Original Article

The Effect of *Curcuma longa* Extract on Interleukin 6, Procalcitonin, Microbial Count, and Histopathology of the Lungs in a Rat Model Infected with *Streptococcus pneumoniae*

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Abstract

Background: *Streptococcus pneumoniae* causes 95% of cases of community-acquired pneumonia (CAP). Interleukin 6, procalcitonin, microbial count, and lung histopathology can help in determining indicators of inflammatory processes and prognosis. Curcumin, which acts as an anti-inflammatory and anti-microbial, can be used as an adjunctive therapy in infectious diseases.

Methods: This was a laboratory experimental study. A sample of 30 white rats (*Rattus norvegicus*) infected with S*treptococcus pneumoniae* was carried out in the experimental animal laboratory at the Faculty of Medicine, Universitas Sebelas Maret in November 2022 with incidental sampling. The control group received 1cc of aquadest, the first treatment group received 30 mg/200 g of *curcuma longa* extract, the second treatment group received 50 mg/200 g of *Curcuma longa* extract, the third treatment group received 30 mg/200 g of *Curcuma longa* extract and 30 mg/200 g of amoxicillin, and the fourth treatment group received 30 mg/200 g of amoxicillin. Interleukin 6 and procalcitonin were measured on the third and twelfth days after the rats were infected with *Streptococcus pneumoniae*. The microbial count and histopathology of the lungs were assessed after the twelfth day.

Results: There was a significant difference (P<0.05) in the decrease in levels of interleukin 6, procalcitonin, and microbial count in the treatment group compared to the control group. There was no significant difference (P>0.05) in the improvement in the histopathology of the lungs in the treatment group compared to the control group.

Conclusion: *Curcuma longa* extract can significantly reduce levels of interleukin 6, procalcitonin, and microbial count, but not significantly improve the histopathology of the lung.

Keywords: histopathology of the lungs, interleukin 6, microbial count, procalcitonin, *Streptococcus pneumoniae*

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INTRODUCTION

Streptococcus pneumoniae (*S. pneumoniae*) caused 95 percent of pneumonia cases before antibiotics were invented. Pneumococci are the most commonly identified cause of community-acquired pneumonia (CAP).[1] Cillóniz et al stated that *S. pneumoniae* is the most common bacterium that causes CAP based on the season.[2]

Curcumin is the main component of the yellow pigment and the main bioactive substance in *Curcuma longa* (*C. longa*). Curcumin is a bright yellow pigment compound that has antimicrobial, anti-inflammatory, antioxidant, and antitumor activities.[3] Alikiaii et al conducted an in vivo study of the effect of curcumin on severe pneumonia using a curcumin formulation dissolved in water in rats inoculated with *Klebsiella pneumoniae* (*K. pneumoniae*). Curcumin administration resulted in a significant reduction in mortality, the number of bacteria in the blood and lungs, lung injury, inflammation, and oxidative stress.[4]

Interleukin 6 (IL-6) is involved in various hematopoietic, immune, and inflammatory responses because it enhances T cell differentiation through IL-2 induction.[5] Liu et al pointed out that the direct response to infection in IL-6 is better than C-reactive protein (CRP) and procalcitonin (PCT). PCT levels are increased in cases of bacterial infections and relatively low in viral infections. Procalcitonin levels can be used to differentiate between bacterial and viral infections. Interleukin 6 can be a predictor of treatment failure and death.[6]

Procalcitonin was first used as a marker of bacterial infection in 1993 when concentrations of immunoreactivity similar to calcitonin were detected at high levels in patients with extrathyroidal disease.[7] Levels of PCT decreased rapidly after antibiotic therapy, and calcitonin levels returned to normal in all subjects. Levels of PCT increase during bacterial septic conditions, and serum concentrations correlate with the severity of microbial invasion.[8]

Trans-nasal infection in rats induced with *S. pneumoniae* resulted in various tissue lesions and immune cell infiltration characteristic of aerogenic bacterial pneumonia. The infection model shows lesions extending to the periphery of the lung lobes with inflammation surrounding the airways and blood vessels.[9] Pneumococcal dissemination causes an initial immune response characterized by predominant intrabronchial and intra-alveolar neutrophil infiltration.[10]

The process of neutrophil infiltration triggers lobular suppurative bronchopneumonia with consolidation of the affected lung area. Necrotic coagulation and liquefaction are characterized by cellular fragmentation, decay, and loss of cellular detail, as well as accumulation of cellular debris with hemorrhage. The perivascular interstitium becomes more extensive with edema due to vascular leakage with massive extravasation of neutrophils into the perivascular space.[10]

METHODS

This was a laboratory experimental research. The researcher treated the sample that had been determined, namely the experimental animal in the form of a male white rat (*Rattus norvegicus*), in the laboratory. The research subjects were male Wistar rats (*Rattus norvegicus*) aged 3–4 months with a body weight of ±200 grams, with a population of 30 white rats. The research procedure has been ethically approved by the research ethics commission of the Faculty of Medicine, Universitas Sebelas Maret, Surakarta. The research was conducted at the Experimental Animal Laboratory, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, in November 2022.

The sampling technique was purposive sampling. The number of samples used was 30 male white rats for the induction of intranasal *S. pneumoniae*. The group consisted of 6 groups: the control group (K) received aquadest, while the treatment groups were infected with *Streptococcus pneumoniae* on day 5. Treatment group 1 was given *C. longa* extract at a dose of 30 mg/200 g (P1), treatment group 2 was given *C. longa* extract at a dose of 50 mg/200 g (P2), treatment group 3 was given *C. longa* extract at a dose of 30 mg/200 g and amoxicillin at a dose of 30 mg/200 g (P3), and treatment group 4 was given amoxicillin at a dose of 30 mg/200 g (P4).

Treatment is given on days 5 to 12. Interleukin 6 and PCT were measured on the third and twelfth days after the rats were infected with *S. pneumoniae*. The microbial count and histopathology of the lungs were assessed after the twelfth day. Microbial count was calculated using the Quebec colony counter in units of CFU/ml after 24 hours of incubation. Histopathological examination of the lungs was performed using hematoxylin-eosin staining, and lung injury was assessed using Marianti score. The Marianti score is used to determine the degree of lung damage based on the alveolar membrane, alveolar lumen, and interalveolar connections. Before blood sampling, induction of *S. pneumoniae*, and euthanasia, the rats were anesthetized using a combination of intraperitoneal injections of ketamine and xylazine.

We used the comparative analysis of unpaired different tests for >2 samples. The data were normally distributed and homogeneous; results were considered statistically significant at $P<0.05$. Followed by the Least Significant Difference (LSD) post-hoc test to determine the different groups. If the data distribution was not normal, the Kruskal-Wallis test was used, followed by the Mann-Whitney test to find out the different groups. We also used a comparative analysis of a paired difference test for 2 pre- and post- samples. The data were normally distributed and homogeneous, so the paired t-test

was used at a significance level of *P*=0.05 to find out the difference between the pretest and posttest. If the data distribution was not normal, the Wilcoxon rank test was used to find out the difference between the pretest and posttest.

RESULTS

Analysis of significance with One-Way ANOVA (Table 1) showed that the average serum IL-6 level in the pretest group was almost equivalent to the value of *P*=0.060. The mean IL-6 value of the group after drug treatment was different, with *P*=0.020, which means there is a significant difference from the average value of the group. In group 3, the combination of amoxicillin and *C. longa* extract reduced IL-6 the most in a rat model infected with *S. pneumoniae*.

Table 1. One-Way ANOVA Test Serum IL-6 Levels

Groups	IL-6 (pg/ml) (Mean±SD)			
	Pretest	Posttest	*P*	Difference
K	107.71±11.15	105.57±11.95	0.019	–2.14±1.52
P1	110.14±11.54	102.64±12.81	<0.001	–7.50±1.71
P2	112.94±7.68	98.08±7.60	<0.001	–14.86±2.81
P3	123.85±10.61	86.84±9.76	<0.001	–37.01±4.36
P4	113.88±2.89	92.81±4.33	<0.001	–21.07±1.91
P	0.060[a]	0.020		<0.001

Note: [a]ANOVA Test (unpaired difference test data meets normality assumption); [b]Paired t-test (paired difference test data meets normality assumption); *significant at *P*<0.05

The results of the post-hoc test (Table 2) also showed that between the treatment groups (P1, P2, P3, and P4), there were significant differences (*P*<0.05), which means that each treatment provided different effectiveness, where the one that reduced IL-6 levels the most was the combination group of *C. longa* extract and amoxicillin 30 mg/200 g (P3), while the one that reduced IL-6 the least was the *C. longa* extract group at a dose of 30 mg/200 g (P1).

Table 2. LSD Post-Hoc Serum IL-6 Levels

Comparison of Differences in Mean IL-6 Levels (pg/ml)	*P*
K (–2.14) vs P1 (–7.50)	0.002
K (–2.14) vs P2 (–14.86)	<0.001
K (–2.14) vs P3 (–37.01)	<0.001
K (–2.14) vs P4 (–21.07)	<0.001
P1(–7.50) vs P2 (–14.86)	<0.001
P1(–7.50) vs P3 (–37.01)	<0.001
P1(–7.50) vs P4 (–21.07)	<0.001
P2(–14.86) vs P3 (–37.01)	<0.001
P2(–14.86) vs P4 (–21.07)	<0.001
P3(–37.01) vs P4 (–21.07)	<0.001

Analysis of significance with One-Way ANOVA (Table 3) showed that the average serum PCT level in the pretest group was almost equivalent to the value of *P*=0.684. The mean PCT value of the group after drug treatment was different, with *P*=0.017, which means that there is a significant difference from the average value of the group. In group 3, the combination of amoxicillin and *C. longa* extract reduced PCT the most in a rat model infected with *S. pneumoniae*.

Table 3. One-Way ANOVA Test Serum PCT Levels

Groups	PCT (ng/ml) (Mean±SD)			
	Pretest	Post-test	*P*	Difference
K	558.22±37.62	540.17±42.55	0.004	–18.05±8.85
P1	564.26±39.87	513.55±36.44	<0.001	–50.71±7.76
P2	594.75±75.46	506.73±70.54	<0.001	–88.01±9.30
P3	600.16±63.99	411.81±78.22	<0.001	–188.35±42.39
P4	587.62±74.22	480.59±72.59	<0.001	–107.03±5.84
P	0.684	0.017		<0.001

The results of the post-hoc test (Table 4) also showed that among the treatment groups (P1, P2, P3, and P4), there were significant differences. This could mean that each treatment provided different effectiveness, where the one that reduced PCT levels the most was the combination group of *C. longa* extract and amoxicillin 30 mg/200 g (P3), while the one that reduced PCT the least was the *C. longa* extract group at a dose of 30 mg/200 g (P1).

Table 4. LSD Post-Hoc Serum PCT Levels

Comparison of Differences in Average PCT Levels (ng/ml)	*P*
K (–18.05) vs P1 (–50.71)	<0.001
K (–18.05) vs P2 (–88.01)	<0.001
K (–18.05) vs P3 (–188.35)	0.001
K (–18.05) vs P4 (–107.03)	<0.001
P1(–50.71) vs P2 (–88.01)	<0.001
P1(–50.71) vs P3 (–188.35)	0.003
P1(–50.71) vs P4 (–107.03)	<0.001
P2(–88.01) vs P3 (–188.35)	0.013
P2(–88.01) vs P4 (–107.03)	0.021
P3(–188.35) vs P4 (–107.03)	0.034

Analysis of significance with One-Way ANOVA (Table 5) pointed out that microbial count obtained a *P*-value of <0.001 (*P* <0.05). Microbial count after treatment in rat models infected with S*. pneumoniae* obtained significantly different results. In group 3, the combination of amoxicillin and *C. longa* extract

reduced microbial count the most in a rat model infected with *S. pneumoniae*.

Table 5. One-Way ANOVA Test Microbial Count

Group	Microbial Count (CFU/ml) (Mean±SD)	*P*
K	420.33±48.27	
P1	343.50±36.68	
P2	335.83±34.24	<0.001
P3	46.50±23.53	
P4	174.67±17.65	

The results of the post-hoc test also showed that the treatment groups (P1, P2, P3, and P4) had significant differences, meaning that each treatment had a different effectiveness. The group P1 vs. P2 obtained results that were not significantly different or had the same effectiveness at *P*>0.05. In this study, the group that reduced the number of germs the most was the combination of *C. longa* extract and amoxicillin 30 mg/200 g (P3), while the group that reduced the number of germs the least was the *C. longa* extract group at a dose of 30 mg/200 g (P1). Based on the description above, *C. longa* extract has the effect of reducing the microbial count in rats infected with S*. pneumoniae*, but it would be better if it were combined with amoxicillin.

Table 6. Kruskal-Wallis test for Marianti Lung Damage Score

Group	Score (Mean±SD)	*P*
K	2.67±0.52	
P1	2.50±0.55	
P2	2.50±0.55	0.972
P3	2.50±0.55	
P4	2.50±0.55	

Analysis of the significance of the average lung injury from the results of histopathological examination was carried out using the non-parametric Kruskal-Wallis test (Table 6). The Kruskal-Wallis test results obtained *P*=0.972. Lung histopathology results after treatment in a rat model infected with *S. pneumoniae* were not significantly different.

The results of the post-hoc test also stated that the treatment groups (P1, P2, P3, and P4) had no significant differences. Treatment with *C. longa* extract doses of 30 mg/200 g, *C. longa* extract doses of 50 mg/200 g, the combination of *C. longa* extract and amoxicillin 30 mg/200 g, and amoxicillin 30 mg/200 g in this study were less effective in repairing lung damage.

DISCUSSION

Streptococcus pneumoniae is a gram-positive bacterium also known as a pneumococcus. *Streptococcus pneumoniae* can survive under aerobic and anaerobic conditions.[11] *Streptococcus pneumoniae* caused 95% of pneumonia cases before antibiotics were discovered. Pneumococci are the most widely identified cause of CAP.[1] *Streptococcus pneumoniae* has several properties that make it undetectable by the host immune system and can survive against normal flora in the nasopharynx.[12]

Curcuma longa contains several secondary metabolites such as curcuminoids, sesquiterpenes, and steroids. Curcuminoid is the main component of the yellow pigment and the main bioactive substance.[13] Curcumin has different anti-microbial mechanisms, such as distribution of bacterial cell membranes, inhibitory action on bacterial DNA replication, decreased motility, and changes in bacterial gene expression.[1] Administration of curcumin causes a significant reduction in mortality, the number of bacteria in the blood and lungs, lung injury, inflammation, and oxidative stress.[14]

The results of this study are similar to a study conducted by Valizadeh et al, where patients were given 160 mg of nano-curcumin therapy for 14 days, and it showed a decrease in IL-1β and IL-6 expression compared to the control group. However, the administration of nano curcumin did not decrease the expression of IL-18 and TNFα compared to the control group. Administering nano-curcumin can reduce symptoms of fever, cough, and shortness of breath.[15] A study by Wang et al on rats infected with *Staphylococcus aureus* (*S. aureus*) revealed that *C. longa* extract reduced mortality in rats that were observed for 3 days.[16]

Trigo-Gutierrez et al have examined the antimicrobial effect of *C. longa* extract, which is effective for both gram-positive and gram-negative bacteria and infections in humans that show antibiotic resistance. The mechanism of *C. longa*'s antimicrobial action involves damage to cell walls

and cell membranes, interference in cellular processes by targeting DNA and proteins, and inhibition of bacterial quorum sensing. This mechanism can, in turn, lead to bacterial cell death.[17] Adeyemi et al study indicated that *C. longa* has been shown to affect L-tryptophan activity in gram-positive bacteria but not in gram-negative bacteria, resulting in lipid peroxidation and increased DNA fragmentation in both bacteria.[18]

The results of this study were similar to a study conducted by Krausz et al on rats infected with *Pseudomonas aeruginosa* (*P. aeruginosa*) with *C. longa* extract therapy at a dose of 25 mg, which explained a decrease in the number of microbial counts compared to the control group.[19] Research by Song et al on rats infected with *Escherichia coli* (*E. coli*) with *C. longa* extract therapy at a dose of 20 mg showed a decrease in the number of microbial counts compared to the control group.[20] This proves that *C. longa* extract has antimicrobial activity by reducing the number of microbial counts.

Streptococcus pneumoniae appears clearly as bluish to purple dots measuring approximately 1 micrometer (µm) in standard HE. *Streptococcus pneumoniae* is mostly located on the pleural surfaces, mediastinal adipose tissue, and within the pulmonary perivascular spaces. Kosai et al investigated the immunological mechanisms underlying the virulence of *S. pneumoniae* influenza virus co-infection using pneumonia rats.[21] The study by Hraiech et al reported that rats inoculated with influenza virus, followed 2 days later with *S. pneumoniae* inoculation, had been associated with increased mortality compared with a single infection with influenza virus or *S. pneumoniae*. The mechanism occurs due to decreased neutrophil function when there is an influenza virus infection before the occurrence of S. pneumoniae infection.[22]

LIMITATION

The limitations of this study were the short interval of administration of *C. longa* extract, the absence of a healthy control group, and the absence of other biomarkers. Longer intervals of *C. longa* extract administration are needed to obtain optimal results to determine the repair effect of lung damage based on lung histopathology. The addition of a healthy control group is needed to find out the results of the comparison between the treatment group and the healthy group, so that it is more objective. The addition of other biomarkers is needed to provide more accurate results regarding the role of *C. longa* extract in this study.

CONCLUSION

Curcuma longa extract had a significant effect on reducing IL-6, PCT levels, and microbial count in a rat model infected with *S. pneumoniae*. *Curcuma longa* extract had no significant effect on the improvement of lung histopathology in a rat model infected with *S. pneumoniae*. Further research can be carried out to find out more about the right dose of *C. longa* extract so that it has an effect comparable to amoxicillin, administration of *C. longa* extract earlier after the pretest data is taken, longer duration of administration of *C. longa* extract in improving the histopathology of the lung, and design research on *C. longa* extract in preventing pneumonia.

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