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The Effect of Nanoparticles *Andrographis paniculata* (Sambiloto) Extract on IL-10 and TNF-α Levels in Wistar Rats Infected with *Listeria monocytogenes*

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ABSTRACT

Introduction: World Health Organization (WHO) determines Listeria monocytogenes as one of the four major bacteria that cause foodborne disease. During L. monocytogenes infection, TNF-α with IFN-γ activates macrophages for the inflammatory process. In the middle of infection, NK cells produce IL-10 to prevent excessive inflammation. Andrographis paniculata (sambiloto in Indonesia) has been known as a traditional medicine for generations. It has been previously known that administration of A. paniculata to mice with rheumatoid arthritis showed an increase in IL-10 cytokine activity in spleen samples. In addition, it also downregulates the production of inflammatory mediators such as Nitric Oxide (NO) and TNF-α. The extracts are converted into nano-sized particles to be easily absorbed by cells, thus allowing the delivery of active substances to the targeted location. This study aims to determine A. paniculata's effect on IL-10 and TNF-α cytokines in rats infected with L. monocytogenes.
Methods: This study used 30 male Wistar rats infected with L. monocytogenes (except in the normal group). The animals were divided into 6 groups, i.e. Normal group, Negative Control group (K-), EAP200, nEAP100, nEAP200, and nEAP400. Crude extract of A. paniculata 200 mg/kg BW, nanoparticles of A. paniculata extract 100 mg/kg BW, 200 mg/kg BW, and 400 mg/kg BW were administered for 7 days to the EAP200, nEAP100, nEAP200, and nEAP400 groups, respectively.
Results: The IL-10 and TNF-α levels in EAP200, nEAP100, nEAP200, and nEAP400 were significantly lower than the K- group. The lowest levels of IL-10 and TNF-α were in the nEAP400 group.
Conclusion: Administration of A. paniculata extract nanoparticles decreased IL-10 and TNF-α levels in infected Wistar rats. The lowest levels of IL-10 and TNF-α were found in the nanoparticle group which received 400 mg/kg BW.

Keywords: Listeriosis; Sambiloto; Nanoparticle; IL-10; TNF-α

INTRODUCTION

Listeria monocytogenes is a Gram-positive bacteria that causes listeriosis in humans. Infection occurs through contaminated food, causing local infections in the digestive tract, central nervous system infections, mother-to-child infections, and sepsis[1]. *L. monocytogenes* can survive for long periods on food industry equipment and environments, increasing the risk of contamination and possibly causing outbreaks[2].

WHO determines *L. monocytogenes* as one of the four major bacteria that cause foodborne disease, which can cause serious illness, along with *Escherichia coli*, *Salmonella*, and *Staphylococcus*

aureus[3]. Considering that Listeria more specifically infects people with immunodeficiency, treatment may not be sufficient just by administering antibiotics. Immunostimulating agents in the case of listeriosis have a good potential to increase recovery.

A. paniculata or *Sambiloto* is a herbal plant from the Acanthaceae family that has been known for its benefits for generations, including as an immunostimulant[4] and anti-inflammatory[5]. The most common active substance in *A. paniculata* is andrographolide, found in all parts of the plant, especially the leaves[6]. Andrographolide has the effect of downregulating the production of inflammatory mediators such as TNF-α and Nitric Oxide (NO) in peritoneal macrophages[7].

Natural substances such as flavonoids, tannins, terpenoids, and saponins have difficulty penetrating lipid membranes due to their large size, resulting in reduced bioavailability and efficacy[8]. These molecules also experience high systemic clearance, requiring repeated administration or large doses, resulting in less effective therapeutic use[9]. To address these problems, administering nano-based herbal medicine can be a solution[10]. Currently, nanotechniques are being widely developed in the fields of medical biology and disease treatment[11].

This study aims to test the anti-inflammatory effect of *A. paniculata* extract nanoparticles on the IL-10 and TNF-α levels in Wistar rats infected with *L. monocytogenes*.

METHOD

The ethical clearance for this research was obtained from the Health Research Ethics Commission of Universitas Diponegoro (No. 055/EC-H/KEPK/FK-UNDIP/VI/2024).

This was a laboratory experimental study with a randomized controlled trial design to compare the intervention and control groups. The study was conducted in March 2024, in the Laboratorium sentral Faculty of Medicine Universitas Diponegoro and the animal laboratory of Universitas Muhammadiyah Semarang.

The crude extract of *A. paniculata* in this study was obtained from the Balai Besar Penelitian dan Pengembangan Tanaman Obat dan Obat Tradisional (B2P2TOOT) Karanganyar, Central Java. *A. paniculata* extract was diluted in 70% methanol. The extract was mixed into a 1% chitosan solution, then Sodium tripolyphosphate (STPP), and then dissolved in distilled water to a concentration of 1.5%. The size reduction process was carried out with a sonicator at a frequency of 20 kHz for 60 minutes. The mixed solution was centrifuged at 10,000 rpm for 10 minutes to separate nanoparticles (pellets) and supernatant[12].

The bacteria used in this study was *L. monocytogenes* ATCC 7644. *L. monocytogenes* suspension for the infection was made using phosphate-buffered saline (PBS), and dissolved until the turbidity was equivalent to 5 McFarland standards (1.5x109 Cells/mL). A solution containing 0.5 mL of *L. monocytogenes* was injected intravenously into each rat.

The sample size (30 Wistar male rats) was calculated using Federer's formula and divided into 6 groups by simple random sampling, namely the Normal group, Negative Control group (K-) (infected with *L. monocytogenes* without intervention), EAP200 (infected with *L. monocytogenes* and administered with 200 mg/kg BW crude extract of *A. paniculata* + 1% chitosan), nEAP100 (infected with *L. monocytogenes* and administered with 100 mg/kg BW nanoparticles of *A. paniculata* extract), nEAP200 (infected with *L. monocytogenes* and administered with 200 mg/kg BW nanoparticles of *A. paniculata* extract), and nEAP400 (infected with *L. monocytogenes* and administered with 400 mg/kg BW nanoparticles of *A. paniculata* extract).

Blood sampling was carried out after 7 days of treatment. The serum was obtained by centrifugation at 3000 rpm for 15 minutes. The serum was analyzed with ELISA kit to determine IL-10 and TNF-α levels. Data analysis used Kruskall Wallis, and then the post hoc test used Mann-Whitney for significance.

RESULT

This study evaluated the effects of *A. paniculata* extract on the levels of IL-10 and TNF-α in Wistar rats infected with *L. monocytogenes*. Cytokine levels were measured after 7 days of treatment to assess the anti-inflammatory response. The data demonstrated differences between treatment groups, with notable reductions in both cytokines, particularly in nanoparticle groups.

IL-10 levels

Figure 1 showed that the mean of IL-10 levels was lower in groups treated with crude and nanoparticles of *A. paniculata* extract. The smallest value of the group was obtained in the nEAP400 group.

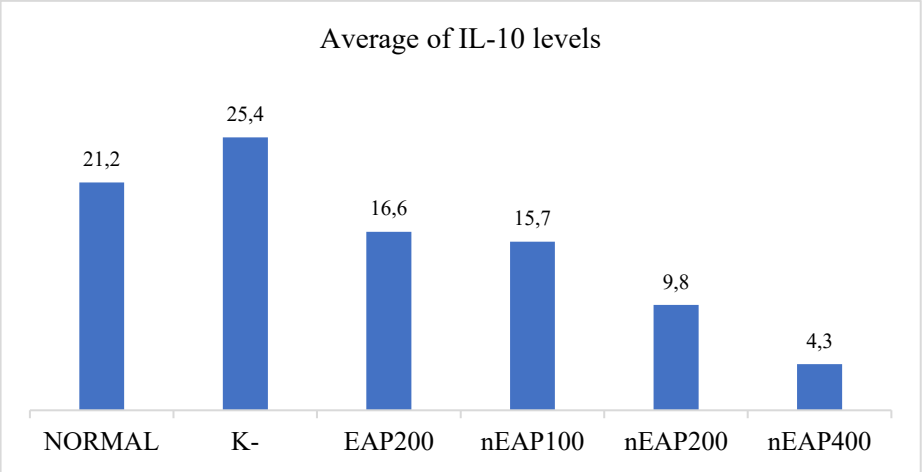

Figure 1. Diagram of Average IL-10 levels

Table 1 showed that IL-10 levels in the EAP200, nEAP200, and nEAP400 groups were significantly lower than in the Negative Control group.

Table 1. Mann-Whitney test result

IL10	NORMAL	K-	EAP200	nEAP100	nEAP200	nEAP400
NORMAL		0,421	0,310	0,151	0,056	0,016*
K-	0,421		0,032*	0,056	0,008*	0,008*
EAP200	0,310	0,032*		1,000	0,095	0,008*
nEAP100	0,151	0,056	1,000		0,222	0,008*
nEAP200	0,056	0,008*	0,095	0,222		0,95
nEAP400	0,016*	0,008*	0,008*	0,008*	0,95	

*statistically significant

TNF-α levels

Figure 2 showed that the mean of TNF-α levels were lower in groups treated with crude and nanoparticles of *A. paniculata* extract. The smallest value of the group was obtained in the nEAP400 group. Table 2 showed that TNF-α levels in the nEAP100, nEAP200, and nEAP400 groups were significantly lower than in the Negative Control group.

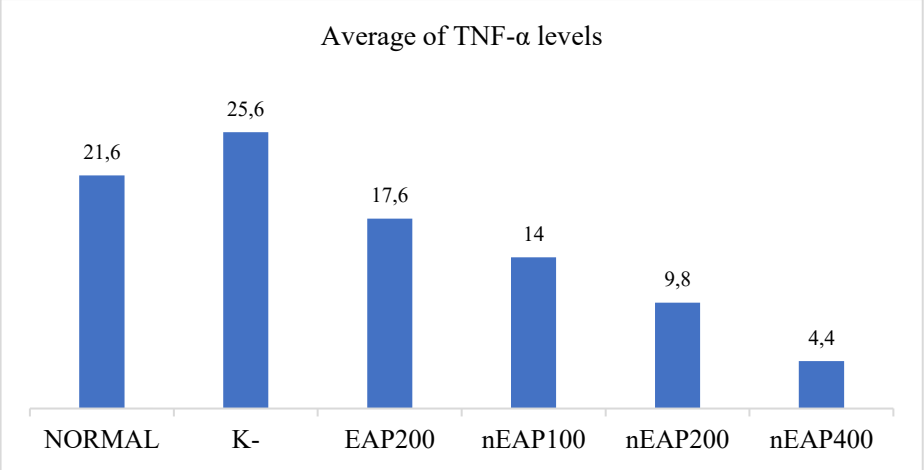

Figure 2. Diagram of Average TNF-α levels

Table 2. Mann-Whitney test result

TNF-α	NORMAL	K-	EAP200	nEAP100	nEAP200	nEAP400
NORMAL		0,421	0,222	0,095	0,095	0,016*
K-	0,421		0,056	0,016*	0,008*	0,008*
EAP200	0,222	0,056		0,548	0,035*	0,008*
nEAP100	0,095	0,016*	0,548		0,421	0,016*
nEAP200	0,095	0,008*	0,035*	0,421		0,151
nEAP400	0,016*	0,008*	0,008*	0,016*	0,151	

*statistically significant

DISCUSSION

This study showed that IL-10 levels (anti-inflammatory cytokines) were lower when nanoparticles of *A. paniculata* extract were given at doses of 200 mg/kgBW and 400 mg/kgBW, as well as the crude extract of 200 mg/kgBW. This may occur due to decreased levels of the pro-inflammatory cytokine TNF-α. Research by D'Orazio in rats shows that IL-10 production is influenced by high levels of IL-12 and TNF-α, resulting in a mechanism to prevent chronic inflammation and tissue damage[13]. Abbas et al. also stated that the production of anti-inflammatory cytokine IL-10 by macrophages and dendritic cells inhibits pro-inflammatory macrophages (classical pathway of macrophage activation).

During the late stages of infection, IL-10 secretion plays an important role in reducing excessive inflammatory reactions. IL-10 circulates in the peripheral blood 4 days post-infection[13]. The main sources of IL-10 are macrophages, endothelial cells, and T cells. γδ T cells produce IL-10, thereby preventing tissue damage due to immune reactions in the liver[14]. In this study, *A. paniculata* extract nanoparticles did not increase IL-10 production. It is necessary to investigate more, whether the levels of IL-10 are also influenced by other factors, such as the antibacterial effect of the active substance, therefore the activity of inflammatory mediators such as TNF-α did not increase much.

TNF-α levels were lower in the nanoparticles *A. paniculata* extract dose groups of 100 mg/kg BW, 200 mg/kg BW, and 400 mg/kg BW compared to the negative control group. TNF-α plays a crucial role in the clearance of *L. monocytogenes*, which is produced through the activation of macrophages and other cells, including T cells, B cells, and NK cells. The primary function of TNF-α in the immune system is to initiate the inflammatory process, resulting in an increase in systemic TNF-α levels during *L. monocytogenes* infection. Specifically, TNF-α production in neutrophils is driven by Sox2, a

cytosolic sensor that is activated upon binding to *L. monocytogenes* DNA. The mechanism of the active substances in *A. paniculata* may be linked to decreased gene expression in the inflammatory cascade.

Based on the results of this study, *A. paniculata* nanoparticles may have anti-inflammatory effects, as seen from the low levels of TNF-α (as a pro-inflammatory cytokine) in the group given *A. paniculata* extract nanoparticles and high levels of TNF-α in the Negative Control group. This was probably caused by the lower level of TNF-α which resulting in less stimulation of IL-10 production. However, this can also occur due to the strong antibacterial effect of the active substance present, causing most bacteria have been eliminated, resulting in low TNF-α production. The antibacterial substance that may interfere with TNF-α production in this study was probably *A. paniculata* itself. Yu et al. stated that andrographolide in *A. paniculata* has the potential to reduce biofilm formation and virulence of *L. Monocytogenes*[2].

We can also state that the low level of TNF-α was not only caused by the anti-inflammatory effect of the IL-10 (because of the low level of this cytokine). To ensure an accurate anti-inflammatory effect, it is necessary to measure IL-10 and TNF-α levels several times at the right time points because the production of cytokines is dynamic according to the inflammatory process. Several measurements may be needed, for example at the beginning of infection, on the 3rd-4th day of infection (when TNF-α levels are high and IL-10 begins to increase), and at the end of infection. Aside from that, the research has other limitations; further studies are necessary. This study has not demonstrated cytokine kinetic profiling and bacterial counting to reveal changes in cytokine levels.

A. paniculata extract nanoparticles may be used as supportive therapy for infectious diseases to regulate excessive inflammation, to prevent massive tissue damage and severe illness. The results of this study suggest modifying the method to test the anti-inflammatory effect of *A. paniculata* extract nanoparticles by multiple time series measurements of IL-10 and TNF-α.

CONCLUSION

Administration of *A. paniculata* extract nanoparticles reduced TNF-α levels in infected Wistar rats. IL-10 levels decreased with decreasing TNF-α levels (the extract did not increase IL-10 levels as an anti-inflammatory cytokine). The lowest levels of IL-10 and TNF-α were in a group that received 400 mg/kg BW.

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CONFLICT OF INTEREST

There is no conflict of interest in this research.

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