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Original Research Article

Effect of *Cinnamomum burmannii* Bark Oil on Bacterial Count, Nuclear Factor of Kappa Beta (NF-K*β*/p65), Interleukin-6, and Chest Radiography in *Rattus norvegicus* with Pneumonia

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ABSTRACT

Pneumonia is the second most common lung infection caused by Multidrug-Resistant *Klebsiella pneumoniae*. This study aimed to analyze the effect of *Cinnamomum burmannii Bark oil* (*Cb*BO) as an antibacterial (decreasing bacterial counts) and anti-inflammatory agent through the inhibition of Immunoreactive Score Nuclear Factor Kappa Beta (IRS *NF-Kβ*/p65) and Interleukin 6 (IL-6), and chest radiography/CXR analysis of male Wistar rats' model *Klebsiella pneumoniae* (*Kp*). The experimental animals were divided into seven groups consisting of normal control group (C), negative control (NC, Kp 100 µL), Treatment 1 (T1: *Kp* + Carboxymethylcellulose sodium 3 mL), Treatment 2 (T2: *Kp* + Levofloxacin 13.5 mg/200 gram BW of rats), Treatment 3-5 (T3, T4, T5: Kp+ *Cb*BO 0.36; 0.72; and 1.44 µL/200 gram BW of rats). The treatment was given for the first, second, and third days post-inoculation. All parameters on day 4 were examined. The bacterial count, IRS NF-K*β*/p65, and IL-6 are parametric data evaluated using Kruskall Wallis and post hoc tests. The CXR, a non-parametric data, was analysed with a chi-square test. The results showed that T3 and T4 decreased in all parameters. There was a significant difference test between NC compared T4 on the bacterial count (p-value=0.033), NC compared T3 (*p*-value=0.05), and T5 (*p*-value=0.037) on the IRS NF- K*β*/p65, and T1 compared T4 (*p*-value=0.045) on CXR. This study concluded that *Cb*BO doses of 0.36 and 0.72 µL/200 gram BW of rats reduce all parameters.

***Keywords*:** antibacterial, anti-inflammatory, *Cinnamomum burmannii bark oil*, pneumonia, wistar rats .

Introduction

Pneumonia is an infection or acute inflammation of the lung tissue caused by microorganisms, such as bacteria, viruses, parasites, fungi, and exposure to chemicals or physical damage to the lungs. However, it often affects people with low immune systems, especially children and older people. Based on a retrospective study in America of 25,000 subjects with community-acquired pneumonia (CAP), those over 65 years old have a 14.7 times higher risk of developing CAP than those under 45 years old. In 2015-2018, confirmed cases of pneumonia in children under five years of age increased by 500,000 per year, reaching 505,331 patients, 425 of whom died.[1] Pneumonia is divided into three types based on the source of infection, namely (CAP), hospital-acquired pneumonia (HAP), and ventilator-associated pneumonia (VAP). However, CAP is the most common, with the highest incidence of mortality.

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Klebsiella pneumoniae (*Kp*) is a gram-negative bacterium belonging to the family Enterobacteriaceae, which most often causes multidrug resistance (MDR) community-acquired pneumonia (CAP). Over the past few years, there has been an increase in infections caused by resistant *Kp*, causing severe consequences, especially in the hospital setting, such as more extended hospital stays, lack of treatment options, and increased morbidity and mortality rates.[2] The incidence of CAP caused by *Kp* from eight countries in Asia demonstrated a mortality rate of 28 days for CAP and nosocomial pneumonia, accounting for 27.9% and 36.9%, respectively. *Klebsiella pneumoniae* in CAP has the highest proportion of hypervirulent strains (*HvKp* strains).[3] *In vitro* and *in vivo* studies involving different antibiotics, herbal ingredients, or combinations of antibiotics and herbal ingredients have been conducted to mitigate the MDR nature of *Kp*. Extracts of *Cinnamomum Cassia* with anti-inflammatory activity and used in treating wounds have also been investigated.[4]

Studies on *Cinnamomum burmannii* Bark oil (CbBO) showed that it has antimicrobial properties and could benefit health when used as an alternative antibacterial agent in medical applications and antibacterial supplements in health products. Its use could lead to reduced drug costs, with no acute or chronic toxicity, mutagenicity or genotoxicity, and no carcinogenicity detected in mammalian studies.[4,5] *Cinnamomum burmannii* Bark oil has the highest concentrations of Cinnamaldehyde (65-80%) and Eugenol (5-10%) compared to other herbal essential oils, which exhibits antibacterial and anti-inflammatory properties.[6,7] Eugenol causes disruption of the cytoplasmic membrane, increases nonspecific permeability, causes ion leakage, and excessive loss of other cellular components, including intracellular proteins, leading to cell death.[8] *Cinnamaldehyde* inhibits the ATPase enzyme and damages

the outer cell membrane.[5,8] The authors reported that cells exposed to CbBO expressed higher levels of oxidative stress, damaging the bacterial membrane through interactions with the lipid bilayer.[2,5]Several studies on the antibacterial effect of *Cb*BO are *in vitro* studies that examined its MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration). Low MIC and MBC values indicate that *Cb*BO has potent antibacterial activity.[8–10] The study on the anti-inflammatory effect of *Cb*BO was done using *in vivo* assay methods, where *Cb*BO was administered topically on the thighs of edematous rat models. The results of the studies showed that administering *Cb*BO as a topical medication to edema wounds reduced the size of the lesions [11,12]

This study aims to analyze the inhibitory effect of *Cb*BO on bacterial counts and the expression of NF-K*β*/p65/Immunoreactive scores (IRS), Interleukin-6, and chest radiography of rats with pneumonia. The novelty of this study is to identify the effect of *Cb*BO as an antibacterial and anti-inflammatory agent against pneumonia caused by *Kp in vivo*, not previously in the literature. The study involves direct inhibition of bacterial growth or damage to bacterial structures (cell walls, cytoplasm, cell nucleus, bacterial metabolism) and the expression of NF-K*β* with the reduction in the expression of pro-inflammatory cytokines (namely IL-6) [5,13] with an associated reduction in lung damage which occurs due to an inflammatory process (infiltration of inflammatory cells), which showed on chest radiography images as consolidation of macrophages and polymorphonuclear cells in the lung parenchyma in the form of opaque lesions or infiltrate. [14–16]

Materials and Methods

Ethical Approval

Ethical approval for this study was obtained from the ethics committee of the Universitas Muhammadiyah Yogyakarta, Yogyakarta, with Ethical Clearance Ref. No.: KE/FK/0342/EC/2023.

Plant Material and *Cb*BO

The *Cinnamomum burmannii* Bark was collected from Merapi Farmaherbal in March 2022. It was identified by a botanist at the Pharmaceutical Biology department, Gadjah Mada University, Yogyakarta, with voucher number 11.27.7/UN1/FFA.2/E1/PT/2022.

Preparation of Cinnamomum burmannii Bark Oil/CbBO

*Cb*BO was extracted by hydrodistillation using the method described previously.[17,18] Briefly, the Cinnamon (*Cinnamomum burmannii*) bark (3 kg) was ground using a mortar and pestle. *Cinnamon bark essential oil* is made using the hydrodistillation method. Hydrodistillation is a direct distillation method where the raw materials used are in direct contact with the heated solvent. The hydrodistillation method is often referred to as a distillation method by boiling, the method is to soak the plant (in this case cinnamon bark) in a large vessel, then boil the vessel and its contents. The equipment used includes a heat source (fire), a condenser which will convert the steam from the vessel into liquid, and a bottle to collect the condensate which also acts as a separator between oil and water.[19] The oil yield (*rendeman*) (15 mL) was recorded.[20]

Analysis CbBO Using Gas Chromatography-Mass Spectrometry (GC-MS)

The GC-MS analysis of *Cb*BO was done at the UGM Mathematics and Natural Sciences (MIPA) Laboratory, Yogyakarta, using an Agilent 7890A GC System equipped with an inert MS 5975C MSD XL EI/CI detector with a circulating automatic sampler. A CombiPAL automatic autosampler (Bruker) was used for this experiment with an ionization energy of 70 eV to detect electron ionization. Helium gas was used as a carrier with a constant 1 mL/minute flow rate. The temperature was set at 270°C with the capillary column specifications used being Agilent 19091S–433: 1548, 52849 HP-5MS 5% Phenyl Methyl Silox 30 m 250 µm × 0.25 µm HP-5MS. The oven temperature was programmed from 80°C to 300°C, with a dilution (1/100 v/v, in Hexane) of 2 µL injected. [19] GC-MS results showed Cinnamaldehyde (86.05%) and Eugenol 0.36%) as the major components.

Dilution and Calculation of CbBO Doses

*Cb*BO dosage, based on the Food Drug Association (FDA) specification, was 4-6 grams/day for adults.[21] The *Cb*BO was diluted with Carboxymethylcellulose sodium (Na CMC). The doses administered were computed based on the experimental animals' average body weights (200 g). The calculation resulted in 4 doses: 0.36 µL/ 200-grams BW Rat Wistar; Dose II: 0.72 µL/ 200-grams BW Rat Wistar, and Dose III: 1.44 µL/ 200 gram BW Rat Wistar.[22]

Making Model Rats and Treatment of Wistar Rats

The experiment involves a pre-test-post-test control group with thirty-five male Wistar rats, aged 6-8 weeks and weighing 200-250 grams, that meet the inclusion and exclusion criteria. The rats were inoculated intranasally with 100 µL of 0.5 McFarland *Klebsiella pneumoniae* solution using a 1-100 µL micropipette without anaesthesia *Kp* solution from patient isolates at the Yogyakarta City Regional General Hospital (RSUD Yogyakarta) to induce pneumonia.[22,23] The inoculation process was carried out at the Biosafety Level 3 Laboratory, Institute of Life Sciences and Engineering, Airlangga University, Surabaya, Indonesia. The animals were divided into seven groups: normal control group (C), negative control (NC, *Kp* 100 µL), Treatment 1 (T1: *Kp* + Na CMC 3 mL), Treatment 2 (T2: *Kp* + Levofloxacin 13.5 mg/200-gram BW of rats), Treatment 3-5 (T3, T4, T5: *Kp* + CbBO 0.36; 0.72; and 1.44 µL/200-gram BW of rats). Before treatment, the animals were acclimatized for 2 weeks at the BSL-3. The experimental animals were certified healthy with a health certificate issued by the East Java Animal Husbandry Service Animal Hospital Work Unit Veterinarian. During the acclimatization and treatment periods, the animals were fed with standard pellets (15-25 grams per day) and had free access to sterile water (50 -100 mL per day). The animals were housed in plastic cages at room temperature (25-28℃) and relative humidity (40-60%) and exposed to 12-hour light and dark cycles.[23,24] The normal control (Group C) was sacrificed on day 0 post-treatment and the treatment groups on day 4 post-treatment.

Rat Interleukin 6 ELISA Kit

This experiment employed a Rat IL6 ELISA Kit (Quantitative Cat#E0135Ra, size 96T, Bioassay Technology, China) designed to quantify Rat IL6 protein in serum, plasma, and tissue homogenates. The Assay time is 2.0 h, and the detection range is 0.1-40 ng/L with 0.052ng/L sensitivity. Enzyme-linked immunosorbent assay (ELISA) IL-6 at the ITD Airlangga University.

Immunohistochemistry (IHC) and NF-Kβ Antibody Assay

Immunohistochemistry was carried out with a BSM-33117M kit (100 µL, Bioss USA). The antibodies used included an antibody against the p65 (7G6) subunit of NF-k*β* (BIOSS, USA), a monoclonal Antibody. The examination of NF-κ*β*/p65 was carried out at the Pathology Anatomy Laboratory of Medicine Faculty of Airlangga University.

Bacterial Count

After treatment, the animals were sacrificed, and the main bronchus were surgically removed and divided for microbial examination at the Microbiology Laboratory of the Institute of Tropical Diseases (ITD) Airlangga University. Examination of germ numbers from lung tissue specimens (main bronchi) was carried out at the ITB UNAIR microbiology laboratory. Then calculate the number of germ colonies using the total plate count method, spread method on Mc Conkey media with gradual dilution. The number of germ numbers is calculated using the formula for the number of colonies on McConkey media times the dilution factor. [24]

Radiological Study

Radiological examination was carried out at the veterinary teaching hospital (RSHP UNAIR). Radiology imaging of the rats lung, the rats were anaesthetised with 0.2 ml of Xylasil: Ketamine (3:7) before being placed on a radio-graphic sensor (Krystal X easy digital, Owandy software) at a distance of 80 cm from the X-ray source.[25,26] Chest radiographic assessment was done by using an X-ray machine (Intra Scan DC Skan Ray, with IV- 1 phase 230 V, IF- 50 Hz, Momentary &

Stand by current -0.25A, Made in India) with a 48 kVp exposure for 0.5 mAs.

Determining the Suitability of Preparation Reading Assessment (Kappa Test)

Readings for assessing the degree of inflammation of IRS NF-k*β*/p65 expression. Histopathological examination and chest radiography were carried out by two examiners who are experts in their fields (anatomical pathology specialist and veterinarian/radiologist). Before reading/assessing the degree scores in preparation, Kappa coefficient qualification is carried out to determine the suitability of the assessments of reader/rater I and reader/rater II. Kappa value = 0.918 with p value: 0.000 ($p<0.05$) which means that the level of balance between examiner 1's results and examiner 2's results is very strong and statistically significant, so the results of this study do not contain any bias or subjectivity on the part of the researcher.[27]

Data Analysis

The data are presented as the Mean ± SD. The bacterial count, IRS NF-K*β*/p65, and IL-6 are parametric data with the Kruskall Wallis test and post hoc, and CXR is non-parametric data with chi-square test using laptop Asus ZenBook14, with SPSS IBM 22 software. In all the groups, differences were considered statistically significant at $p < 0.05$. Excel 2016 was used to obtain the graphs.

Result and Discussion

Plants have been known to play a significant role in man's healthcare since time immemorial, particularly in the last decade, attaining significant recognition in both developed and developing countries. Extracts of medicinal plants have exhibited different pharmacological activities, including anti-inflammatory, antibacterial, antidiabetic, and anticancer, and some have been used as insect repellants, etc. Community-acquired pneumonia has been recognized for its severity and the most common cause of pneumonia-related deaths. *Klebsiella pneumoniae* (*Kp*) is a Multidrug Resistance (MDR) gram-negative bacterium belonging to *Enterobacteriaceae*, which most often causes CAP. Over the past few years, there has been an increase in infections caused by resistant *Kp*, causing severe consequences, especially in the hospital setting, leading to longer hospital stays, lack of treatment options, and increased morbidity and mortality rates.[5] The incidence of CAP caused by *Kp* from eight countries in Asia revealed a mortality rate of 27.9% and 36.9%, respectively, for CAP and nosocomial pneumonia caused by *Kp*. Pneumonia or lung parenchymal infection due to *Kp* produces endotoxin and lipopolysaccharide, bound in Pathogen Associated Molecular Patterns (PAMPs) and triggering Tall Like receptor 4 (TLR-4), which affects the My-88 adapter, triggering NF-κ*β*/p65 for translocation into the nucleus. This NF-K*β* translocation will increase intracellular proinflammatory cytokines, i.e., TNF-α, IL-6, IL-8, and IL-1β. This prolonged inflammatory process will cause histological changes in the lung parenchyma and chest radiographic changes. The presence of *Kp* that enters the lung parenchyma causes an increase in the count of bacteria.[14,15]

Statistical analysis results of the effects of administration of CbBO to experimental animals infected with *Kp* are shown in Tables 1-2. Results of this study show that CbBO exhibited antibacterial (reduction in bacteria count) and anti-inflammatory activity by down-regulation of IRS NF-K*β* and IL-6. The antibacterial and anti-inflammatory effects of CbBO are believed to be through two mechanistic pathways: the first is direct inhibition of bacterial growth or structural damage (cell walls, cytoplasm, cell nucleus, bacterial metabolism) through partial degradation of the bacterial cell wall, increased membrane permeability, leakage of cytoplasmic material, bacterial cell shrinkage and prominent distortion, and changes in secondary, and tertiary bacterial protein architecture[12] and by inhibiting NF-κ*β* which automatically influences the expression of pro-inflammatory cytokines.[5,28]

The differences in the average values of the bacterial count, IRS NF-K*β*, and IL-6 are shown in Table 1. The Kruskall-Wallis Test shows a strong agreement and statistical significance in the data set with $p<0.05$. There is an indication that the extract reduces macrophage activation and lung inflammation induced by *Kp* infection by inhibiting the NF-K*β* signalling pathway. Accordingly, the data was subjected to the Mann-Whitney Test, as shown in Table 2. The table shows the test results difference between groups that showed significant results regarding the administration of the CbBO dose from the parameters examined, namely: (1). Bacterial count results from the normal control group (healthy rats) and the group infected with *Kp* (pneumonia rats) which received CbBO at a dose of 0.72 µL /200-gram BW Wistar Rat (group C vs T4); (2). IRS NF-K*β*/p65 results in the negative control (NC) group (untreated pneumonia rats), with the group of pneumonia rats that received CbBO at a dose of 0.36 microliters/200-gram BW of rat (group NC vs T3) and a group given CbBO at a dose of 1.44 µL/ 200-gram BW Wistar Rat (group NC vs T5). (3). The results of IL-6 expression in the pneumonia rats group that received 3 mL of Na CMC solution (group T1) compared to the group of pneumonia rats that were given CbBO 0.72 µL/200-gram BW Wistar Rat (group T1 vs T4) and the group of pneumonia rats that were given standard antibiotics, Levofloxacin 13.5 mg/200 gram BW of rat (group T2) compared to the group of pneumonia rats given CbBO at a dose of 1.44 µL/200-gram BW (group T2 vs T5). The results (Table 2) show a significant difference in bacterial count between the normal/healthy rats and those of pneumonic rats that received CbBO at a dose of 0.72 µL/200-gram BW (group T4). However, there was a noticeable presence of bacteria growth in the T4 group, evidenced by the presence of *Kp* in the lung tissues compared to the normal control (group C) without infection. The lung is part of the lower respiratory tract, usually free from microbial growth, unlike the upper respiratory tract, including the nose and the skin around it. The normal flora that grows in this area is *Staphylococcus aureus*,

Table 1: Test for the difference in average values (mean) with the Kruskall Wallis Test on bacterial count, IRS NF-K*β*, and Interleukin 6)

Group	Bacterial count Colony Forming Unit/CFU/ml)	*P* value	IRS NF-K*β*/p65 *(Score 1-12)*	*P* value	Expression of IL-6 (µL/ml	P value
C	0.00 ± 0.00		1.00 ± 0.00		4003.80 ± 2605.19	
NC	100.00 ± 0.00		9.00 ± 0.00		6192.60 ± 930.72	
T1	97.60 ± 4.34		8.20 ± 1.30		5957.00 ± 463.48	
T2	40.03 ± 54.76	0.034*	4.00 ± 1.90	0.003	4243.40 ± 921.85	0.011*
T3	22.60 ± 43.44		6.00 ± 1.90		4460.80 ± 172.13	
T4	412.00 ±92.00		5.00 ± 0.70		4100.00 ± 352.28	
T5	26.200 ± 21.73		4.40 ±1.70		630.00 ± 346.43	

Note: Asterix sign (*): $p<0.05$, which indicates a significant relationship.

Streptococcus Sp, *Haemophilus*, *Neisseria*, *Candida, and Micrococcus*.[26,29]

The negative control group (Group NC) showed differences with the T3 and T4 treatment groups regarding NF-K*β* expression. The negative control group (pneumonic rats but untreated with CbBO) means that *Kp* will release NF-K*β* with iK*β,* which causes NF-K*β* translocation into the nucleus, triggering pro-inflammatory cytokines, including IL-6 with an increase in the NF-K*β*/p65 immunoreactive score (IRS). The group treated with CbBO (groups T3 and T5) shows a decrease because the extract inhibits the release of NF-K*β*/p65, with a corresponding decrease in pro-inflammatory cytokines, including IL-6.[27]

The T1 vs T4 group also showed significant differences in IL-6 parameters with $p<0.05$. Group T1 (pneumonic rats, given Na CMC solution) showed high levels of IL-6, whereas T4 that received CbBO showed decreased expression of IL-6, which means CbBO possesses potent anti-inflammatory effects.[16] Similarly, Figure 1 shows the bacterial counts from *Kp* isolate culture preparations on Mac Conkey's media using the Total Plate Count Spread method with multilevel dilution. The count was computed by multiplying the number of bacterial colonies growing in the petri dish by the dilution factor.

The CXR analysis employs the chi-square test in which the Chest radiography is the dependent variable with an ordinal measurement scale. While, the treatment group is the independent variable with a numerical measurement scale. The p-value of the relationship between giving CbBO and CXR gave significant results (p-value=0.000). *Kappa* value of chest radiography = 0.918 with p-value=0.000 ($p<0.05$), which means that the level of agreement between the two independent results was very strong and statistically significant. That is, the results of this study do not contain any bias or subjectivity on the part of the research.

The result of the antibacterial effects of CbBO extract in the pneumonic rats is shown in Figure 3a as a plot of bacteria count (CFU/mL) versus the treatment groups. The result showed that Levofloxacin as a standard drug significantly reduced the bacterial count (Group T4). CbBO at oral doses of 0.36 and 0.72 µL/200 g BW show a good reduction in bacterial count. On the other hand, Figure 3b demonstrates that pneumonic rats without treatment exhibit high IRS (Group NC and T1). Meanwhile, groups T1 and T2, given the standard antibiotic Levofloxacin, showed decreased IRS. Groups T3, T4, and T5, which received CbBO at oral doses of 0.36, 0.72, and 1,44 µL /200 g BW, showed a lower IRS reduction than the Levofloxacin treated group (T2). Figure 3c Interleukin-6 showed an increase in the group inoculated with *Kp* without any treatment (Group NC) and Group T1, which received 3 mL orally of Na CMC on days 1, 2, and 3 post *Kp* inoculation. Groups T3 and T4, given CbBO at doses of 0.36 and 0.72 µL/200 g, showed a decrease in IL-6 values. Group T5, given CbBO 1.44 µL/200 g BW, resulted in an increase in IL-6 compared to Group NC and Group T1. Figure 3d shows the CXR scores in the pneumonic rats treated with Na-CMC solution (diluent) and the groups without treatment. The effect of standard antibiotics on reducing the CXR score is also indicated by improvement in the lesions in the infected animals. Groups T3 and T4 showed that the lesion improved with a decreasing score. T5, with the highest dose of CbBO (1.44 µL /200 g BW showed an increased score, although this was lower than T3 with a CbBO dose of 0.36 µL /200 g BW.

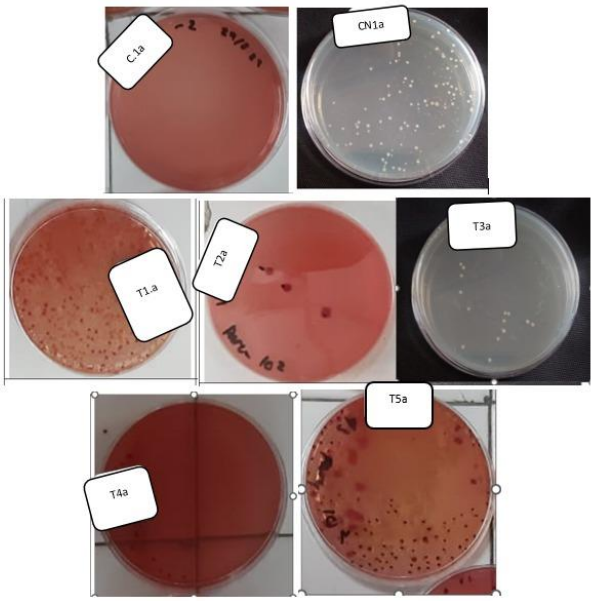

Figure 1: Culture results of lung tissue preparations on Mac Conkey's selective media (bacterial Count)
C1a: normal control (no bacterial growth); NCa: Negative control, rats were inoculated with *Kp* 100 µL (670,000 CFU/gram); T1a: Kp + NaCMC 3 ml po (950,000 CFU/gram); T2a: *Kp* + Levofloxacin 13.5 mg (300 CFU/gram); T3a: *Kp*+ CbBO 0.36 µL (14,000 CFU/gram); T4a: *Kp*+ CbBO 0.72 µL (900 CFU/gram); T5a: *Kp*+ CbBO 1.44 µL 7600 CFU/gram).

Table 2: Kruskall Wallis Test Between Groups, continued Post Hoc Mann Whitney if the *p* value is significant: Bacterial Count, IRS NF-K*β*/p65, IL-6

Group Pair	Bacterial Count *p* value	IRS NF-Kβ/p65 *p* value	IL-6 *p* value
C vs T3	1.000	1.000	1.000
C vs T4	0.033*	1.000	1.000
C vs T5	1.000	1.000	0.115
NC vs T3	1.000	0.05*	1.000
NC vs T4	1.000	1.000	1.000
NC vs T5	1.000	0.037*	1.000
T1 vs T3	0.760	0.935	1.000
T1 vs T4	1.000	1.000	0.045*
T1 vs T5	1.000	1.000	1.000
T2 vs T3	1.000	1.000	1.000
T2 vs T4	1.000	1.000	1.000
T2 vs T5	1.000	1.000	0.018*

Note: Asterix sign (*): The difference test between groups showed significant results

The results revealed that the administration of CbBO at doses of 0.36 µL/200 g BW and 0.72 µL/200 g BW showed a decrease in the bacterial count, CXR score, and inflammatory parameters (IRS NF-K/p-65 and IL-6). However, the administration of CbBO at a dose of 1.44 µL/200 g rats showed an increase in all parameters except IRS NF-K*β*/p-65, though this effect below that of the negative control group (Group NC and Group T1) except for IL-6, which had a titer above that of the untreated group (Group NC and Group T1). This may be due to conditions related to the pharmacokinetic process of drugs in the body (non-linear pharmacokinetics). It was expected that doubling the drug dose would be followed by an increase in the plasma drug concentration. However, the body's pharmacokinetic fate of drugs is influenced by absorption, distribution, metabolism, and excretion (ADME). Non-linear pharmacokinetic events are caused by enzyme saturation processes in the ADME process in drugs or herbal ingredients and pathological drug changes in the ADME process.[30,31]Several studies relating to non-linear pharmacokinetic effects demonstrated that administering antimicrobials by giving multiple doses with variations in the frequency of administration within 2-3 times a day showed a small effect. However, the more frequent administration in a day showed a better effect. An example of a high dose of Amoxicillin/Clavulanic acid tablets within a twice-daily regimen was less beneficial than a regimen with a lower dose but higher frequency of administration. [22,23]

This study reported that CbBO administration significantly reduced the expression of NF-K*β* at both the transcript and protein levels. [25] *Cinnamomum burmannii Bark Oil*, therefore, likely further attenuates inflammation following bacterial pneumonia via modulation of the intecan inhibiting the expression of proinflammatory cytokines, i.e., IL-6 and IL-1β, repairing damage to lung tissue from histopathological examination, and improving the degree of lesions on chest radiography. [14–16] Bacterial infection in pneumonia spreads through the respiratory tract until symptoms appear. The initial symptoms usually include an increase in body temperature of infection (the initial cause) lasting less than three days. The next phase is called (non-early presenters) with a time of more than three days. The results of this study showed that from 1 hour to 48 hours after bacterial inoculation, there was a drastic increase in bacterial growth, a systemic inflammatory reaction characterized by an increase in procalcitonin, C-reactive protein, inflammatory cytokines (IL-6, IL-1β) that occur in animal models in pneumonia.[14,31] Other research mentions that the initial infection process is followed by an increase in the IHC TNF-alpha and NF-K*β* Immuno Reactive Score examination.[15]

The decrease in the number of bacteria colonies indicates that CbBO contains an antibacterial containing Cinnamaldehyde, which inhibits the ATPase enzyme and damages the outer cell membrane *Kp*. It also contains Eugenol, causing disruption of the cytoplasmic membrane, increasing nonspecific permeability, causing ion leakage and excessive loss of other cellular components, including intracellular proteins, leading to cell death.[5,12]. As an antibacterial agent, Cinnamon bark oil has an anti-quorum sensing (QS) effect, a compound that interrupts QS and influences motility and biofilm formation. Quorum Sensing is an inter-cell communication system used by Gram-positive and Gram-negative bacteria based on the secretion and detection of external signaling molecules. Quorum sensing also affects motility and biofilm formation.[5,28]

Increasing the dose of CbBO causes an increase in bacteria colonies, possibly due to several factors. According to studies, it is stated that bacterial growth is influenced not only by the host or environmental conditions but also by bacterial resistance to CbBO. Bacterial colonization of organs is strongly influenced by internal and external factors.[8,28] According to research by El-Farmawi *et al*. (2021), the decrease in the number of bacterial colonies in organs is also influenced by the bactericidal effect of CbBO (The time-kill curve study showed that *Cinnamon*), which has an optimal bactericidal effect within 6-8 hours. Bacterial colonies will survive (survival rate <1%) within 4 hours after culture in the media, and some survive for more than 24 hours.[16]

Furthermore, the highest CbBO dose of 1.44 µL/200 g BW showed increased bacterial colony growth. It occurs because an overdose of an herbal medicine sometimes does not provide an optimal therapeutic effect but a counter effect. Thus, we must consider the synergistic nature of herbal ingredients as antibacterial and antimicrobial agents. This combination can increase efficacy, reduce toxicity, reduce side effects, increase bioavailability, reduce the required dose, and the emergence of antimicrobial resistance. In addition, we need to conduct further research regarding this combination of CbBO with antibiotics to overcome the MDR properties of *Kp*. The synergistic effect of combining *Cinnamomum cassia L* with Polymyxin B antibiotics has been tested on Carbapenemase-producing bacteria, namely *Klebsiella pneumoniae* and *Serratia marcenscens.* Administration of this essential oil within 4 hours affects oxidative stress and hence contributes to cell viability loss, revealing that the growth of these bacteria decreased. [28,32]

According to the Food and Drug Administration (FDA), the maximum dose of Cinnamon bark extract is 6 g/day or 6000 mg/day. Based on the calculation of the yield of CbBO 5 mL/1000 g, the maximum dose of CbBO in 200 g is 0.00054 mL or 0.54 µL. The doses of CbBO given in this study were 0.36 µL, 0.72 µL, and 1.44 µL. The dose of CbBO needed to reduce IL-6 is 0.72 µL, indicating that the effective dose of CbBO required for the treatment of pneumonia is between 0.36 - 0.72 µL/200 g BW. [31]

Based on several studies, CbBO has side effects of irritation to the skin, oropharynx mucosa, hepatotoxic reactions, and allergic reactions. Therefore, there is a need for caution in administration of CbBO, and attention should be paid to the dose and length of administration. It should not be given directly but dissolved or diluted with a carrier such as olive oil, coconut oil, or Na CMC solution used in this study.[28]

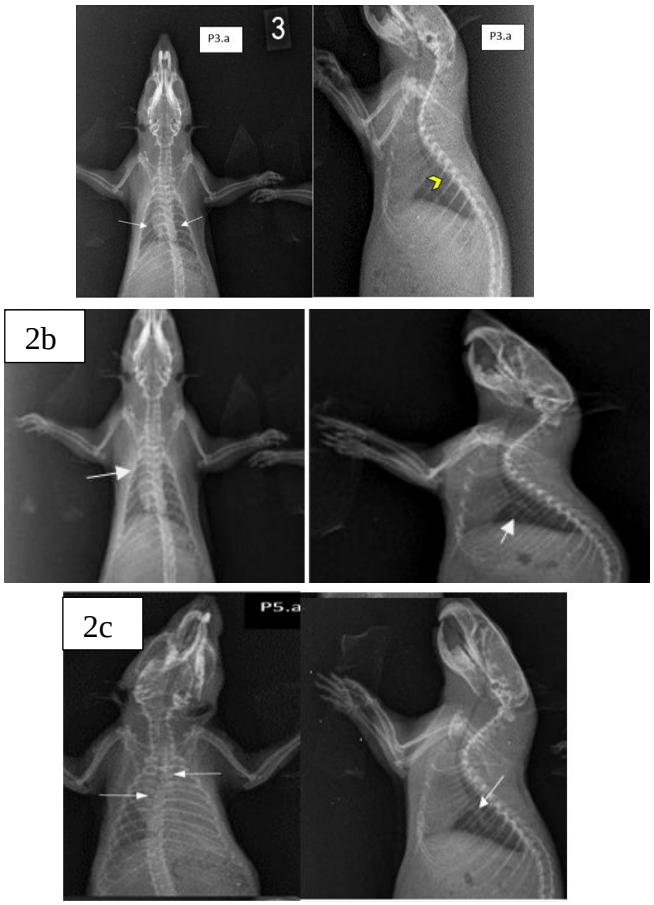

Figure 2a. Chest Radiography in the T3 (rat pneumonia+ CbBO 0.36 µL/200-gram BW). Visible faint consolidation at the right apical lung (Score 1); **2b**. **T4**: pneumonia rat). Opacity in the right lung, especially superior lobe: Pneumonia, (Score 1). **2c NC**. Opasity in the right lung and right posterior lobe (Score 4)

The score lesion inflammation CXR:
Score 0: Chest X-Ray is normal.
Score 1: Lesion of 1-2 lung segments affecting the right or left lung
Score 2: Lesion in 3 lung segments, affecting the right or left lung.
Score 3: Lesions > 2 lung segments involving the right and left lungs.

CbBO also inhibits proinflammatory cytokines such as IL-6, IL-1β, and Tumor Nuclear Factor-*α* (TNF-*α*), causing the repair of inflammatory lesions or edema of the alveoli. Chest radiography showed improved lesions and decreased consolidation in the right lung (Figure 3d CXR of the negative control group) (group NC), especially the superior lobe. After CbBO therapy (Group 5), it showed improvement and reduced lesions (score 1). Another studied the improvement of lung lesions on chest radiography using ginger extract (*Zingiber officinale rhizome*) in a rat pneumonia model with *Pseudomonas aeruginosa* inoculation. The group given ginger extract (Zingiber officinale rhizome) showed improvement as indicated by a reduction in lung lesions or consolidation.[34]

Conclusion

The results of this study conclude that the bioactivity of CbBO may be due to the phytochemicals it contains (mainly cinnamaldehyde and eugenol), which have been shown to exhibit antibacterial by reducing bacteria count and anti-inflammatory through the reduction of NF-K*β*/p65 and IL-6 expression with a decrease in chest lesions in the pneumonic rats as shown in the chest radiographs at CbBO doses of 0.36 and 0.72 µL/200 g body weight in Wistar rats.

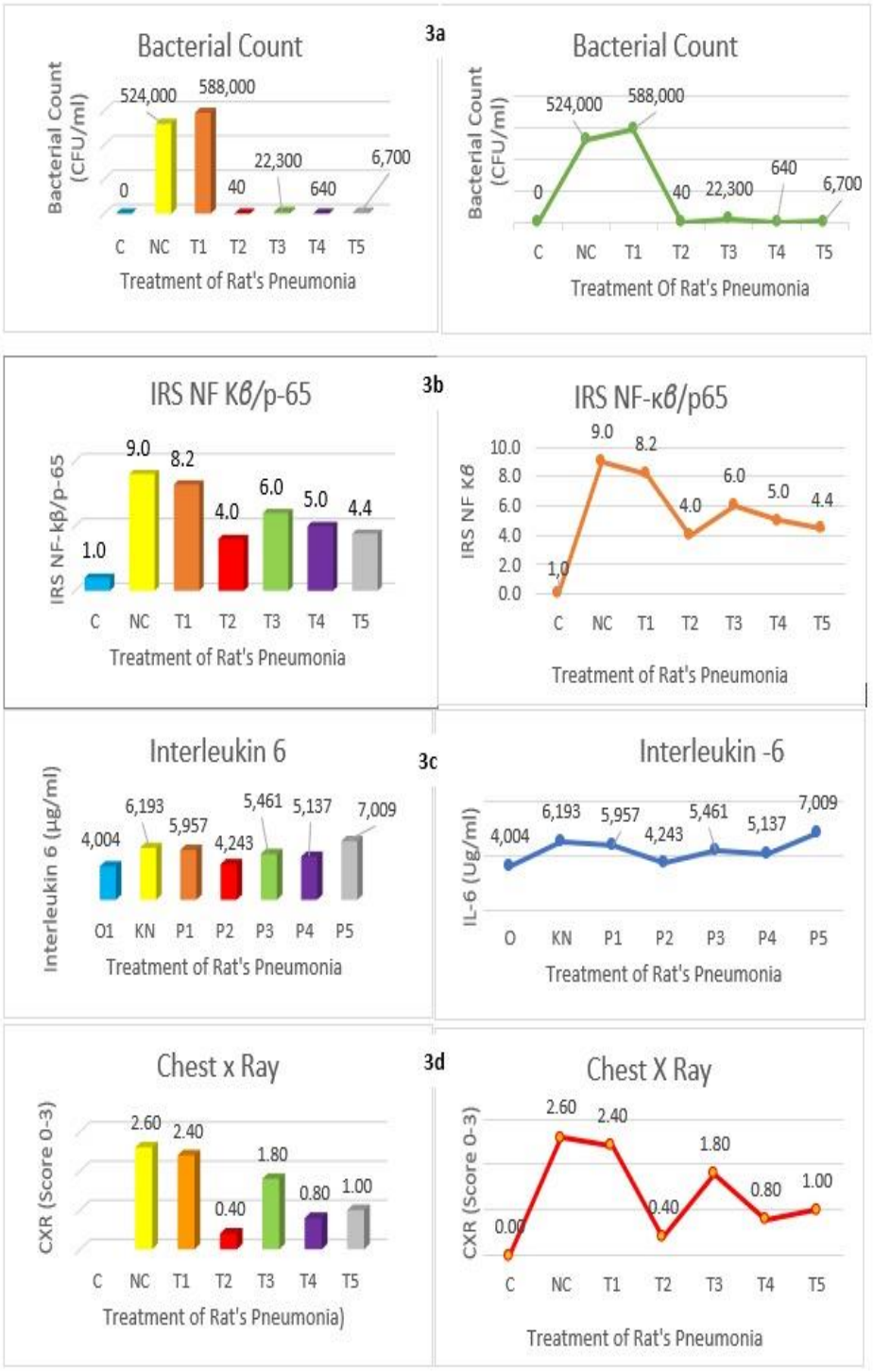

Figure 3: Graph of bacterial count (**3a**); Graph of Imuno Reactive Scores/IRS (**3b**); Graph of IL-6 (**3c**); Graph of Chest X-Ray/CXR (**3d**)

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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