have a regulatory role in many inflammatory and neoplastic diseases (Nold et al., 2010; Ding et al., 2016), and the mechanism of immune regulation of this cytokine is still not fully known. The mechanism of non-specific immunosuppression by IL-37 depends on specific immunity. Moretti et al found in 2014 that IL-37 inhibits the activation of Th17, Th2 cells in mice with allergic bronchial infection resulting from infection with Aspergillus, which shows its effect on acquired immunity.

A study of Luo et al 2014 demonstrated that IL-37 stimulates tolerance in dendritic cells, thereby suppressing acquired immune responses and that these cells play an important role in the body as antigen-presenting cells that receive signals from many cells and transmit them to many Immunological cells, and thus play a role in acquired immune responses and in the case of immune tolerance (Pulendran et al., 1999).

As for the effect of propolis, especially with its low concentrations, it is considered one of the compounds known for its biological activity, including anti-inflammatory and anti-bacterial activity, because it contains many active compounds such as multiple phenols, terpenes, steroids and acids, and its content of these substances varies according to the geographical location and the type of plants in the region and has a regulating effect on macrophages (Moreno et al., 2000).

The results of the current study agreed with (Bailly et al., 1993) regarding the effect of antibiotics on the production of interleukins, as they work to modify their production, and that quinolones work to reduce the production of IL-1, IL-6, TNF, while erythromycin increases the production of interleukins 6 indicating the effect of antibiotics on the body's immune response. The study of (Williams et al., 2005) showed that the use of antibiotics may affect the gene expression of cytokines, and therefore it affects the gene expression of cytokines by (T-helper) cells, such as the expression of interleukin 4 when used to treat ulcers. Ciprofloxacin reduces the production of IL-4 and IFN-γ, and increasing the concentration of the antagonist Clarithromycin may increase the production of some interleukins.

Propolis inhibits the production of IL-6, a cytokine that plays an important role in inflammation as a mediator (De Figueiredo et al., 2014). A study of (Okamoto et al., 2012) conducted in Brazil showed the ability of propolis to modify the immune activity in laboratory mice with arthritis, as its use caused inhibition of IL-17 and IL-6 production in experimental mice. Our results are in agreement with the results of (Szliszka et al., 2013) about the ability of alcoholic propolis extract to inhibit the production of some interleukins produced in inflammatory conditions. The results of (Jia et al., 2018; Wang et al., 2018; Li et al., 2019) were similar to the results obtained in the current study in that IL-37 is a factor that suppresses the immune response and has a role in protecting the body against toxic shock, autoimmune disease, and cardiovascular disease.

Conclusion

Immunization with propolis with its low concentrations caused a significant increase in the level of cytokine IL-17, which was higher than the increase that occurred when immunization with high concentrations, and the low and high concentrations of propolis caused an inhibition in the level of IL37 compared to healthy mice**.**

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