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*Research Article*

# Propolis as an Immunomodulatory Agent: Evaluation of Antioxidant Activity, Cytokine Modulation, and Hematological Parameters in Albino Rats Infected with *Escherichia coli*

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**ABSTRACT**

**Background:** Propolis, a natural substance produced by honeybees, has a rich history in traditional medicine due to its diverse composition of phenolic compounds, including flavonoids, aromatic acids, and benzopyran. This study aimed to assess the effects of propolis on the immune system, blood parameters, antioxidant levels, and antimicrobial properties against Escherichia coli in male albino rats.

**Methods:** The experiment involved thirty male albino rats divided into three groups: control, positive control, and propolis-treated group infected with E. Coli. The rats were administered propolis extract (400mg/kg) once daily for four weeks.

**Results:** The results demonstrated that propolis exhibited immunomodulatory effects by increasing lymphocyte percentages and enhancing the production of antibodies (IgM and IgG). It also improved hematological parameters such as hemoglobin, hematocrit, red blood cell count, and platelets. Propolis showed higher nitric oxide levels and phagocytosis percentages than the infected group. Additionally, it reduced serum levels of TNF-α, IL-6, IL-10, and IL-1β while significantly decreasing eosinophil levels. Propolis lowered serum malondialdehyde levels but decreased superoxide dismutase, catalase, and glutathione levels in rats infected with *E. coli*.

**Conclusion:** These findings indicate that propolis holds promise as an alternative or complementary therapy for managing bacterial infections, providing a natural and potentially effective solution. However, further research and clinical trials are required to fully comprehend propolis's therapeutic benefits and its applications in human health.

## INTRODUCTION

In recent times, there has been considerable interest in propolis, a sticky material that honeybees gather from the buds of different plants and trees. It has garnered attention due to its wide range of medicinal properties. Honeybees use propolis to seal openings and keep their hives clean, and humans have been using it in traditional medicine for many centuries. Propolis is made up of different substances like flavonoids, phenolic acids, terpenoids, and essential oils, and it demonstrates diverse biological and pharmacological effects (Sforcin, 2016; Bankova et al., 2014). Research has demonstrated this substance's efficacy in combating Gram-negative and positive bacteria. This includes its ability to target antibiotic-resistant strains like methicillin-resistant Staphylococcus aureus effectively. (Kurek-Górecka et al., 2013). Propolis has also demonstrated antifungal activity against Candida species and other fungi (Capoci et al., 2015).

Furthermore, propolis has been documented to possess antiviral attributes, including its effectiveness against viruses such as herpes simplex virus (HSV) and influenza virus, as reported by Schnitzler et al., (2010). Propolis has been shown to possess anti-inflammatory properties by inhibiting the synthesis of cytokines that promote inflammation and other inflammatory mediators (Orsi et al., 2005). Propolis has promising therapeutic implications for inflammatory conditions like arthritis and asthma. Additionally, propolis has been noted to possess immunomodulatory properties, enhancing the immune system's ability to respond to infections and other challenges (Sforcin & Bankova, 2011).

Due to its high content of flavonoids and phenolic compounds, propolis displays potent antioxidant activity. This property safeguards cells against damage caused by free radicals and oxidative stress (Kumazawa et al., 2004). This property may contribute to its potential role in preventing or treating chronic diseases associated with

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oxidative stress, such as cardiovascular diseases and neurodegenerative disorders.

Propolis has been used as a topical agent to promote wound healing and treat various skin conditions. Its antimicrobial, anti-inflammatory, and antioxidant properties may accelerate wound healing, reduce inflammation, and improve tissue regeneration (Bankova, 2005). Additionally, propolis has been shown to have potential applications in dermatology for treating conditions like acne and atopic dermatitis (Silici & Kutluca, 2005; Kurek-Górecka et al., 2020). Research findings have suggested that propolis possesses immunomodulatory properties, including its ability to enhance the activity of different interleukins. These interleukins encompass interleukin-1 (IL-1) as a pro-inflammatory cytokine, interleukin-2 (IL-2) as a growth factor for T cells, interleukin-4 (IL-4) as an immunoregulatory cytokine, interferon-gamma (IFN-γ) which can function as both pro-inflammatory and immunoregulatory, interleukin-6 (IL-6), and interleukin-1β (IL-1β) as pro-inflammatory cytokines. Additionally, propolis has been shown to induce higher concentrations of many immune cells and different immunomodulatory cytokines essential for homeostasis maintenance (Al-Hariri, 2019; Zulhendri et al., 2022). According to Bouchelaghem et al. (2022), Propolis exhibits diverse biological properties, making it a promising candidate for developing effective and affordable antimicrobial agents.

In conclusion, propolis is a natural substance with significant medical importance due to its diverse biological and pharmacological activities. These properties make propolis a promising candidate for further research and potential therapeutic applications. Therefore, the current study aimed to assess the impact of propolis on hematological parameters, antioxidant, some proinflammatory and anti-inflammatory cytokines levels, and immune system enhancement in male albino rats infected with Escherichia coli (E. coli); to investigate whether propolis could serve as a viable alternative or complementary treatment for bacterial infections, offering a natural and potentially effective solution.

## MATERIALS AND METHODS

### Experimental Design

*Extraction of Propolis:* The propolis used (Egyptian propolis) was sourced from the Apiary of the Beekeeping Research Section, which is part of the Plant Protection Research Institute at the Agriculture Research Center in Dokki, Giza, Egypt.

Forty grams of propolis were cut into small pieces and immersed in a 1000-mL solution of 70% ethanol. Following the combination of ingredients, the mixture was incubated for 48 hours at a temperature of 50 °C while being continuously shaken. After incubation, the solution was concentrated using a rotary evaporator and a freeze-dryer. This process formed a dry propolis extract (Askari et al., 2016; Rahimi et al., 2017). To prepare the treatment solution, 2000 mg of the propolis extract was dissolved in 5 mL of normal saline**.** Administration of Propolis: The rats were administered the propolis solution at a dosage of 0.1 mL per 100 g of body weight. This corresponded to a dosage of 400 mg/kg of propolis. The solutions were prepared daily to maintain efficacy (Askari et al., 2018).

*Inoculum preparation and infection method:* For the experiment, Escherichia coli cultures were obtained from the Laboratory of Microbiology at the Biology Department, Science College, Jazan University, KSA (Essa et al., 2017). The isolate was obtained from a sludge sample from Egypt numbered Z3 and identified by 16s rDNA gene sequencing. Fierer et al. (2002) described the infection method. Rat infection was initiated by orally administering 2 ml of sterile phosphate-buffered saline through a gastric cannula *containing* $1\times 10^9$ colony-forming units of E. coli per gram.

*Bacterial infection and treatment with propolis:* Thirty male albino rats were housed in the Biology Department, Faculty of Science animal facility at Jazan University in Saudi Arabia. The rats weighed between 160 and 180 grams. They were provided with clean and hygienic cages and had unrestricted access to standard rodent chow, water, and food. The rats were kept under controlled conditions with a 12-hour light-dark cycle, a temperature maintained at 20±4°C, and a relative humidity of 50±5%. The study involved dividing the rats into three groups, as follows:

Group 1 (G1), consisted of ten control rats.

Group 2 (G2), Ten rats were included in the study and intentionally infected with a bacterial suspension.

Group 3 (G3), Ten rats were subjected to the bacterial infection and treated with propolis extract. These animals were given propolis extract orally by intragastric gavage (Singla et al., 2014). Each animal received a daily dosage of 1.0 ml for a duration of four weeks. In contrast, groups 1 and 2 were administered a saline solution (0.9% NaCl) in the same volume. After a duration of four weeks, all animals were euthanised with light ether anaesthesia. Following euthanasia, blood samples were collected immediately. The blood samples were divided into two portions. The first portion was collected in EDTA tubes for haematological analysis. The second portion was collected in plain tubes to obtain blood serum. The tubes containing blood serum were centrifuged at 1059 xg for 15 minutes to separate the serum. The separated serum samples were stored at -20 °C until they were used for immunological analysis in the study.

*Assessment of immunoglobulins:* The determination of total Immunoglobulin M (IgM) was conducted using a quantification ELISA kit (Cat. No. E4482-100, BioVision's Quick Detect Kit, USA) designed for IgM (Rat), following the manufacturer's instructions. The measurement of total Immunoglobulin G (IgG) was carried out using a quantification ELISA kit (Cat. No.

E4478-100, BioVision's Quick Detect Kit, USA) intended for IgG (Rat), as per the manufacturer's instructions.

*Assessment of cytokines***:** Interleukin-1 beta (IL-1β) was assessed using an ELISA kit (Cat. No. E-EL-R0012, Elabscience, USA) intended for rat IL-1β, as per the manufacturer's instructions. Total Interleukin-6 (IL-6) was quantified using an ELISA kit (Cat. No. ERA31RB, Invitrogen, USA) intended for Rat IL-6, as per the manufacturer's instructions. The measurement of total Interleukin-10 (IL-10) was performed using an ELISA kit (Cat. No. BMS629, Invitrogen, USA) designed for Rat IL-10, following the instructions provided by the manufacturer. Determining total Tumor Necrosis Factor-alpha (TNF-α) using an ELISA kit (Cat. No. KRC3011, Invitrogen, USA) designed for Rat TNF-α, following the manufacturer's instructions.

*Assessment of antioxidant***:** The malondialdehyde (MDA) assessment was performed using an ELISA kit (Cat. No. MBS268427, MyBioSource, USA) intended for rat malondialdehyde, following the instructions provided by the manufacturer .The assessment of superoxide dismutase (SOD) was conducted using an ELISA kit (Cat No. MBS266897, MyBioSource, USA) designed for rat superoxide dismutase, following the manufacturer's instructions.

The measurement of catalase (CAT) was performed using an ELISA kit (Cat. No. MBS726781, MyBioSource, USA) for rat catalase, following the instructions provided by the manufacturer .To determine the levels of glutathione (GSH), an ELISA kit (Cat. No. MBS261448, MyBioSource, USA) designed for rat glutathione was used, following the instructions provided by the manufacturer. The assessment of nitric oxide was carried out using the Biovision Kit Cat. No. k262 for the nitric oxide colourimetric assay.

*Evaluation of phagocytic activity:* According to Lu et al. (2014), the phagocytic function was assessed.

**Haematological studies**

The collected blood samples were utilised to determine specific haematological parameters, which included measuring the RBC count using the method described by Math et al. (2016). The calculation of the red cell indices was done utilising the following formulas:

$$\text{MCV (fl)} = \frac{haematocrit(\%)}{haemoglobin\ (g/dl)} \times 10$$

$$\text{MCH (pg)} = \frac{Hb\ (g/dl)}{RBCs(millions\ /mm3)} \times 10$$

$$\text{MCHC (\%)} = \frac{haemoglobin\ (g/dl)}{haematocrit\ (\%)} \times 100$$

The packed cell volume (PCV) and platelet count were performed following the protocol outlined by Bain et al. (2006). The hemoglobin concentration in the blood was evaluated using Drabkin's solution and the colorimetric method, as described by Tietz (1995).

The determination of white blood cell count (WBCs) was done according to Bain et al. (2006). The differential leukocyte count was conducted on thin blood films prepared on slides using the spread technique described by Bain et al. (2006).

**Statistical analysis**

The data were assessed by ANOVA using the SPSS (version 21) software package for Windows. The dissimilarities between the groups were evaluated. The data are expressed as the means ± SD. The least significant (Tukey) test was used to compare the groups. The statistical significance was set at $P < 0.05$.

# RESULTS

**The effect of propolis on immunoglobulins and pro-inflammatory cytokines**

The data from Table 1 and Figures 1 and 2 demonstrated a notable increase in serum IgG levels (ug/ml) in both G2 and G3 compared to the control group. Additionally, G3 exhibited a significant rise in serum IgG with a percentage change of 17.3% compared to G2, which served as the positive control. On the other hand, concentrations of IgM (ng/ml) were significantly higher in G2, displaying a percentage change of 87.23% compared to G1. In contrast, rats infected with bacteria and treated with Propolis EtOH extract in G3 exhibited a significant reduction (*p <.05)* with a recorded percentage change of -43.2% compared to G2, the positive control.

The data presented in Table 1 and Figure 2 revealed notable variations in serum IL-6, TNF-α, IL-1β, and IL-10 levels in different groups. In the infected group (G2), there was a striking increase in serum IL-6 levels, showing a percentage change of 59.85% compared to the control group (G1). However, in G3, which received treatment with Propolis EtOH extract, there was a significant decrease in IL-6 levels, with a percentage change of -22.52% compared to G2. Similarly, concentrations of TNF-α were significantly higher in G2, exhibiting a percentage change of 61.1% compared to G1. Conversely, rats infected with bacteria and treated with ethanolic Propolis (EtOH) extract in G3 showed a significant decrease in TNF-α levels, with a recorded percentage change of -34.67% compared to G2. The estimation of IL-1β levels revealed a significant increase in the infected group (G2) as a percentage change of 128.7% compared to the control group.

In contrast, G3 exhibited a significant decrease in IL-1β levels, with a percentage change of -44.57% compared to G2. Furthermore, serum concentrations of IL-10 were significantly increased in G2, showing a percentage change of 65.38% compared to G1. On the other hand, G3 displayed a significant decrease in IL-10 levels, with

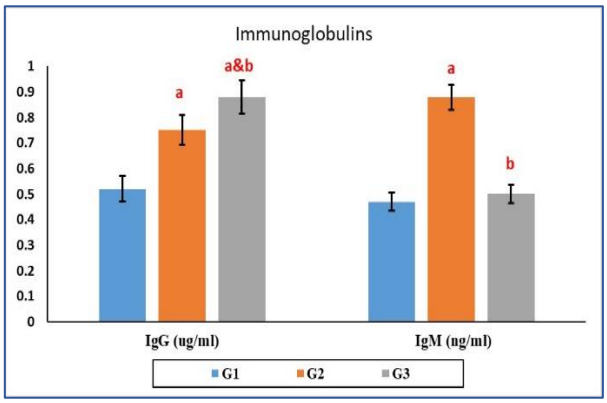


**Figure 1***: Effect of Propolis EtOH extract on serum immunoglobulins IgG and IgM in treated rat group compared with negative and positive control groups.*

a percentage change of -29.12% compared to G2**.**

**Table 1***: Effect of Propolis EtOH extract on serum on immunoglobulins and pro-inflammatory cytokines.*

| Groups / Parameters | G1 | G2 | G3 |
|---|---|---|---|
| | Mean ± SD | | |
| IgG (ug/ml) | 0.52 ± 0.051 | 0.75 ± 0.058a | 0.88 ± 0.065a,b |
| % of change | | %↑44.23 | 69.23%↑ &17.3%↑ |
| IgM (ng/ml) | 0.47 ± 0.035 | 0.88 ± 0.049a | 0.50 ± 0.035b |
| % of change | | %↑87.23 | 6.38%↑ & 43.2%↓ |
| IL-6 (pg/ml) | 88.27 ± 6.84 | 141.1 ± 7.97a | 109.33 ± 9.1a,b |
| % of change | | 59.85%↑ | 23.85%↑ &-22.52%↓ |
| TNF-α (pg/ml) | 73.77 ± 7.14 | 118.83 ± 11.12a | 77.62 ± 10.33b |
| % of change | | 61.1%↑ | 5.22%↑ &-34.67%↓ |
| IL-1β (pg/ml) | 62.45 ± 3.52 | 142.83 ± 13.72a | 79.17 ± 10.44a,b |
| % of change | | 128.7%↑ | 26.77%↑ & -44.57%↓ |
| IL-10 (pg/ml) | 75.78 ± 5.23 | 125.33 ± 16.86a | 88.83 ± 6.27b |
| % of change | | 65.38%↑ | 17.22% ↑ & -29.12%↓ |

*The values are presented as the Mean ± SD. (*a) indicates a significant difference (P<0.05) compared to G1, while (*b) signifies a significant difference (P<0.05) compared to G2.*

**The effect of Propolis on MDA, SOD, CAT, GSH, NO, and phagocytic function.**

The results presented in Table (2) and Figures (3, 4, and 5) demonstrated a statistically significant rise in serum malondialdehyde (MAD) levels (nmol/ml) in group G2, with a percentage change of 78.7% compared to the control group (G1). Conversely, a significant decrease of -42.48% was observed in group G3 compared to group G2. Although there was a decrease in serum superoxide dismutase (SOD) levels (U/m) in G2, with a percentage change of -46.41% compared to G1, a significant increase of 81.1% was noted in G3 compared to G2. Additionally,

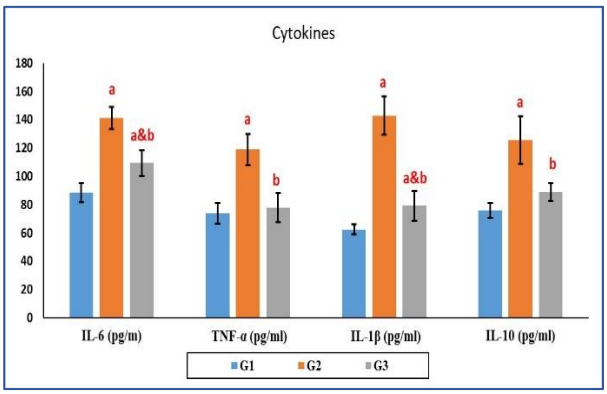


**Figure 2***: Effect of Propolis EtOH extract on serum pro-inflammatory cytokines in treated rat group compared with negative and positive control groups.*

both G2 and G3 exhibited a decrease in serum catalase (CAT) levels (ng/ml) compared to the control group, but G3 showed a significant increase of 103.8% compared to G2. Serum glutathione (GSH) levels (µg/ml) experienced a significant decrease of -72.6% in G2 compared to G1, while G3 showed a highly significant elevation of 203.3% compared to G2.

Moreover, the findings from Table 2 and Figure 4 demonstrated a notable rise in serum nitric oxide (NO) levels in both G2 and G3 groups when compared to the control group. Particularly, G3 exhibited a significant increase of 12.7% compared to G2. The assessment of phagocytic cell activity through the nitro blue tetrazolium (NBT) dye reduction test revealed enhanced phagocytosis in G3, where Propolis EtOH extract was utilized. These results are presented in Table 2 and Figure 4. In contrast, G2 displayed a significant decrease of -8.7% in phagocytosis percentage compared to the control group (G1). Conversely, G3 exhibited a significant increase of 13.78% compared to G2.

The data extracted from Table 3 and Figures 6 and 7 revealed notable changes in various hematological parameters across the different groups.

**The effect of Propolis on hematological parameters.**

In G2, a significant decrease of -21.75% in hemoglobin (Hb) concentration was observed compared to the control group. However, in G3, there was a significant increase of 29.27% in Hb concentration compared to G2. Furthermore, G2 exhibited a significant decrease in red blood cell count (RBCs) with a change percentage of-22.45% relative to G1. In contrast, G3 showed a significant increase of 24.47% in RBC count compared to G2. Regarding mean corpuscular volume (MCV) levels, a slight decrease of 1.99% was observed in G2 compared to G1, while G3 exhibited a slight increase of 3.41% compared to G2. Similarly, there were a slight increase in mean corpuscular hemoglobin (MCH) levels in G2 and G3, with change percentages of 0.6% and0.73%,

**Table 2:** *Effect of Propolis EtOH extract on MDA, SOD, CAT, GSH, NO, and phagocytic function.*

| Parameters \ Groups | G1 | G2 | G3 |
|---|---|---|---|
| | Mean ± SD | | |
| MDA(nmol /ml) | 1.08 ± 0.15 | 1.93 ± 0.2[a] | 1.11 ± 0.14[b] |
| % of change | | 78.7%↑ | 2.78↑ &-42.48%↓ |
| SOD (U/ml) | 7.39 ± 0.54 | 3.96 ± 0.74[a] | 7.17 ± 0.85[b] |
| % of change | | 46.41%↓ | -2.977%↓ &81.1%↑ |
| CAT (ng/ml) | 2.69 ± 0.44 | 1.03 ± 0.27[a] | 2.1 ± 0.22[a,b] |
| % of change | | 61.71%↓ | -21.93% ↓&103.8%↑ |
| GSH (ug/ml) | 3.32 ± 0.43 | 0.91 ± 0.27[a] | 2.76 ± 0.51[b] |
| % of change | | -72.6%↓ | -16.87%↓ & 203.3%↑ |
| NO (µl/nmol) | 12.72 ± 0.99 | 19.3 ± 0.79[a] | 21.75 ± 2.041[a,b] |
| % of change | | 51.72%↑ | 70.99%↑ &12.7↑ |
| Phagocytosis% | 81.58 ± 3.41 | 74.5 ± 4.04[a] | 84.77 ± 2.32[b] |
| % of change | | 8.7↓ | 3.91%↑ &13.78↑ |

*The values are presented as the Mean ± SD. (*a) indicates a significant difference (P<0.05) compared to G1, while (*b) signifies a significant difference (P<0.05) compared to G2.*

respectively, compared to the control group. However, G3 displayed a slight decrease of -4.03% compared to G2. Regarding mean corpuscular hemoglobin concentration (MCHC) levels, G2 showed a slight increase of 2.71% compared to the control group. Additionally, G2 experienced a significant decrease of -23.89% in hematocrit (HCT) levels compared to G1.

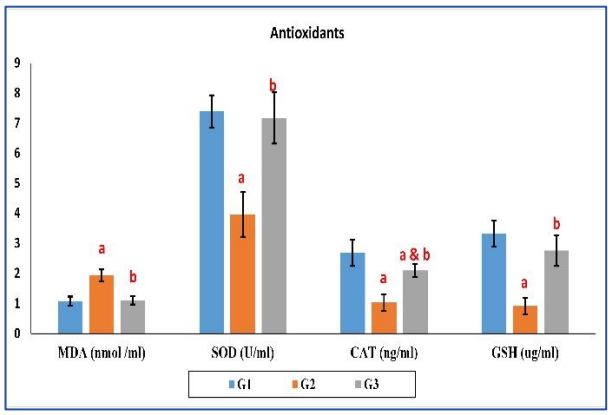


**Figure 3:** *Effect of Propolis EtOH extract on serum antioxidants (MDA, SOD, CAT, and GSH) levels in treated rat group compared with negative and positive control groups.*

Conversely, G3 exhibited a significant increase of 28.55% in HCT levels compared to G2. The results presented in Table 4 and Figures 8, 9, 10, and 11 demonstrated a noteworthy rise in the total leukocyte count

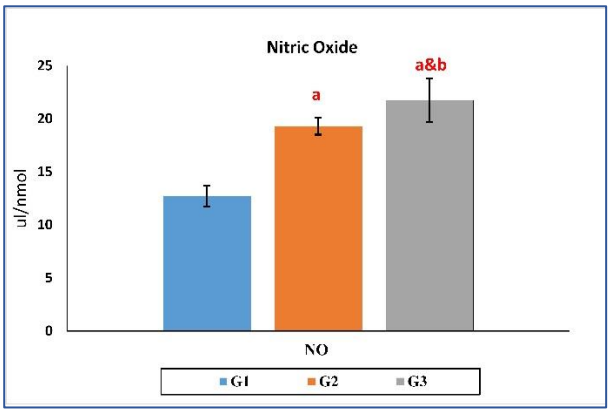


**Figure 4:** *Effect of Propolis EtOH extract on serum Nitric Oxide (NO) level in treated rat group compared with negative and positive control groups.*

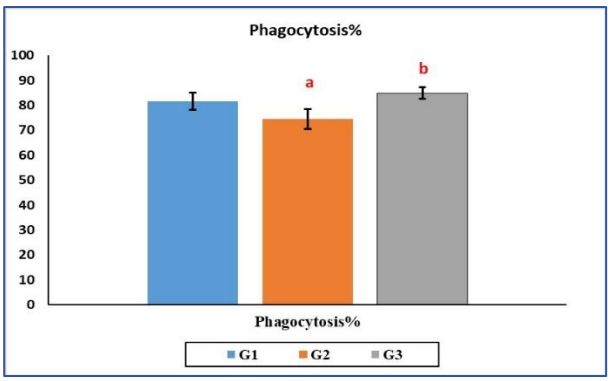


**Figure 5:** *Effect of Propolis EtOH extract on Phagocytosis (%) in treated rat group compared with negative and positive control groups.*

(TLC) in G2, exhibiting a percentage change of 91.1% when compared to the control group (G1). In contrast, G3 displayed a significant decrease in TLC, with a percentage change of -39.8% compared to G2. Additionally, there was a significant increase in neutrophil count in G2, showing a percentage change of 144.83% compared to the control group. Conversely, G3 exhibited a substantially reduced neutrophil count, as shown in a percentage change of -54.27% compared to G2. Moreover, a significant decrease in lymphocyte count was observed in G2 (-46.8%) compared to the control group. Conversely, G3 significantly increased lymphocyte count (81.8%) compared to G2. The data also indicated a slight increase in monocyte count in G2 (80.7%) compared to G1 and G3. However, a remarkable elevation in eosinophil count was observed in G2 (600%) compared to G1, while G3 exhibited a significant reduction compared to G2. Furthermore, there was a significant decrease in platelet count in G2 (-62.72%) compared to the control group. However, in G3, the data showed a significant increase with a percentage change of 138.64% compared to G2.

**Table 3:** *Effect of Propolis EtOH extract on Hb-Concentration, RBC count, MCH, MCV, MCHC indices and PCV%.*

| Groups / Parameters | G1 | G2 | G3 |
|---|---|---|---|
| | Mean ± SD | | |
| Hb (g/dl) | 12.87 ± 0.6 | 10.07 ± 0.27a | 12.42 ± 0.51b |
| % of change | | -21.75%↓ | -3.49%↓ & 23.33↑ |
| RBCs (×106/µl) | 4.32 ± 0.17 | 3.35 ± 0.055a | 4.17 ± 0.18b |
| % of change | | 22.45%↓ | -3.47%↓ & 24.47%↑ |
| MCV (fl) | 88.8 ± 0.61 | 87.03 ± 1.56 | 90 ± 1.66b |
| % of change | | 1.99%↓ | 1.35% ↑& 3.41%↑ |
| MCH (Pg) | 29.8 ± 0.42 | 29.98 ± 0.35 | 30.2 ± 1.29 |
| % of change | | 0.6% | 1.34% ↑ & 0.73↑ |
| MCHC% | 33.55 ± 0.5 | 34.46 ± 0.54a | 33.07 ± 0.44b |
| % of change | | 2.71%↑ | -1.43%↓ & -4.03%↓ |
| PCV% | 38.33 ± 1.37 | 29.17 ± 0.75a | 37.5 ± 1.52b |
| % of change | | 23.89%↓ | -2.165%↓ & 28.55%↑ |

*The values are presented as the Mean ± SD. (*a) indicates a significant difference (P<0.05) compared to G1, while (*b) signifies a significant difference (P<0.05) compared to G2.*

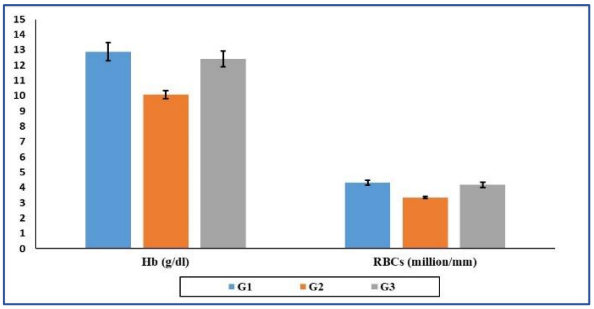


**Figure 6:** *Effect of Propolis EtOH extract on Hb (g/dl) and RBCs (×10⁶/µl) in treated rat group compared with negative and positive control groups.*

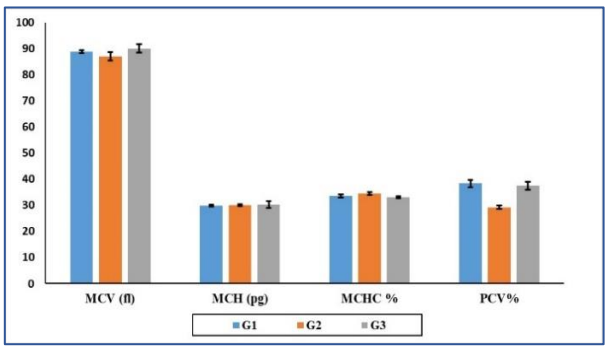


**Figure 7:** *Effect of Propolis EtOH extract on RBCs indices (MCH, MCV, MCHC) and PCV (%) in treated rat group compared with negative and positive control groups.*

**Table 4:** *Effect of Propolis EtOH extract on TLC, neutrophil, lymphocyte, monocyte, and eosinophil percentages and platelets count.*

| Group / Parameters | G1 | G2 | G3 |
|---|---|---|---|
| | Mean ± SD | | |
| TLC (X103 /µl) | 7866.7 ± 838.25 | 15033.3 ± 1201.11a | 9050 ± 831.26b |
| % of change | | 91.1%↑ | 15.04% ↑&-39.8%↓ |
| Neutrophil | 22.33 ± 2.42 | 54.67 ± 4.08a | 25 ± 3.41b |
| % of change | | 144.83%↑ | 11.95%↑ &-54.27%↓ |
| Lymphocyte | 75.83 ± 2.92 | 40.33 ± 3.78a | 73.33 ± 3.98b |
| % of change | | 46.8%↓ | -3.29%↓ &81.8%↑ |
| Monocyte | 0.83 ± 0.75 | 1.5 ± 0.55 | 0.83 ± 0.75 |
| % of change | | 80.7%↑ | 0 |
| Eosinophil | 0.50 ± 0.55 | 3.5 ± 1.05a | 0.83 ± 0.75b |
| % of change | | 600%↑ | 66%↑ &-76.3↓ |
| Platelets count (x103/ µl) | 325 ± 49 | 121.17 ± 18.32a | 289.17 ± 52.67b |
| % of change | | 62.72↓ | -11.02%↓ &138.64%↑ |

*The values are presented as the Mean ± SD. (*a) indicates a significant difference (P<0.05) compared to G1, while (*b) signifies a significant difference (P<0.05) compared to G2.*

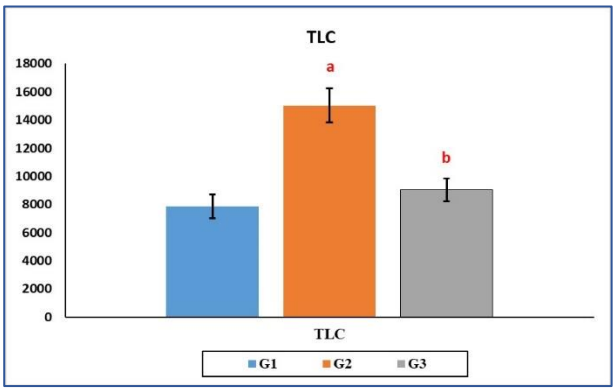


**Figure 8:** *Effect of Propolis EtOH extract on TLC (X10³ /µl) in treated rat group compared with negative and positive control groups.*

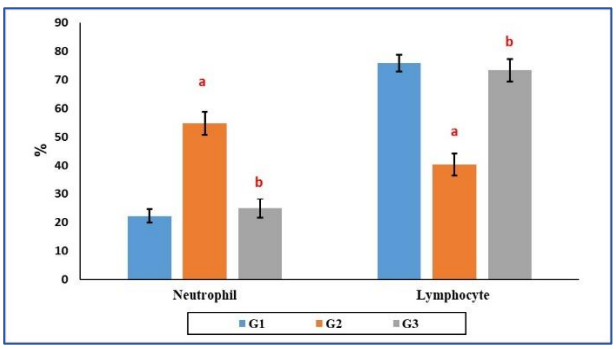


**Figure 9:** *Effect of Propolis EtOH extract on neutrophils and lymphocytes (%) in treated rat group compared with negative and positive control groups.*

## DISCUSSION

The evaluation of natural products for their immunomodulatory effects and antimicrobial properties has gained significant attention in recent years.

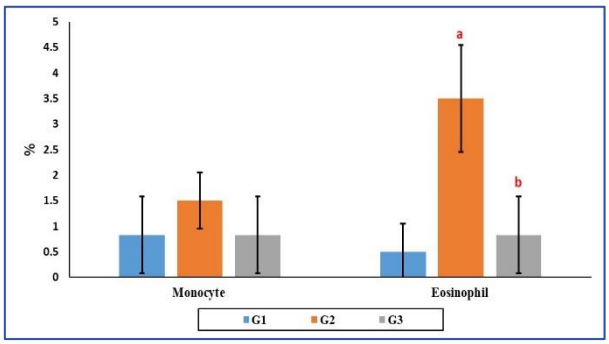


**Figure 10***: Effect of Propolis EtOH extract on Monocyte and Eosinophil (%) in treated rat group compared with negative and positive control groups.*

Propolis, a sticky substance gathered by bees from diverse plant sources, has emerged as a promising candidate for therapeutic properties. This study's primary objective was to assess propolis's immunomodulatory effects on the immune system, hematological parameters,

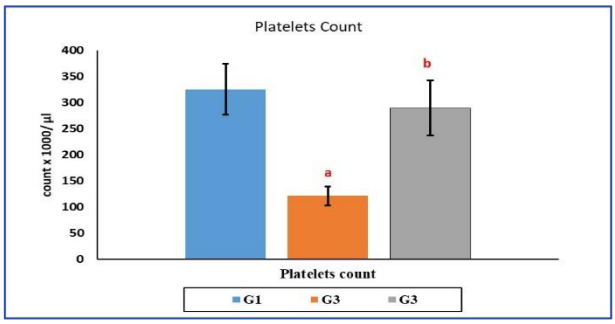


**Figure 11***: Effect of Propolis EtOH extract Platelets count in treated rat group compared with negative and positive control groups.*

and antioxidants, along with investigating its antimicrobial properties in male albino rats infected by *Escherichia coli*. Through the finding results, we found that the levels of antibodies immunoglobulins (IgG and IgM) increased after infection with the bacteria. The immune response plays a vital role in combating infectious agents and maintaining the overall health of an organism. One of the critical components of the immune system is the production of antibodies, specifically IgG and IgM, which serve as crucial mediators in the defence against pathogens.

Understanding the dynamics of antibody production following infection is essential for comprehending the immune response and the development of potential therapeutic interventions. The current results showed a notable increase in the levels of both IgG and IgM antibodies following rat infection by *E. coli*, particularly after 4 weeks. These findings suggest that the immune system of the rats responded to the *E. coli* infection by mounting an antibody-mediated immune response. The observed increase in IgG and IgM antibody levels implies the activation and engagement of B cells in producing these specific immunoglobulins. This response indicates the immune system's recognition and subsequent targeting of the *E. coli* pathogen. These findings align with the studies conducted by (Mahajan and Mehta (2009) and Amin et al. (2019). These studies underscore the importance of immunoglobulin molecules synthesized by plasma cells after B-lymphocyte activation in the antibody-mediated response. They highlight the crucial roles of immunoglobulins G and M in complement fixation, opsonization, and neutralizing toxins.

Moreover, a notable inverse relationship was observed between the levels of IgG and IgM, indicating that as IgG levels increased following the treatment, there was a concurrent decrease in IgM levels. Ma *et al.* (2022) stated that administering propolis flavonoid to pigs vaccinated with an inactivated vaccine against anti-porcine parvovirus (PPV) significantly increased serum levels of IgG subclasses. Additionally, propolis flavonoid administration led to enhanced T lymphocyte proliferation. The elevated levels of IgG subclasses were in line with the increased serum levels of interferon-gamma (IFN-γ) and interleukin-2 (IL-2).

Furthermore, the findings of Turunen *et al.* (1983) corroborate these results, as they observed an increase in immunoglobulins in rats treated with propolis compared to the control group of healthy rats. Specifically, they noted that IgM levels rose initially, followed by an increase in Immunoglobulin A (IgA). Also, Çetin *et al.* (2010) found that adding propolis to the diet stimulates IgM and IgG production. According to the Draganova-Filipova et al. (2010) study, propolis impacts humoral immunity in rats immunized with bovine serum albumin. The research suggests that propolis, as a whole, exerts a synergistic effect by enhancing antibody production in rats. This indicates that the combined components of propolis contribute to its immunomodulatory effects on humoral immunity. Oxidative stress arises from an imbalance between producing and accumulating reactive oxygen species (ROS) within cells and tissues. This imbalance can arise from factors such as microbial infection or metabolic by-products. Excessive ROS production can harm lipids, proteins, nucleic acids, and other macromolecules, causing damage to cellular components. A simultaneous compensatory mechanism activates antioxidant molecules like glutathione (GSH) and enzymes in response to the increased ROS levels. These antioxidant molecules and enzymes are immediately upregulated to counteract the detrimental effects of ROS. Their heightened activities aim to neutralize ROS and prevent further harm to cellular structures (Rana & Kumar, 2022). This interplay between the overproduction of ROS and the compensatory increase in antioxidant molecules and enzymes underscores the importance of maintaining a delicate balance. It highlights the need for an efficient antioxidant defence system to mitigate the damaging effects of oxidative stress on cells and tissues. Serum MDA level significantly decreased in the group treated with propolis. Meanwhile, the data observed a significant increase in SOD, CAT, and GSH levels. These findings suggest that the antioxidant effect of propolis is contingent upon its specific chemical composition. The findings presented

align with the results reported by Jasprica *et al. (2007).* Studies have presented evidence indicating that the administration of propolis resulted in a decrease in malondialdehyde (MDA) levels, which is a marker of oxidative stress. Additionally, propolis administration led to an increase in the activities of antioxidant enzymes, such as superoxide dismutase (SOD) and catalase (CAT). Additionally, Sobocanec *et al.* (2006) noted increased CAT activity due to propolis administration. The findings indicate that the flavonoids present in propolis have the potential to augment the activities of antioxidant enzymes and mitigate the levels of reactive oxygen species (ROS). Additionally, Kanbur *et al.* (2009) observed a decrease in both plasma and liver tissue levels of malondialdehyde (MDA) and the restoration of antioxidant enzyme parameters (such as SOD, CAT, and GSH) in animals subjected to propolis treatment. According to the study by Kurek-Górecka *et al.* (2013).

Propolis comprises a wide array of biologically active compounds, including phenolic acids and flavonoids. These substances exhibit strong antioxidant properties and have been observed to effectively inhibit the activity of different enzymes, including xanthine oxidase, adenosine triphosphatase (ATPase), protein kinase C, lipoxygenase, cyclooxygenase, ascorbic acid oxidase, and cAMP phosphodiesterase. Moreover, they interfere with the reactions involved in lipid peroxidation, a process associated with generating free radicals, thus effectively reducing the production of reactive oxygen species (ROS). This showcases the ability of propolis to counteract the creation of free radicals by inhibiting key enzymes and interrupting the lipid peroxidation process.

Concerning the impact of propolis extract on cytokines, our findings indicate that the levels of IL-6 in the serum of groups treated with propolis extract were significantly lower than those infected with bacteria. This outcome is consistent with Al-Qarni *et al.* (2019), who suggested that propolis extracts possess anti-inflammatory properties by inhibiting pro-inflammatory cytokines and modulating the activity of lipopolysaccharide (LPS) in macrophage cells, thereby exhibiting an immunomodulatory effect.

Furthermore, the active components found in green propolis, namely phenolic acids and flavonoids, function as free radical scavengers and inhibitors of nitric oxide and inflammatory cytokine production by macrophages or neutrophils. This information is supported by studies conducted by Teixeira *et al. (2010) and Chan et al.* (2013), highlighting the role of these biologically active molecules in propolis in mitigating inflammation and oxidative stress. As reported by Xool-Tamayo *et al.* (2020), propolis has been demonstrated to lower the levels of pro-inflammatory cytokines such as IL-6 and TNF-α. This indicates that propolis exhibits an anti-inflammatory effect by modulating various mediators, including NF-kB and IL-1β. The study suggests that propolis can potentially mitigate inflammation through these pathways. IL-6 is a crucial pro-inflammatory cytokine that plays a role in the immune system's response to tissue injury, as mentioned in a study by Zhang *et al.* (2021). While an increase in IL-6 levels results from the immune system's defensive reaction to aggression, prolonged secretion of IL-6 by macrophages can perpetuate an inflammatory process, potentially leading to chronic inflammatory diseases, as Gabay (2006) noted.

The data from this study demonstrated a notable reduction in TNF-α, IL-1β, and IL-10 levels in the group that received treatment with propolis extract. The results of the present study concur with the observations made by Machado *et al.* (2012), who reported an immunomodulatory effect of Brazilian green propolis extracts in animal models of acute and chronic inflammation. Their study demonstrated a decrease in the production of pro-inflammatory cytokines, including TNF-α, in animals treated with propolis extracts. Furthermore, various studies, such as those conducted by Bachiega *et al. (2012),* Hori (2013), and Zamarrenho et al. (2023), have shown that propolis possesses the capacity to decrease the levels of pro-inflammatory cytokines *IL-6* TNF-α, and IL-1β in macrophage models. Additionally, propolis has been observed to increase the levels of the anti-inflammatory cytokine IL-10 in these studies. In addition to the effects mentioned above, Araujo *et al.* (2012) have further documented that propolis exhibits effective anti-inflammatory activity by inhibiting prostanoids, specifically prostaglandin E2 (PGE2), and reducing cytokine levels. Their study also revealed additional mechanisms of action, including the modulation of inflammatory cell activity, such as cell migration and macrophage activation, suppression of nitric oxide synthesis, attenuation of enzymatic activity during the healing process, and inhibition of TNF-alpha. These findings further support the diverse range of anti-inflammatory mechanisms associated with propolis. According to the study conducted by Zulhendri *et al.* (2022), propolis exhibits anti-inflammatory properties by inhibiting and reducing the activity of multiple components involved in inflammation. The inflammatory response involves various components, including Toll-like receptor 4, myeloid differentiation primary response 88, IL-1 receptor-associated kinase 4, Toll/IL-1 receptor domain-containing adaptor protein inducing interferon-β (TRIF), NOD-like receptor protein (NLRP) inflammasomes, and nuclear factor kappa B (NF-κB). These components interact to regulate the production of pro-inflammatory cytokines such as IL-6, IFN-γ, TNF-α, and IL-1β. Moreover, propolis has demonstrated its ability to effectively reduce the migration of immune cells such as macrophages and neutrophils. This effect may be attributed to the downregulation of chemokines. Monokine induced by interferon-gamma (MIG) and interferon gamma-induced protein 10 (IP-10) are additional cytokines stimulated by interferon-gamma. These findings suggest that propolis exerts its anti-inflammatory effects through multiple pathways and contributes to the regulation of various immune responses. The findings align with a study conducted by Bueno-Silva (2017); it was shown that propolis reduced the expression of various inflammatory cytokines in LPS-activated peritoneal macrophages isolated from C57BL6 mice. These cytokines encompass IL-1, IL-4, IL-6, Interleukin-12p40, IL-1β, IL-12p70, IL-13, granulocyte-macrophage colony-stimulating factor (GM-CSF) and monocyte chemoattractant

protein-1. According to Kitamura *et al.* (2018), various in vivo studies have provided evidence of propolis' ability to modulate the immune system, promoting a regulatory profile and creating an anti-inflammatory environment.

Hegazi et al. (2021) also studied newborn Egyptian-Nubian goat kids. They found that supplementation with propolis resulted in a significant increase in serum levels of immunoglobulins IgG and IgA. Additionally, the addition of propolis resulted in decreased levels of pro-inflammatory cytokines (such as IL-6, IL-1β, TNF-α, and IFN-γ) in the serum. These findings suggest that propolis has an immunomodulatory effect and can contribute to decreased inflammation and enhanced immune function in animal models. In the study conducted by Wang *et al.* (2018), it was observed that propolis had a significant impact on reducing inflammatory markers associated with colonic inflammation. Specifically, the levels of IL-1β, IL-6, and monocyte chemoattractant Protein-1(MCP-1), which are known to be involved in the inflammatory response, were found to be decreased by the administration of propolis.

The administration of propolis resulted in a notable decrease in nitric oxide (NO) levels in the treated group compared to the positive control group. This finding aligns with the research conducted by Ojo, Osukoya, and Ajiboye (2017), who emphasized the significant role of inflammation in various metabolic abnormalities. Inflammation markers such as C-reactive protein (CRP), NO, and inducible NO synthase (iNOS) are crucial indicators in assessing inflammation, and their suppression offers promising strategies for combating inflammatory processes. Propolis exerts its anti-inflammatory effects through multiple mechanisms. According to Pahlavani *et al.* (2020), propolis has been found to inhibit cyclooxygenase (COX) activity. This inhibition prevents the production of inflammatory prostaglandins, molecules involved in the inflammatory response. Propolis may help reduce inflammation in the body by inhibiting COX activity (Magnavacca *et al.* (2022). Propolis also acts as a scavenger of free radicals, reducing oxidative stress and inflammation.

Furthermore, it inhibits nitric oxide synthesis, a molecule involved in inflammatory processes. Additionally, propolis reduces the concentration of inflammatory cytokines and exhibits immunosuppressive activity, regulating immune responses and alleviating inflammation. These combined actions contribute to propolis's overall anti-inflammatory properties.

According to Selamoglu Talas (2014), propolis was found to alleviate oxidative stress in rats with hypertension induced by L-NAME. This effect was attributed to a reduction in malondialdehyde (MDA) expression. Fuliang *et al.* (2005) conducted a study on the effects of propolis on diabetic rats. They found that it led to a reduction in various blood parameters associated with diabetes, such as nitric oxide (NO), malonaldehyde (MDA), and nitric oxide synthetase (NOS). The results obtained from the positive control group in this study corroborate the findings reported by Tripathi (2007), who proposed that activated macrophages possess the ability to impede pathogen replication through the release of various effector molecules, including nitric oxide (NO). Nitric oxide plays a crucial role as a potent defensive molecule in combating infectious microorganisms. It regulates diverse immune cell types' functions, growth, and survival, including T lymphocytes, basophils, eosinophils, neutrophils, natural killer cells, antigen-presenting cells (APCs), macrophages, and mast cells. The findings suggest that propolis EtOH extract possesses anti-inflammatory properties. The in vitro assessment of phagocytic cell activity using the nitro blue tetrazolium (NBT) dye reduction test indicated an enhancement in phagocytosis when the propolis EtOH extract was used. The phagocytosis % in the treated group increased compared to the positive control group. The results are in agreement with those of Yuan et al. (2012) and Al-Hariri (2019), who also reported that propolis has the potential to enhance the efficacy of vaccines as an adjuvant. It can improve the protective index by increasing phagocytic activity, eliciting a sustained and higher production of antibodies, promoting mucosal immunity, and enhancing cellular immune responses.

Additionally, Berretta *et al.* (2020) some studies have shown that propolis can act as an immunostimulant, with the ability to improve the immune response. Ghosh *et al.* (2022) suggested that propolis may influence antibody production, including neutralizing antibodies. Berretta *et al.* (2020) Propolis, also called bee glue, exhibits antibacterial effects against the human tubercle bacillus, although its efficacy against Gram-negative bacilli is usually limited. These antimicrobial properties are believed to be attributed to a significant number of flavonoids in propolis. According to Bankova *et al.* (1995), propolis has exhibited antibacterial effects against *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *E. coli* bacteria. The recent study revealed noteworthy improvements in red blood cell count, hemoglobin concentration, and packed cell volume (PCV) levels in the group that received propolis treatment compared to the positive control group. Moreover, the group treated with propolis demonstrated substantially elevated platelet levels when compared to the positive control group. These findings are consistent with previous studies conducted by Talas *et al.* (2013) and Zulhendri *et al.* (2021), which also reported the beneficial effects of propolis on various blood parameters. The findings are consistent with the research conducted by Orsolic and Basic (2005), who observed significant improvements in red blood cell, white blood cell, hemoglobin, platelet, and PCV% (packed cell volume percentage) counts in rats administered with propolis. Propolis administration led to a notable increase in the proliferation of hematopoietic cells within bone marrow and spleen.

Furthermore, studies have demonstrated that it can improve the absorption of dietary iron and enhance the process of hemoglobin regeneration, especially during the recuperation period of anemia (Haro *et al.*, 2000). The high concentration of flavonoids in propolis has also been found to accelerate the production of erythrocytes and hemoglobin (Dong *et al.*, 2005).

Furthermore, studies by Suwalsky *et al. (2008) and Moreria et al.* (2011) have suggested that propolis administration can reduce the osmotic fragility of erythrocytes, potentially due to the phenolic and flavonoid components of propolis interacting with membrane phospholipids and stabilizing the erythrocyte membrane, thereby decreasing erythrocyte hemolysis in the spleen. These various factors contribute to an increase in the count of red blood cells and hemoglobin concentration. These findings align with the results reported by Cristina *et al.* (2007), who demonstrated that propolis supplementation leads to an increase in hemoglobin concentration, which correlates positively with packed cell volume (PCV). Meanwhile, the results in the propolis-treated group were all similar to those with the negative control. According to the data of RBC indices, there were no significant differences in MCH (mean corpuscular hemoglobin) between the propolis-treated group and the negative or positive control groups. However, the propolis-treated group showed a significant increase in MCV and MCHC compared to the positive control group. On the other hand, MCHC (mean corpuscular hemoglobin concentration) was significantly decreased in the propolis-treated group compared to the positive control group. These findings align with a study conducted by Alishahi and Jangeran Nejad (2012), which also reported no statistically significant differences in MCV, MCH, and MCHC in groups treated with propolis ethanolic extract. Additionally, the results indicated a significant decrease in hemoglobin concentration and packed cell volume (PCV%), along with a significant decrease in erythrocyte count in the positive control group as compared to the negative control and treated groups. This decline may be attributed to the breakdown of erythrocytes caused by hemolysis enzymes produced by *E. coli.* Consequently, reducing erythrocyte count leads to decreased packed cell volume (%) and hemoglobin concentration (Justice *et al.*, 2006). Moreover, there was a significant decrease in thrombocytes (platelets) in the infected group. The decrease in platelet count observed in this study may be associated with infection, as Venkata et al. (2013) suggested. Platelets can be destroyed in large numbers due to antigen-antibody reactions on the platelet surface membrane, which is likely responsible for a significant portion of idiopathic thrombocytopenia in animals.

The study's findings indicate that E. coli infection in rats has multiple effects on blood parameters. Specifically, the infected group demonstrated a substantial increase in total white blood cell count when compared to the control group. This increase in total leucocyte count may be attributed to the *E. coli* infection, which is consistent with the observations made by Coles (1986). This rise in leucocytes is primarily associated with increased neutrophils, a characteristic of pathological leukocytosis. The increase in neutrophils could be either relative, resulting from an elevated percentage of neutrophils, or absolute, due to the overall increase in the total leukocyte count (Zamely & Falh, 2011). Neutrophilia, characterized by an increased neutrophil count, can occur in systemic infections such as Colibacillosis and Salmonellosis (Dale & Liles, 1998). In the infected group, there was a notable decrease in lymphocytes compared to the control group. This decline in lymphocyte count could be attributed to the stress induced by the infection. Stress has been recognized as a factor that can lead to lymphopenia, characterized by a substantial decrease in the absolute count of lymphocytes.

In contrast, the propolis-treated group exhibited a significant decrease in total leukocyte count ($\times 10^3/\mu l$) compared to the control group but a non-significant increase compared to the negative control group. These findings align with the results reported by Çetin *et al.* (2010), who found that dietary supplementation with propolis did not significantly impact white blood cell count. The results indicate that propolis acts as an effective natural immunomodulatory agent by reducing the percentage of neutrophils and increasing the percentage of lymphocytes in the treated group, thereby enhancing the production of IgM, IgG, and IgA antibodies. Propolis has been shown to have an anabolic effect, stimulate immune responses, induce mitosis, promote lymphocyte proliferation, and enhance the size of immune organs (Fan *et al.*, 2014). These results confirm the immune-suppressive effect of *E. coli*.

Lymphocytes are integral to the immune system as they play a vital role in determining the specificity of the immune response towards infectious microorganisms. (Alberts *et al.*, 2002). T cells play a role in the formation of lymph kinase, which facilitates the movement of phagocytic cells towards areas of inflammation. On the other hand, B cells are responsible for producing immunoglobulins (Abo-Zaid & Hamdi, 2022). In the present study, the group designated as the positive control exhibited a noteworthy rise in the proportion of eosinophils when compared to both the negative control group and the group treated with propolis. The findings are consistent with the research conducted by Linch et al. (2009), which suggested that elevated levels of eosinophils in IL-5 transgenic mice contribute to their improved ability to clear bacterial infections.

Hogan et al. (2013) mentioned that eosinophils possess toll-like receptors, produce antibacterial proteins, and contribute to forming neutrophil extracellular traps (NETs). According to Linch et al. (2012), eosinophils might function differently in intestinal inflammatory diseases induced by bacterial antigens. One potential role is their involvement in tissue repair and remodeling. Various experimental studies suggest that cytokines derived from eosinophils, such as IL-13, play a significant role in fibrotic responses. Eosinophils possess the essential receptors of the innate immune system to recognize bacteria, as well as cytolytic granule proteins that exhibit potent bactericidal activity (Hogan *et al.*, 2013). DeChatelet *et al.* (1978) demonstrated that while eosinophils and neutrophils have similar rates of phagocytosis, eosinophils are less effective in killing bacteria due to the inability of eosinophil peroxidase to catalyze specific reactions. In the absence of neutrophils, eosinophils are unable to clear bacteria effectively. However, in contrast, Yazdanbakhsh et al. (1986) did not find significant differences in the bactericidal activity between neutrophils and eosinophils. According to Ondari *et al.* (2021), eosinophils play a role in promoting TH2 responses. When

eosinophils respond to stimuli and secrete cytokines, such as IL-4, IL-5, IL-13, and others, they generate a type II immune response, which can further facilitate the development of TH2 responses.

## CONCLUSION

In conclusion, this study's findings highlight propolis's therapeutic potential as a natural approach to boosting immunity and combating bacterial infections. The observed improvements in blood parameters and enhanced immunity in rats infected with bacteria demonstrate the efficacy of propolis in neutralizing infections. This study's findings support the use of propolis as a valuable option for managing bacterial infections, either as an alternative or complementary therapy. Propolis offers a natural approach that has the potential to be effective in combating such infections. However, further research and clinical trials are necessary to fully understand and explore the complete range of therapeutic benefits that propolis may offer. Additionally, these investigations are crucial for determining the potential application of propolis in promoting human health.

## AUTHOR CONTRIBUTION

MAZ Designed the study, conducted the experiments, collected data, analysed it, wrote the manuscript, and finalised it.

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## DECLARATIONS

### Ethical Considerations

The Jazan University Institutional Review Board approved this study before starting.

### Participants Consent

Not Applicable

### Source of Funding

None.

### Conflict of Interest

All authors have declared that no financial support was received from any organization for the submitted work. All authors have declared that no other relationships or activities could appear to have influenced the submitted work.

## OPEN ACCESS



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