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Original article

THE IMMUNOMODULATORY ROLE OF CLOVE AND CINNAMON EXTRACTS ON *KLEBSIELLA PNEUMONIAE* INFECTED RATS

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Summary

Sadeq, Z. E., I. J. Lafta, F. M. K. AL-Rekabi & R. A. Faraj, 2025. The immunomodulatory role of clove and cinnamon extracts on *Klebsiella pneumoniae* infected rats. *Bulg. J. Vet. Med.* (online first).

This study aimed to investigate the ability of clove and cinnamon extracts to make pathogenic multidrug-resistant (MDR) *Klebsiella pneumoniae* more sensitive to the host's immune system and thereby interrupt the bacterial infection process in the rat model. Therefore, 60 Wistar male rats were used in this study. The phytochemical constituents of the plant extract were analysed using the gas chromatography-mass spectrometry (GC-MS) technique. Then, the capability of the plant extracts, as prophylactic and treatment, against *K. pneumoniae* in rats was studied by estimating the complete blood counts (CBCs) and the serum concentrations of interleukin-4 (IL-4) and interferon-gamma (IFN-γ) before and after the treatment. The results showed that the main phytoconstituent present in the aqueous ethanolic extract of cinnamon was cinnamaldehyde, and eugenol for clove. Concerning the haematological parameter, significant alterations in white blood cell counts, lymphocytes %, monocytes %, granulocytes %, and plateletcrit % were observed in the blood of animals. Increased IL-4 and IFN-γ levels occurred following the administration of cinnamon and clove, and that increase was dose-dependent. To conclude, clove and cinnamon ethanolic extracts exerted potent immunomodulatory effects against *K. pneumoniae.* The study recommends further exploration of these plant extracts separately or in combination to develop alternative therapies against MDR *K. pneumoniae* infections.

Key words: cinnamon, clove, complete blood counts, IL-4 and IFN-γ, *Klebsiella pneumoniae*

INTRODUCTION

Klebsiella pneumoniae, a member of the *Enterobacteriaceae* family, is a noteworthy human pathogen. This bacterium is the cause of blood and urinary tract infections, meningitis, pneumonia, and other infectious illnesses (Yoon *et al.,* 2022). Immunocompromised patients and those with serious illnesses are hospitalised

primarily due to this opportunistic pathogen (Abbas *et al.,* 2024). The ability of *K. pneumoniae* to resist the host innate immune responses is linked to several virulence factors, which contribute to its pathogenicity. The virulence factors of *K. pneumoniae* comprise adhesins, lipopolysaccharides, mucoviscosity-associated exopolysaccharides, capsules, and iron absorption systems (Riwu *et al.,* 2022). The MDR and *K. pneumonia*'s capacity to produce nosocomial infections in people are factors that exacerbate the infection (Aminul *et al.,* 2021). This bacterium has developed numerous mechanisms to evade β-lactam drugs, involving penicillins, cephalosporins, and carbapenems. One example of a drug resistance strategy is the creation of extended-spectrum β-lactamase, the production of metallo-β-lactamase, carbapenemase formation, production of AmpC β-lactamase, in addition to porin loss (Karampatakis *et al.,* 2023). Furthermore, this microbe has additional resistance mechanisms represented by efflux pumps, which increase resistance to macrolides, β-lactam antibiotics, chloramphenicol, and quinolones (Pages *et al.,* 2009).

Due to repeated antibiotic therapies, many countries have experienced an increase in drug-resistant bacteria in recent decades. This has made treatment and prophylaxis more challenging and increased the incidence of recurrent infections (Kaur & Kaur, 2021). Therefore, creating treatments that would be effective against bacterial infections over the long term is more crucial than ever. The current treatments put a lot of pressure on bacteria to evolve resistance mechanisms since they mostly involve the use of antibiotics, which kill off invasive bacteria and remove them from the body. It should theoretically be feasible to develop treatments that reduce the forces that favor resistance by avoiding consequences that limit growth (LaSarre & Federle, 2013). Numerous studies have used medicinal plants as an antibacterial agent (Shahata *et al.,* 2011; Ibrahim, 2021; Sadeq *et al.,* 2024; Zouine *et al.*, 2024). Thus, the creation of nontoxic, immunomodulatory, and plant-based medications has proven immensely helpful recently. From what is mentioned above, this study aimed at assessing the capability of clove and cinnamon ethanolic extracts, at the minimum inhibitory concentration (MIC), as prophylactic and therapeutic agents against *K. pneumoniae* infection in rats.

MATERIALS AND METHODS

Klebsiella pneumoniae

A highly virulent MDR isolate of *K. pneumoniae* obtained from the study of Sadeq & Lafta (2024) was used in this research.

Plant extracts

This study used two medicinal plants that were bought from a local market in Baghdad, Iraq. These plants included dried clove flower buds (*Syzygium aromaticum* L), which were purchased in late summer, August, which represented the main harvesting period. The other plant was cinnamon bark (*Cinnamomum cassia* L), which was purchased in June from the harvest that took place in May. The Directorate of Seed Testing and Certification, Ministry of Agriculture, Baghdad, Iraq, identified and verified the herbs. Ethanolic plant extracts were prepared using the method outlined by Abubakar & Haque (2020). In short, 500 mL of 70% ethanol was combined with 50 g of the pulverised plants in a glass beaker. The beaker was then incubated for the entire night at room

temperature in a shaker incubator (Lab Companion, Korea). Following the use of a Buckner funnel under negative pressure to filter the solution through a filter paper No. 0.4 mm (CHM, Spain), the filtrate was then stored in a container at room temperature until the liquid evaporated and a powder was formed (Anesini & Perez, 1993). The cinnamon ethanolic extract yielded 9 g of the powder, which had a dark reddish-brown colour and a pleasant, pungent smell, with an extraction yield of 18%. The ethanolic extract of clove resulted in 15.50 g powder, ranging in colour from brown to black, with a pleasant and pungent aroma and an extraction yield of 62%. Ultimately, 1 mg of each plant extract was dissolved in 1 mL of sterile phosphate-buffered saline (PBS, pH 7.4) to create a stock (1000 мg/mL) from the extracts.

Phytoconstituent analysis

The phytoconstituent analysis of the plant extracts was done by using the gas chromatography-mass spectrometry (GC-MS) technique, performed by Industrial Research and Development Authority, Ibn Al-Bitar Research Center, Iraqi Ministry of Industry and Minerals, Baghdad, Iraq. The GC-MS analysis was conducted via the use of 5977E Gas Chromatograph (Agilent Technologies, USA) connected to Mass Spectrometer (Humax, Germany). Identification of each component was achieved based on its retention indices and MS solution software provided by the supplier to control the system and to acquire the data.

MIC determination

To determine the MIC of the plant extracts, the agar-well diffusion assay described by Al-Sarai (2010) was applied. Firstly, *K. pneumoniae* was grown on the Brain Heart Infusion agar (Microgen /India) overnight at 37 °C. The next day, the culture was harvested with PBS, and a suspension equivalent to McFarland tube no. 0.5 (1×10^8 CFU/mL) was made. Meanwhile, different concentrations (400, 200, 100, 50, 25, 12.5, 6.25, and 3.125 μg/mL) of the plant extracts were set out. Mueller Hinton agar (Himedia, India) was prepared, and the bacterial inoculum was spread by a cotton swab on this medium at different directions. Then, 4 wells, each of 6 mm diameter, were punched into each agar plate to be filled with 100 μL of each plant extract concentration. The plates were incubated at 37 °C for 48 h to inspect the occurrence of growth inhibition at the minimum concentration of the plant extracts.

Ethical approval

The work on the animals used in this research obtained an ethical approval according to the Animal Utilisation Protocol Certification, number 1078/PG, issued by the Research Ethics Committee of the College of Veterinary Medicine, University of Baghdad.

Laboratory animals

Totally, 60 adult Wistar male rats purchased from the College of Veterinary Medicine, University of Baghdad were used in the experiment. These rats were between 4–8 months of age and weighed between 150 g to 225 g. The animals were kept in cages at the animal house of the same college, mentioned above. They were housed in an air-conditioned room at a temperature of 20–25 °C and exposed to a light cycle of 12 light/12 dark. They were fed on a locally made standard diet as pellets, composed of ground corn (40%), dehulled soybean (30%), dried beet pulp (5%), fish meal (5%), ground

oats (5%), and cane molasses (5%). In addition, the pellets contained brewers dried yeast (2%), alfalfa meal (2%), wheat middling's (2%), salt (1%), calcium carbonate (1%), DL-methionine (0.5%), vitamin D3 (0.02%), folic acid (0.002%), menadione (vitamin K) (0.002%), biotin (0.0002%), thiamin (0.0002%), alpha-tocopherol acetate (0.0002%), vitamin B12 (0.00002%), riboflavin (0.00002%), ferrous sulphate (0.00002%), manganous oxide (0.00002%), ferrous oxide (0.00002%), and zinc sulphate (0.00002%). The animals had unlimited access to food and water during the experiment and were able to acclimatise to the room conditions before starting the experiment.

Experimental design

The experiment involved 10 groups of rats (G1 to G10), each group with 6 animals. The 1st group (G1) was the negative control, which was given 0.1 mL PBS orally and another dose intranasally (I/N) of the same amount. The 2nd group (G2) or the positive control was infected with 0.1 mL (1×10^8 CFU/mL) of *K. pneumoniae* instilled I/N and another 0.1 mL given orally to the same animals by the stomach tube. The other four treatment groups of rats (G3, G4, G5, and G6) were first infected with *K. pneumoniae* using the same dose and route (as described for G2 above). Then, 6 h after administering the bacteria, G3 was treated orally with 125 mg/kg body weight (bw) of the clove extract, while G4 was treated with 175 mg/kg bw of the clove extract orally too. Similarly, 6 h after bacterial administration, G5 was treated orally with 250 mg/kg bw of the cinnamon extract, and G6 with 350 mg/kg bw of the same extract via the same route. The next day, following 24 h of the infection, the blood was withdrawn from all the animals. The same treatments mentioned above continued once daily and orally for G3, G4, G5, and G6 for 14 days post infection. Afterwards, the blood was collected from the animals of these groups as well as from G1 and G2.

The prophylaxis experiment included the use of four groups (G7, G8, G9, and G10) of rats, each with 6 animals. The G7 was given clove at a dose of 125 mg/kg bw as a single dose daily for 2 weeks, while the G8 was given clove at a dose of 175 mg/kg bw once daily for 2 weeks. Likewise, the G9 and G10 were administered orally the cinnamon extract at a dose of 250 mg/kg bw and 350 mg/kg bw, respectively, once daily for 2 weeks. Then, after 2 weeks, the G7, G8, G9, and G10 were infected with *K. pneumoniae* (1×10^8 CFU/mL) orally and I/N at 0.1 mL each. Following 24 h of infection, the blood was collected from all the infected rats in G7, G8, G9, and G10 along with the negative and positive groups (G1 and G2, respectively). The doses here were chosen based on the above described MIC experiment.

Blood collection

The animals were fasted for about six hours on the previous day of the experiment before the induction of anaesthesia or the collection of blood samples. The blood was collected from all of the experimental animals through heart puncture according to the procedure described by Beeton *et al.,* (2007). Briefly, the rats were anesthetised by injecting a mixture composed of 0.6 mL of xylazine (Xyl-M2 V.M.D., Belgium) and 0.4 mL of 10% ketamine (Panther, England) divided equally into each thigh muscle (each 0.5 mL). Then, approximately 1.5 mL of heart blood was withdrawn into a tube containing EDTA for CBC testing, and another ~ 1.5 mL of blood was put into a gel tube

without anticoagulant (Vacutte, Germany) for serological tests. To acquire the serum, the gel tube with blood was centrifuged (Humax 4, Germany) at 3000 rpm for 10 min, and the serum was separated from the coagulated blood. Finally, the collected sera were maintained in Eppendorf tubes and kept in –20 °C till use.

Complete blood count

The CBC was analysed using Automated Hematology Analyzer MEK 6500 (Nihon Kohden, Holland) to determine the impact of clove and cinnamon extracts on the animals infected with *K. pneumoniae*. In this study, white blood cells, red blood cells, lymphocytes, monocytes, granulocytes, platelets, haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, red cell distribution width, plateletcrit, mean platelet volume, and platelet distribution width were tested simultaneously.

Serum IL-4 and IFN-γ assays

The concentrations of IL-4 and IFN-γ before and after treatment of rats with clove and cinnamon extracts were measured by the enzyme-linked immunosorbent assay (ELISA) using kits specific for rats, manufactured by Bioassay technology laboratory (BT LAB, China).

Statistical analysis

The collected data for CBC, IL-4 and IFN-γ were analysed by two-way analysis of variance (ANOVA) via the Standard Least Squares procedure of JMP Pro 16.0.0 software (SAS, Institute Inc., Cary, NC, USA). The model accounted for the main effects of treatments and treatment type, as well as their interaction effect. Significant effects and interaction means were analysed and accepted at $P \leq 0.05$ using the least significant difference (LSD) *post-hoc* test.

RESULTS

Phytoconstituents of the plant extracts

A total of 25 compounds were identified in the cinnamon ethanolic extract. The highest peak in the cinnamon extract was that of the compound 2-propenal, 3-phenyl cinnamaldehyde, (E)-2-propenal, 3-phenyl-2-propenal, 3-phenyl. Concerning the clove extract, 25 compounds were also identified with the most abundant chemical was eugenol.

MIC

According to the agar well-diffusion assay, the MIC of cinnamon that inhibited the growth of *K. pneumoniae* was 50 µg/mL while the MIC of clove was 25 µg/mL. Therefore, for cinnamon a dose of 250 mg/kg bw and for clove a dose of 125 µg/kg bw were given to the rats.

Effects of the plant extracts on infected animals

After infection with *K. pneumoniae* (1×10^8 CFU/mL) and before treatment, clinical signs appeared after 24 h, in which all of the infected groups suffered from fever (39 ºC), lethargy with sluggishness, loss of movement, and anorexia. However, 48 h after receiving the plant extracts, the signs started to disappear gradually, and the temperature slightly decreased to 38 ºC. Following 4 days of taking the extracts by the infected rats, their temperature returned to normal with no clinical signs noticed. The prophylactic groups showed a moderate increase in body temperature with no other clinical symptoms.

Complete blood count

The results of this study showed significantly elevated levels (approximately five-fold) of white blood cells in the positive control animals relative to the negative control in all the studied groups (Fig. 1). However, when the animals were treated with clove or cinnamon following 24 h or 14 days of infection, as well as in the prophylactic group subjected to either plant extract, significant medium levels of white blood cells lower than the positive control but higher than the negative control were reported (Fig. 1). No significant difference was reported between different concentrations of the extracts.

In the current study, the lymphocyte percentage was significantly higher in the infected group than in the treatment and prophylactic groups (Fig. 2). The results showed that the plant extracts at different concentrations and various groups caused an increase in the lymphocyte percentage compared to the negative control. This study also demonstrated increased percentages of monocytes in all treated groups as well as the positive control compared to the negative control (Fig. 3).

Regarding red blood cells, no significant differences were observed between their counts in the negative control group and the treatment and prophylactic groups given the cinnamon or clove extracts. Concerning haemoglobin, no significant difference was seen between the negative and positive controls. In the groups of prophylaxis and treatment for 24 h or 14 days after infection, there were neither statistically significant differences. In addition, haematocrit did not differed significantly in most studied groups compared to the control groups. However,

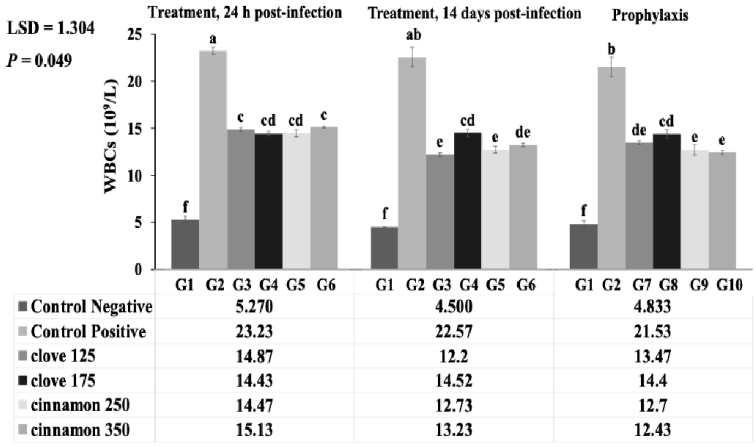


Fig. 1. White blood cell counts (10^9/L) in rats infected with *K. pneumoniae* and given clove or cinnamon extracts as a treatment or prophylaxis. Ten groups (each with 6 rats) are shown: the control groups included G1 (negative control, PBS only) and G2 (positive control, *K. pneumoniae* infection). The treatment groups (G3 to G6) were infected firstly with *K. pneumoniae,* and then treated with clove at 125 mg/kg bw (G3), clove at 175 mg/kg bw (G4), cinnamon at 250 mg/kg bw (G5), or cinnamon at 350 mg/kg bw (G6). Prophylactic groups (G7 to G10) were given firstly the plant extracts for 14 days, and then infected as mentioned above. They were administered clove at 125 mg/kg bw (G7), clove at 175 mg/kg bw (G8), cinnamon at 250 mg/kg bw (G9), or cinnamon at 350 mg/kg bw (G10). The bars represent means, and error bars indicate the standard error of the mean (SEM). Groups labelled with different small letters are significantly different ($P \leq 0.05$).

compared to the negative control, a significant increase in the mean corpuscular volume was observed in the group treated 24 h post-infection and the prophylactic groups, but no significant difference in the groups treated with the plant extracts for 14 days post-infection. The mean corpuscular haemoglobin levels were roughly similar within and among the groups of rats. The study also demonstrated the presence of non-significant differences for the mean corpuscular hemoglobin concentration among the different groups of rats. However, the cinnamon extract at a concentration of 350 mg/kg bw caused a slight decrease in the mean corpuscular haemoglobin compared to the other groups. Platelet counts fluctuated statistically significantly, particularly in the prophylactic group. The treatment group that received clove extract at 175 mg/kg bw had lower platelets than the other groups. Concerning granulocytes, the positive control group demonstrated a higher percentage than the other groups. Furthermore, a significant increase in the value of red cell distribution width was seen in the animals treated with the higher dose of the cinnamon extract 24 h post-infection compared to the other groups. Nevertheless, all the animals in the prophylactic groups showed higher red cell distribution widths than the negative and positive control. Pertaining to the rats treated with the plant extracts after 14 days of infection, only two groups (treated with 125 mg/kg bw of clove extract and with 350 mg/kg bw of cinnamon) demonstrated a significant increase in the red cell distribution width percentage compared to the controls. The results of this study revealed that all groups treated with the plant extracts either as a prophylactic or as a treatment for 14 days post-infection had a significant decrease in the plateletcrit % compared to the infected group (Fig. 4).

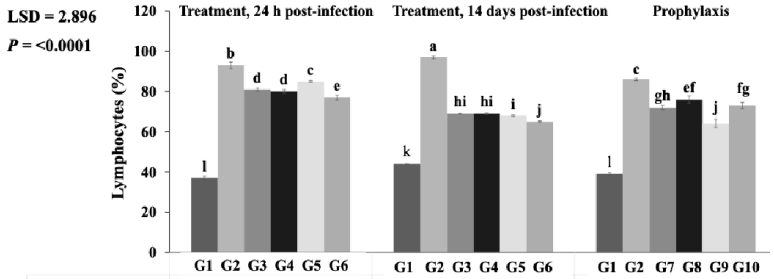
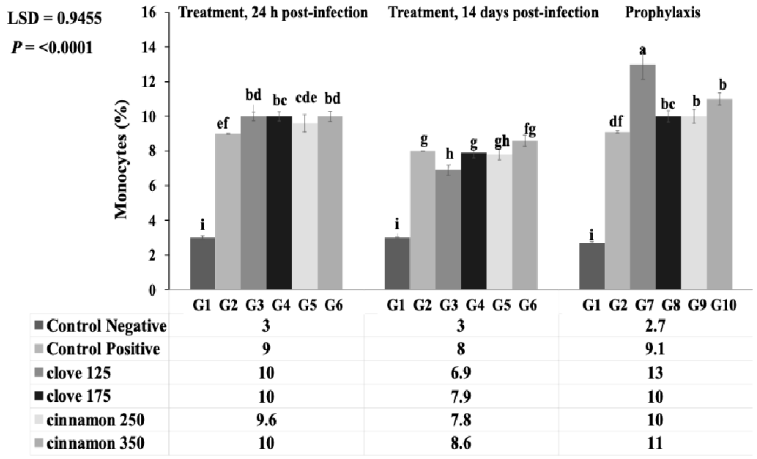


Fig. 2. Lymphocytes percentage in rats infected with *K. pneumoniae* and given clove or cinnamon extracts as a treatment or prophylaxis. Ten groups (each with 6 rats) are shown: the control groups included G1 (negative control, PBS only) and G2 (positive control, *K. pneumoniae* infection). The treatment groups (G3 to G6) were infected firstly with *K. pneumoniae,* and then treated with clove at 125 mg/kg bw (G3), clove at 175 mg/kg bw (G4), cinnamon at 250 mg/kg bw (G5), or cinnamon at 350 mg/kg bw (G6). Prophylactic groups (G7 to G10) were given firstly the plant extracts for 14 days, and then infected as mentioned above. They were administered clove at 125 mg/kg bw (G7), clove at 175 mg/kg bw (G8), cinnamon at 250 mg/kg bw (G9), or cinnamon at 350 mg/kg bw (G10). The bars represent means, and error bars indicate the standard error of the mean (SEM). Groups labelled with different small letters are significantly different (P≤0.05).



Control Negative	3	3	2.7
Control Positive	9	8	9.1
clove 125	10	6.9	13
clove 175	10	7.9	10
cinnamon 250	9.6	7.8	10
cinnamon 350	10	8.6	11

Fig. 3. Monocytes percentage in rats infected with *K. pneumoniae* and given clove or cinnamon extracts as a treatment or prophylaxis. Ten groups (each with 6 rats) are shown: the control groups included G1 (negative control, PBS only) and G2 (positive control, *K. pneumoniae* infection). The treatment groups (G3 to G6) were infected firstly with *K. pneumoniae,* and then treated with clove at 125 mg/kg bw (G3), clove at 175 mg/kg bw (G4), cinnamon at 250 mg/kg bw (G5), or cinnamon at 350 mg/kg bw (G6). Prophylactic groups (G7 to G10) were given firstly the plant extracts for 14 days, and then infected as mentioned above. They were administered clove at 125 mg/kg bw (G7), clove at 175 mg/kg bw (G8), cinnamon at 250 mg/kg bw (G9), or cinnamon at 350 mg/kg bw (G10). The bars represent means, and error bars indicate the standard error of the mean (SEM). Groups labelled with different small letters are significantly different ($P \leq 0.05$).

Nevertheless, the plant extracts had no effects on the plateletcrit % 24 h after infection relative to the positive control.

This study showed that the mean platelet volume was higher in the infected groups than in the negative control or in the other groups treated with the plant extracts. Importantly, using the plant extracts as a prophylaxis or treatment for 14 days post-infection was successful in rendering the level to the normal status. Additionally, platelet distribution width percentages showed no significant differences between the positive and negative control groups. While the rats treated with different concentrations of cinnamon had values similar to those of the control group, clove treatment caused a slightly significant increase 24 h after infection. In addition, the groups treated for 14 days and the prophylactic groups showed not too many differences compared to the control animals.

IL-4 and IFN-γ serum concentrations

This study showed that after 24 h of infection with *K. pneumoniae,* IL-4 levels occurring in the positive control animals were increased compared to the other groups (Fig. 5). Interestingly, in the groups treated for 14 days post-infection and in the prophylactic groups, significantly increased IL-4 levels occurred due to the administration of cinnamon and clove, and that increase was dose-dependent.

Fig. 6 illustrates the levels of IFN-γ in the different groups of the experimental animals. There was a steady increase in the level of this cytokine in all of the treated animals as well as the positive

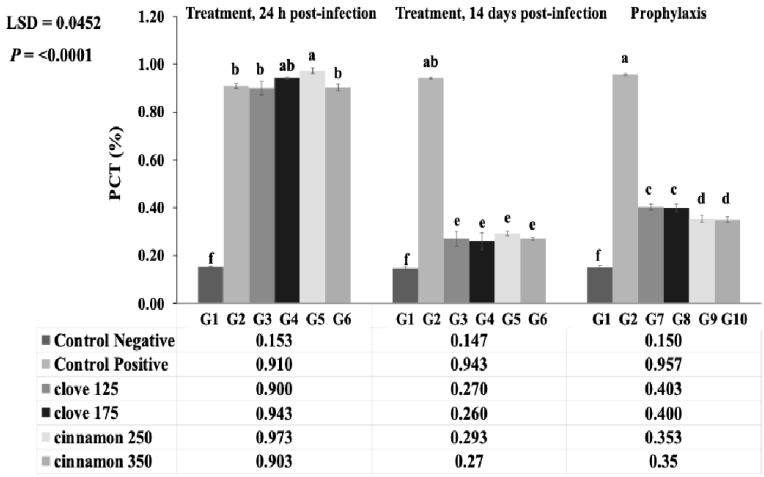


Fig. 4. Plateletcrit percentage in rats infected with *K. pneumoniae* and given clove or cinnamon extracts as a treatment or prophylaxis. Ten groups (each with 6 rats) are shown: the control groups included G1 (negative control, PBS only) and G2 (positive control, *K. pneumoniae* infection). The treatment groups (G3 to G6) were infected firstly with *K. pneumoniae,* and then treated with clove at 125 mg/kg bw (G3), clove at 175 mg/kg bw (G4), cinnamon at 250 mg/kg bw (G5), or cinnamon at 350 mg/kg bw (G6). Prophylactic groups (G7 to G10) were given firstly the plant extracts for 14 days, and then infected as mentioned above. They were administered clove at 125 mg/kg bw (G7), clove at 175 mg/kg bw (G8), cinnamon at 250 mg/kg bw (G9), or cinnamon at 350 mg/kg bw (G10). The bars represent means, and error bars indicate the standard error of the mean (SEM). Groups labelled with different small letters are significantly different (P≤0.05).

control following 24 h of infection relative to the negative control. However, the groups treated for a couple of weeks after infection showed significantly increased levels of IFN-γ in rats treated with either clove or cinnamon compared to the negative and positive controls. Similarly, the same trend was seen in the prophylactic groups, despite lower values were reported in these animals.

DISCUSSION

The current study showed that the most abundant chemical present in the clove extract was eugenol. This result is consistent with another study, where eugenol was found to be the main bioactive component of cloves [2-methoxy-4-(2-propenyl) phenol] (Shan *et al.,* 2005). Eugenol has been suggested to possess antibacterial properties against different types of bacteria, and it may disturb the cell membrane causing bacterial growth inhibition (Ulanowska & Olas, 2021). The presence of peaks of eugenol mixed with phenol,2-methoxy-4-(4(2-propenyl)-acetate was also observed here in clove extract. Phenol is known to possess antimicrobial properties, and it interferes with bacterial cell membranes and proteins leading to their damage (Lobiuc *et al.,* 2023). The highest peak of cinnamon extract was rich mainly in the compound 2-propenal, 3-phenyl cinnamaldehyde, (E)-2-propenal, 3-phenyl-2-propenal, 3- phenyl. This finding agrees with other studies, which stated that the primary component of essential

oil molecules originated from the *Cinnamommum* genus was cinnamonaldehyde (Abdel-Moaty *et al.,* 2016; de Sousa *et al.,* 2023). Cinnamaldehyde was demonstrated to have antibacterial effects against various bacterial strains, including Gram-positive and Gram-negative bacteria (Usai & Di Sotto, 2023).

This investigation revealed that the clove and cinnamon extracts were effective against *K. pneumoniae* infection, in which the clinical signs started to disappear from rats gradually 48 h after receiving the extracts. The response of Wistar rats to *K. pneumoniae* infection following treatment or prophylaxis with plant extracts was studied here based on CBC data. In this study, a five-fold increase in white blood cell counts was reported in the positive control animals relative to the negative controls. The normal range of these cells in rats is 1.96–8.25 $\times 10^9$/L (Giknis, 2008). However, when the animals were treated with clove or cinnamon following 24 h or 14 days of infection, as well as in the prophylactic group subjected to either plant extract, average white blood cell counts significantly lower than that of the positive control but higher vs the negative control were reported. It has been proposed that greater lymphocyte release from lymph myeloid tissue and/or accelerated lymphopoiesis are the causes of an increased total white blood cells (Das & Mukherjee, 2003). The elevated count of these cells may also represent a low-level inflammatory response (Chmielewski & Strzelec, 2018). Both cinnamon and clove may be useful in markedly increasing the total white blood

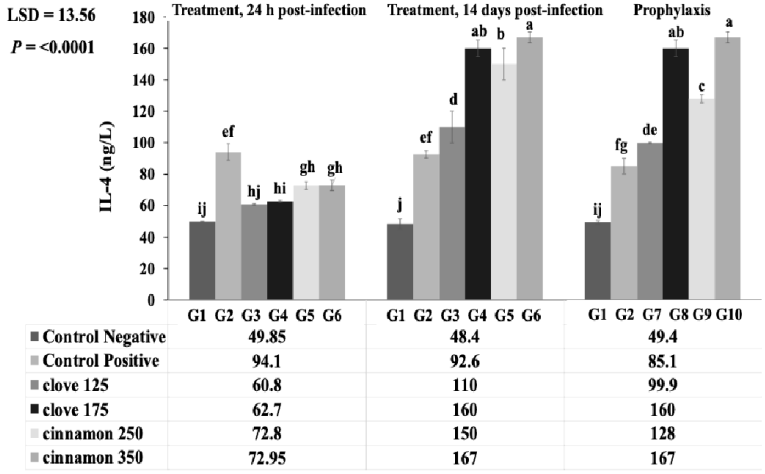
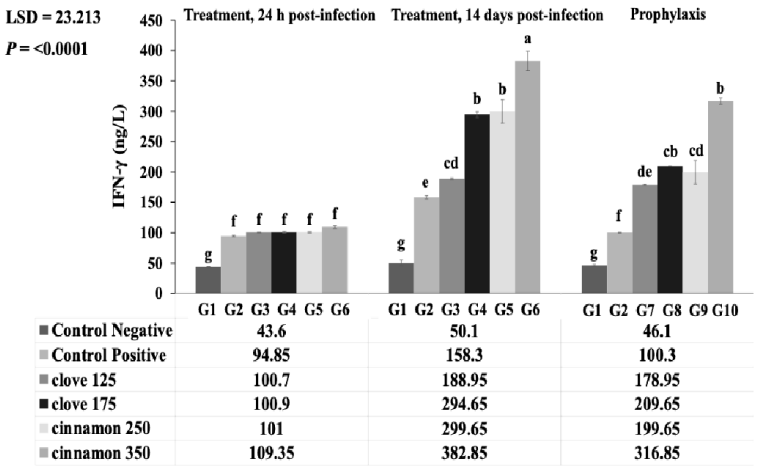


Fig. 5. Serum IL-4 (ng/L) in rats infected with *K. pneumoniae* and given clove or cinnamon extracts as a treatment or prophylaxis. Ten groups (each with 6 rats) are shown: the control groups included G1 (negative control, PBS only) and G2 (positive control, *K. pneumoniae* infection). The treatment groups (G3 to G6) were infected firstly with *K. pneumoniae,* and then treated with clove at 125 mg/kg bw (G3), clove at 175 mg/kg bw (G4), cinnamon at 250 mg/kg bw (G5), or cinnamon at 350 mg/kg bw (G6). Prophylactic groups (G7 to G10) were given firstly the plant extracts for 14 days, and then infected as mentioned above. They were administered clove at 125 mg/kg bw (G7), clove at 175 mg/kg bw (G8), cinnamon at 250 mg/kg bw (G9), or cinnamon at 350 mg/kg bw (G10). The bars represent means, and error bars indicate the standard error of the mean (SEM). Groups labelled with different small letters are significantly different ($P \leq 0.05$).



Control Negative	43.6	50.1	46.1
Control Positive	94.85	158.3	100.3
clove 125	100.7	188.95	178.95
clove 175	100.9	294.65	209.65
cinnamon 250	101	299.65	199.65
cinnamon 350	109.35	382.85	316.85

Fig. 6. Serum IFN-γ (ng/L) in rats infected with *K. pneumoniae* and given clove or cinnamon extracts as a treatment or prophylaxis. Ten groups (each with 6 rats) are shown: the control groups included G1 (negative control, PBS only) and G2 (positive control, *K. pneumoniae* infection). The treatment groups (G3 to G6) were infected firstly with *K. pneumoniae,* and then treated with clove at 125 mg/kg bw (G3), clove at 175 mg/kg bw (G4), cinnamon at 250 mg/kg bw (G5), or cinnamon at 350 mg/kg bw (G6). Prophylactic groups (G7 to G10) were given firstly the plant extracts for 14 days, and then infected as mentioned above. They were administered clove at 125 mg/kg bw (G7), clove at 175 mg/kg bw (G8), cinnamon at 250 mg/kg bw (G9), or cinnamon at 350 mg/kg bw (G10). The bars represent means, and error bars indicate the standard error of the mean (SEM). Groups labelled with different small letters are significantly different ($P \leq 0.05$).

cell counts (Sugihartini *et al.,* 2019).

In the present study, the lymphocyte percentage was significantly higher in the infected group (positive control) than in the treatment and the prophylactic groups. The increase in lymphocytes occurred because of the immune response against the bacterial infection (Cox *et al.,* 2011). The plant extracts at different concentrations and in various groups caused an increase in the lymphocyte percentage compared to the negative control. The increased white blood cells count is believed to be the reason behind the enhanced lymphocyte release from myeloid tissue lymph glands (Cox *et al.,* 2011). Monocytes also showed increased percentages in all treated groups as well as in the positive control compared to the negative control of this study. It has been proposed that a high percentage of monocytes (relative monocytosis) occurs because of the direct involvement of these cells in the phagocytosis of organisms or foreign particles (Araújo *et al.,* 2015).

Regarding red blood cells, no significant differences were observed between their counts in the negative control animals and those given cinnamon or clove as a treatment or prophylaxis. Their counts in all groups were within the normal range of red blood cells in rats, where they account for 7.27–9.65 $\times 10^{12}$/L (Giknis, 2008). Erythrocytes are important for immune system maintenance because when bacteria or pathogens lyse erythrocytes, haemoglobin produces free radicals that damage the pathogen's cell membranes (Pillaia *et al.,* 2011). This might be the cause behind the slight (not signifi-

cant) decrease in the counts of red blood cells in the treated groups relative to the negative control. The haemoglobin and haematocrit showed no significant difference among the various study groups. Their levels were found to directly depend on the changes in red blood cells (Shabnam, 2009). However, the findings of the present research also revealed a significant increase in the mean corpuscular volume in the groups treated 24 h post-infection and the prophylactic groups compared to the negative control. Increased mean corpuscular volume is associated with macrocytic anaemia (Longe *et al.,* 2015). Nevertheless, the groups treated with the plant extracts for 14 days post-infection showed levels similar to the negative control indicating the absence of anaemia in these groups in comparison with the prophylactic groups. Moreover, the current study revealed that the mean corpuscular haemoglobin levels were roughly similar within and among the groups of rats. Because increased mean corpuscular haemoglobin values are indicative of macrocytic anaemia (Longe *et al.,* 2015); thus, the plant extracts used in the present study are not causing anaemia. Pertaining to the mean corpuscular haemoglobin concentration value, this study demonstrated no significant differences among the different groups of rats. These results are consistent with those of Longe *et al.* (2015). Furthermore, the current study found that the higher concentrations of the plant extracts had a negative effect on the platelets than the lower concentrations. Platelets help innate immunity in a variety of ways. Interactions between bacteria and platelets cause platelet activation and antimicrobial peptides secretion (Morera & MacKenzie, 2011).

Regarding granulocytes, the positive control group had a higher percentage than the other groups. Mature granulocyte levels often increase with illness or infection (Chmielewski & Strzelec, 2018). Interestingly, the plant extracts were successful in decreasing the percentage of granulocytes in the treated and prophylactic groups relative to the positive control. Concerning plateletcrit, it became a promising diagnostic marker for identifying bacterial infections because their levels are higher in individuals with bacterial infections than in those with viral infections or non-specific inflammatory disorders (Nishikawa *et al.,* 2016). This is consistent with the results of this study, where all groups given plant extracts either as a prophylaxis or treatment for 14 days post-infection showed a significant decrease in plateletcrit compared to the infected group. Therefore, clinical judgments on the start and stop of antibiotic therapy for bacterial infection may be supported by plateletcrit levels (Schuetz *et al.,* 2012). Nevertheless, the plant extracts had no effects on the plateletcrit following 24 h of infection relative to the positive control. This means that the short time of treatment with these plant extracts was not sufficient to eliminate the bacterial infection. This study showed that the mean platelet volume was higher in the infected groups than in the other groups. Importantly, using the plant extracts as a prophylaxis or treatment for 14 days post-infection was successful in rendering the mean platelet volume to the normal status. This finding means that the plant extracts were able to prevent sepsis caused by *K. pneumoniae* infection, in agreement with the results of Golwala *et al.,* (2016).

When studying the effects of the plant extracts on the animals' immunity, positive impacts have been reported in the current study. Using cinnamon and clove caused induction of the humoral and cellu-

lar immune response by elevating IL-4 and IFN-γ. This finding agrees with that of Niphade *et al.* (2009) who showed that cinnamon at a high dose increased both cell-mediated and humoral immunity, while at low doses showed impacts on the humoral immunity only. Furthermore, cinnamon extract was suggested to control the expression of pro- and anti-inflammatory genes to influence immune responses (Cao *et al.,* 2008). Concerning clove, Dibazar *et al.* (2014) demonstrated that it had varying immunomodulatory properties. The same researchers observed that splenocytes treated with clove extracts at different concentrations (0.1–1000 μg/mL) showed induction of IL-4 and IFN-γ, which has been verified to play an important role in the protection against *K. pneumoniae* infections in mice. Eventually, despite all the positive impacts exerted by the studied plant extracts, this study had some limitations. For instance, the present study, conducted on small mammals (rats), may not accurately reflect the clinical status of farm animals. Furthermore, comprehensive research on farm animals necessitates substantial funding, which is challenging to secure.

CONCLUSION

Both clove and cinnamon extracts significantly enhanced the immune system of rats by improving their blood profile. Each of these extracts exhibited notable anti-inflammatory properties and caused an increase in white blood cell counts, lymphocyte percentage, monocyte percentage, IL-4 as well as IFN-γ serum levels. These enhancements collectively demonstrate their potent immunomodulatory effects against *K. pneumoniae* infection.

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