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Original Article

Antibacterial and Antioxidant Effect from Nanoparticle of *Andrographis paniculata* Extract on Wistar Rat Infected with *Listeria monocytogenes*

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Abstract

Background : Listeria infection triggers the production of ROS (Reactive Oxygen Species) and radical-waste accumulation such as Malondialdehyde (MDA). *Andrographis paniculata*, triggers the production of Superoxide Dismutase (SOD) as antioxidant and suppresses the bacteria multiplication in host's body. However, these active-phytochemical have poor destruction due to their large size and molecular weight. Nanoparticle technology may overcome this problem to increase bioavailability and benefit of herbal pharmacology. This study was aimed to evaluate the effectivity nanoparticle of *A. paniculata* extract to increase bacteria elimination, increase SOD level, and reduce MDA level in rat infected with *L. monocytogenes*.
Methods : Experimental research with post-test only controlled trial design was conducted using white rats (*Rattus novergicus* strain Wistar) into Normal (N), Negative Control (K-), *A. paniculata* extract (EAP-200), and Nanoparticle of *A. paniculata* extract Group (nEAP-100, nEAP-200, and nEAP-400). Colony was measured using total plate count method. SOD & MDA level were measured using colorimetric method
Results : *L. monocytogenes* bacterial count from group with extract *A. paniculata* was significantly lower than those in negative control (p<0.05). SOD level from group with extract *A. paniculata* was very significantly higher than those in negative control (p<0.01), while MDA level from group with extract *A. paniculata* was very significantly lower than those in negative control (p<0.01). Dose of 200 mg/kgBW in nanoparticle form was the most optimum dose.
Conclusion : Extract of *A. paniculata* at various doses and form, increase bacterial clearance, increase SOD level, and reduce MDA level in rat infected with *L. monocytogenes*.

Keywords : Antibacterial, *Andrographis paniculata*, Antioxidant, *Listeria monocytogenes*, Nanoparticle

INTRODUCTION

WHO reports that 600 million people are suffered from foodborne infection with 175.000 people were died in Southeast Asia.[1] Listeria monocytogenes has highest mortality rate among other foodborne pathogen bacteria in United States (16,9%) and Europe (18,8%).[2] Listeria infections cause diseases such as sepsis, meningitis, and die in some individuals such as children and pregnant woman.[3–7]

Free radical such as *Reactive Oxygen Species* (ROS) are side products of aerobic metabolism, which reactive, destructive, and causing oxidative stress. Infection of *L. monocytogenes* increase production of ROS for killing bacteria. However, an excessive of ROS in host body, causes oxidative stress along with the accumulation of radical waste in the host body such as fatty acid radicals or Malondialdehyde (MDA) that resulted from lipid peroxidation in cell membrane or tissues. It presence, provides effects such as DNA damage and protein degradation which causes apoptosis, neuro-inflammation, and cancer.[8–11] The cell requires antioxidant enzyme such as Superoxide Dismutase (SOD), which is able to neutralize ROS into oxygen (O_2) dan hydrogen peroxide (H_2O_2) and also to prevent ROS adverse effect and protect cells from oxidative stress.[12,13]

Various medicine plants which contains natural active compounds have been used and developed in health sector as one of the alternative therapy for patient with antibiotic resistant.[14,15] *A. paniculata* is one of the Indonesia traditional medicines used for antimalarial, antimicrobial,[16] anti-inflammatory,[17] antioxidant, anti-parasitic,[18] antidiabetic, and anticancer agents in Asia.[19] *A. paniculata* contains active phytochemical such as diterpenoid lactone, flavonoid, quinic acid, tannins, and alkaloid.[20,21]

The active pharmacological effects of herbal plants on various disease are still not optimum in host body because poor distribution due to their large size and molecular weight.[22] Nanoparticle are one of the technology-based product utilization which control the optical, physical and chemical properties of the compound to increase drug absorbtion in body (increase bioavailability).[23] Chitosan is a natural polymer biomaterial derived from chitin compounds which low side effects on humans. *A. paniculata* extract with chitosan may also increase antioxidant capacity.[24]

Studies about the benefit of nanoparticle of *A. paniculata* extract still limited as in vitro studies, therefore this research focus on the effectivity of nanoparticle of *A. paniculata* extract to determine the optimal dose for increasing bacterial clearance and SOD level, also reducing MDA level in rat infected with *L. monocytogenes* (in vivo).

METHODS

An experimental study was done with a post-test-only randomized controlled group design. The study was conducted at the Research and Animal Laboratory, Muhammadiyah Semarang University, Semarang for 7 days after obtaining ethical approval from the Health Ethics Committee. The Ethical clearance issued by The Health Research Ethics Committee Faculty of Medicine Universitas Diponegoro. Colony count from the blood and liver samples of rat was measured at Microbiology Laboratory, Faculty of Medicine, Diponegoro University while the analysis of SOD and MDA level was measured at Ecotoxicology Laboratory, Faculty of Biology, Jenderal Soedirman.

Animals and Experimental Groups

The study used 36 male Wistar Rat aged 812 weeks with a body weight of 200300 grams, divided into 6 groups. The research group division was as follows:

Normal group (N) : Healthy Rat (only general feed)

Negative control group (K-) : Rat infected by *L. monocytogenes*

Treatment group I (EAP) : Rat infected by *L. monocytogenes* and treated with 200 mg/kg *A. paniculata* extract

Treatment group II (nEAP-100) : Rat infected by *L. monocytogenes* and treated with 100 mg/kg Nanoparticle of *A. paniculata* extract

Treatment group III (nEAP-200) : Rat infected by *L. monocytogenes* and treated with 200 mg/kg Nanoparticle of *A. paniculata* extract

Treatment group IV (nEAP-400) : Rat infected by *L. monocytogenes* and treated with 400 mg/kg Nanoparticle of *A. paniculata* extract

The experimental animals were inoculated with *Listeria monocytogenes* ATCC 7644 from Gadjah Mada University. 0.5 mL of *L. monocytogenes* in PBS (109 CFU/mL) was intravena injected into each rat on the first day.[25,26] After bacterial infection, nanoparticles of *A. paniculata* dissolved in 1% DMSO were given orally (gastric tube) on treatment groups at the same time of Biorat feeding on each day for a week. After one week, blood (whole blood and serum) and liver tissue was isolated for analysis.[25]

Preparation of A. paniculata extract

The methanol extract of *A. paniculata* was prepared by maceration using 70% methanol as solvent. In this study, the dried *A. paniculata* leaf powder was obtained from *Balai Besar Penelitian dan Pengembangan Tanaman Obat dan Obat Tradisional* (B2P2TOOT), Tawangmangu, Central Java, Indonesia. Approximately 20 g r of simplisia was macerated with 200 mL of 70% methanol (w:v = 1:10). After maceration, this mixture was filtered using

Whatman filter paper number No.1. The filtrate was evaporated using a Vacuum rotary evaporator at 50°C until crude extract was obtained.[27]

Preparation of Nanoparticle of *A. paniculata* leaf extract

The nanoparticle of *A. paniculata* leaf extract was produced by using ionic gelation and ultrasonication method followed by addition of chitosan (polimer) and sodium tripolyphosphate (STPP as crosslinker). The 1% chitosan was dissolved in a 1% acetic acid solution and homogenized. About 0.15 gram of *A. paniculata* extract was diluted in 5 drops of 70% methanol. The diluted *A.paniculata* extract was then added to the chitosan solution. The crosslinker in the form of STPP was dissolved in distilled water until reached 1.5% concentration and a mixture of bitter extract + chitosan was added to the solution and then homogenized. The homogeneous solution was then centrifuged at a speed of 10,000 rpm for 10 minutes and then the natant was separated from the supernatant. The natan solution was then sonicated at a frequency of 20 kHz for 60 minutes.28 Morphological analysis including the size and shape of the nanoparticle compound was measured using a Particle Size Analyzer (PSA) and Scanning Electron Microscope (SEM).[29]

Total Bacteria Colony Count

Blood and liver samples approximately 1 mL/1 gr were diluted in 9 mL of NaCl solution (ratio 1:9).[30] 1 mL of the 10–3 to 10–5 dilution tubes, 1mL was taken and transferred into a petri dish. Next, 20 mL of Nutrient Agar (NA) media was poured into a petri dish and then homogenized. The media was incubated at 37°C for 2x24 hours.[31] The colony formed were then counted using the TPC formula.[32]

$$\text{TPC (CFU/mL)} = \text{total colony x volume sample x } \frac{1}{\text{Dilution factor}}$$

SOD and MDA Level

Measurement of superoxide dismutase (SOD) level was analyzed using the Superoxide Dismutase commercial Kit (Elabscience).[33] A 20 µL of plasma was added 1 mL of SOD buffer solution and 100 µL of xanthine oxidase solution. A blank solution was prepared by adding and mixing 1 mL of SOD buffer solution and 100 µL of xanthine oxidase solution. The absorbance value at λ520 nm was measured using a UV-Vis spectrophotometer. The activity of the SOD enzyme was measured using the following formula.[34]

$$\text{Activity of SOD (U/mL)} = \frac{\text{As}}{Ao} \times \text{Ks}$$

Note: As: absorbance value of sample solution;
Ao: absorbance value of a standard solution;
Ks: standard concentration (30.65).

Measurement of malondialdehyde (MDA) level was analyzed using the Superoxide Dismutase commercial Kit (Elabscience).[33] A 400 µL of plasma was added 400 uL of TCA 20% solution in reaction tube then centrifuge. After centrifuged, 400 uL supernatant of the mixture added 1 mL of TBA 0,67% solution then incubated on water bath for 10 minutes. A blank solution was prepared by adding and mixing 1 mL of TBA 0,67% solution and 400 µL of TCA 20% solution. The mixture was measured by spectrophotometry at λ532 nm.[35]

Statistical Analysis

The data normality was tested using the Shapiro-Wilk test. The average of Listeria colony in blood, SOD, and MDA level were normally distributed, then were analyzed using One Way Anova and followed by Post Hoc LSD. The average of Listeria colony in hepar was not normally distributed, then were analyzed using Kruskall-Wallis test followed by the Mann-Whitney test to determine the differences between groups in this study. The result of *p-value* was considered significant, if $p < 0.05$.

RESULTS

The condition after 7 days experiment, rats in the Negative Control Group showed different behaviors and clinical conditions from the treatment groups. The rat appeared weak and less active as the day went on, the hair was dirty, and the feces became loose, while in the Treatment Group (EAP-200, nEAP-100, nEAP-200, and nEAP-400), the rats were active with clean hair, normal feces, and physically similar to the mice in the Normal Group.

***Listeria monocytogenes* Colony Count**

The total number of bacteria in the blood and liver samples after being treated for 7 days are shown in Figure 1 and Figure 2. Bacterial colony count was carried out with a dilution until 10^{-3} for blood samples and 10^{-6} for liver samples.

***L. monocytogenes* Colony Count in Blood**

The average number of *L. monocytogenes* bacterial colony in the blood (Figure 1), the treated group (EAP-200, nEAP-100, nEAP-200, and nEAP-400) showed a very low graph compared to the Negative Control.

The result of LSD test showed that the average of *L. monocytogenes* colony in blood for treatment group (EAP-200, nEAP-100, nEAP-200, and nEAP-400) was

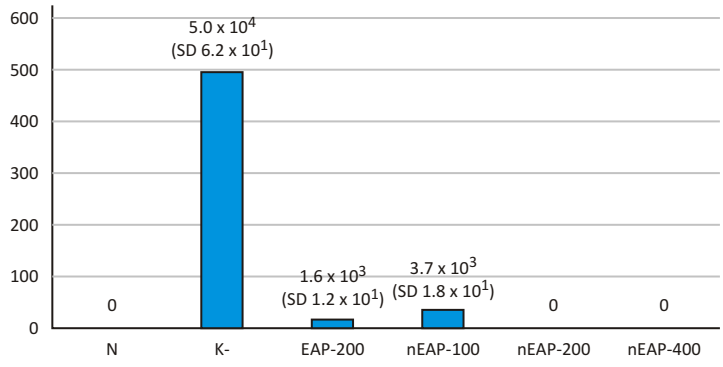

Figure 1. Average Number of *L. monocytogenes* Colony in Blood (CFU/mL)
(N (Normal), K- (Negative Control), EAP-200 (*A. paniculata* Extract 200 mg/kgBW), nEAP-100 (100 mg/kgBW Nanoparticle of *A. paniculata* Extract), nEAP-200 (200 mg/kgBW Nanoparticle of *A. paniculata* Extract), and nEAP-400 (400 mg/kgBW Nanoparticle of *A. paniculata* Extract))

TABLE 1
Statistical Analysis of Average Number of *L. monocytogenes* in Blood

	N	K-	EAP-200	nEAP-100	nEAP-200	nEAP-400
N	–	<0.001*	0.370	0.043	1	1
K-	<0.001	–	<0.001*	<0.001*	<0.001*	<0.001*
EAP-200	0.370	<0.001*	–	0.233	0.370	0.370
nEAP-100	0.043*	<0.001*	0.233	–	0.043*	0.043*
nEAP-200	1	<0.001*	0.370	0.043*	–	1
nEAP-400	1	<0.001*	0.370	0.043*	1	–

(N (Normal), K- (Negative Control), EAP-200 (*A. paniculata* Extract 200 mg/kgBW), nEAP-100 (100 mg/kgBW Nanoparticle of *A. paniculata* Extract), nEAP-200 (200 mg/kgBW Nanoparticle of *A. paniculata* Extract), and nEAP-400 (400 mg/kgBW Nanoparticle of *A. paniculata* Extract); *different results are statistically significant)

significantly lower than of Negative Control group (p<0.01; Table 1). The average of *L. monocytogenes* colony for nEAP-200 and nEAP-400 groups was significantly lower than of EAP200 and nEAP-100 (p<0.05; Table 1), and there was no significant difference in the nEAP-200 group with nEAP-400 (p>0.05; Table 1). The average number of colony in EAP200 was lower than of nEAP-100, but was not statistically significant (p>0.05; Table 1).

L. monocytogenes Colony Count in Liver

The average number of *L. monocytogenes* bacterial colony in the liver (Figure 2), the treated group (EAP-200, nEAP-100, nEAP-200, and nEAP-400) showed a very low graph compared to the Negative Control.

The result of Mann Whitney test showed that the average number of *L. monocytogenes* colony in the blood for EAP-200, nEAP-200, and nEAP-400 groups was very significantly lower than of Negative Control group (p<0.01; Table 2) while the nEAP-100 group was significantly lower than the Negative Control group (p<0.05; Table 2). The average number of *L. monocytogenes* colony in the EAP-200, nEAP-100, and nEAP-200 groups showed there was not statistically significant difference (p>0.05; Table 2).

Superoxide Dismutase (SOD) Plasma Level

Figure 3 shows that the average of SOD plasma level in the treated group (EAP-200, nEAP-100, nEAP-200, and nEAP-400) were higher compared to the Negative Control and Normal Group.

The result of LSD test showed that SOD plasma level in the EAP-200, nEAP-100, nEAP-200, and nEAP-400 groups were very significantly higher than the Normal and Negative Control Groups (p<0.01; Table 3). SOD level in the EAP-200 and nEAP-100 groups were very significantly higher than Negative Control Group,

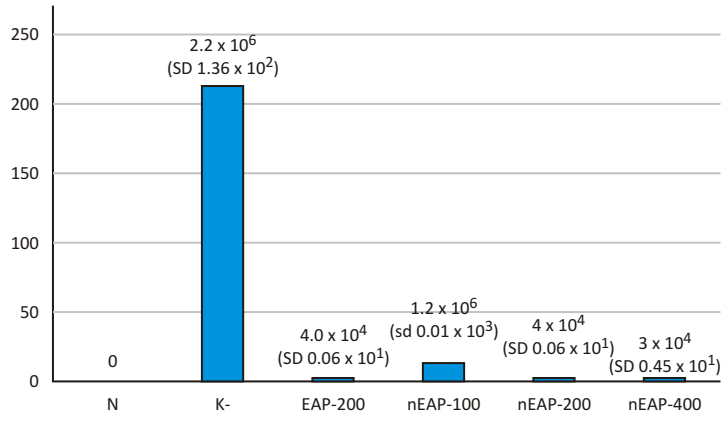

Figure 2. Average Number of *L. monocytogenes* Colony in Liver (CFU/mL)
(N (Normal), K- (Negative Control), EAP-200 (*A. paniculata* Extract 200 mg/kgBW), nEAP-100 (100 mg/kgBW Nanoparticle of *A. paniculata* Extract), nEAP-200 (200 mg/kgBW Nanoparticle of *A. paniculata* Extract), and nEAP-400 (400 mg/kgBW Nanoparticle of *A. paniculata* Extract))

TABLE 2
Statistical Analysis of Average Number of *L. monocytogenes* in Blood

	N	K-	EAP-200	nEAP-100	nEAP-200	nEAP-400
N	–	0.003*	1	0.869	1	1
K-	0.003	–	0.069	0.992	0.069	0.047*
EAP-200	1	0.060	–	1	1	1
nEAP-100	0.869	0.992	1	–	1	1
nEAP-200	1	0.069	1	1	–	1
nEAP-400	1	0.047*	1	1	1	–

(N (Normal), K- (Negative Control), EAP-200 (*A. paniculata* Extract 200 mg/kgBW), nEAP-100 (100 mg/kgBW Nanoparticle of *A. paniculata* Extract), nEAP-200 (200 mg/kgBW Nanoparticle of *A. paniculata* Extract), and nEAP-400 (400 mg/kgBW Nanoparticle of *A. paniculata* Extract); *different results are statistically significant)

but this increase was still significantly lower than of nEAP-200 and nEAP-100 (p<0.05; Table 3). The increase of SOD level between nEAP-200 and nEAP-400 was not statistically significant (p>0.05; Table 3).

Malondialdehyde (SOD) Plasma Level

Figure 4 shows that the average of MDA plasma level in the treated group (EAP-200, nEAP-100, nEAP-200, and nEAP-400) was slightly lower than Negative Control.

The result of LSD test showed that MDA plasma level in all treatment groups (EAP-200, nEAP-100, nEAP-200, and nEAP-400) were significantly lower than of the Negative Control Group (p<0.05; Table 4). The MDA level in nEAP-200 and nEAP-400 groups were significantly lower than of EAP200 group (p<0.05; Table 4). The MDA level between nEAP-200 and nEAP-400 were not statistically significant (p>0.05; Table 4).

DISCUSSION

This study proved that EAP in both crude extracts and nanoparticle form increased the elimination (clearance) of *Listeria monocytogenes* bacteria in wistar rat infected-*L. monocytogenes*. The effect of bacterial clearance were seen on 200 mg/kgBW of crude extract and nanoparticle form. The bacterial clearance effect from active metabolite compounds in EAP might be caused by antibacterial effects and / or the ability to stimulate phagocytosis and intracellular bacterial killing. At this point, we are not sure which mechanism caused effective bacterial clearance in this study.

Previous studies reported that andrographolide (diterpenoid) content in *A. paniculata* has antibacterial activity to inhibit bacterial biofilms form in the host, damage bacterial cell membranes and walls through inhibition of difflux pumps, inhibit the hemolytic

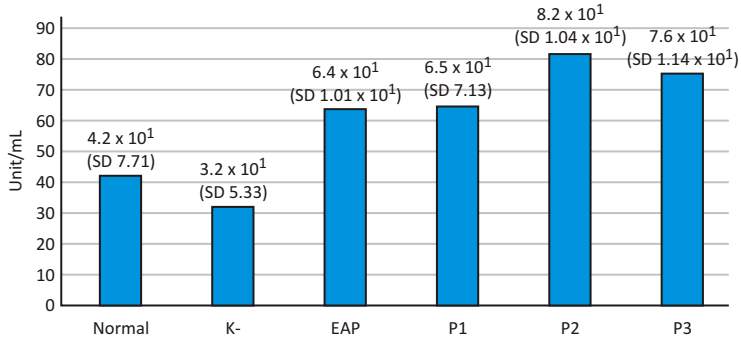

Figure 3. Superoxide Dismutase Serum Level (Unit/mL)
(N (Normal), K- (Negative Control), EAP-200 (*A. paniculata* Extract 200 mg/kgBW), nEAP-100 (100 mg/kgBW Nanoparticle of *A. paniculata* Extract), nEAP-200 (200 mg/kgBW Nanoparticle of *A. paniculata* Extract), and nEAP-400 (400 mg/kgBW Nanoparticle of *A. paniculata* Extract))

TABLE 3
Statistical Analysis of SOD Plasma Level

	N	K-	EAP-200	nEAP-100	nEAP-200	nEAP-400
N	–	0.155	0.001*	<0.001*	<0.001*	<0.001*
K-	0.155	–	<0.001*	<0.001*	<0.001*	<0.001*
EAP-200	0.001*	<0.001*	–	0.676	<0.001*	0.014*
nEAP-100	<0.001*	<0.001*	0.676	–	0.003*	0.035*
nEAP-200	<0.001*	<0.001*	0.001*	0.003*	–	0.299
nEAP-400	<0.001*	<0.001*	0.014*	0.035*	0.299	–

(N (Normal), K- (Negative Control), EAP-200 (*A. paniculata* Extract 200 mg/kgBW), nEAP-100 (100 mg/kgBW Nanoparticle of *A. paniculata* Extract), nEAP-200 (200 mg/kgBW Nanoparticle of *A. paniculata* Extract), and nEAP-400 (400 mg/kgBW Nanoparticle of *A. paniculata* Extract); *different results are statistically significant)

activation of Listeriosin O (LLO), and inhibit communication between bacterial cells.[36–39] The Flavonoid in *A. paniculata* able to damage cell walls bacteria, inhibit synthesis of nucleic acid (through inhibition of the topoisomerase enzyme) and inhibit energy metabolism (through inhibition of NADH-cytochrome C reductase).[40] Previous studies proved that supplementation of silver-nanoparticle *A. paniculata* was effective to inhibit Gram-positive bacteria (*Staphylococcus aureus* with MIC: 25 ug/mL) and Gram-negative bacteria (*E. coli* with MIC: 12.5 ug/mL) in dilution agar method.[41] Paramasivam[42] reported that Aurum(Au)-nanoparticles *A. paniculata* showed a trend of increasing antibacterial effects from a dose of 50 ug/mL to 100 ug/mL using agar diffusion method. On the other hand, andrographolide also triggers lymphocyte proliferation, increases migration of macrophages towards the site of infection, phagocytosis and intracellular killing.[43]

Our findings also showed that *A. paniculata* in various doses and forms increased SOD level very significantly. Dose of 200 mg/kgBW in nanoparticle was significantly more efficaciously increased SOD level compared to dose of 200 mg/kgBW in crude extract form, thus, it can be concluded that the nanoparticle form on dose of 200 mg/kgBW is more effective in increasing SOD level than the crude extract form. There was no significant differences of SOD level between dose of 200 mg/kgBW and 400 mg/kgBW in nanoparticle, implying that the dose of 200 mg/kgBW in nanoparticle form is optimum for increasing SOD level.

SOD is an enzyme which stabilize free radicals of ROS which produced by phagocyte cells in vacuoles to kill bacteria intracellularly and prevent cell/tissue damage due to oxidative stress.[44] According to Bhattacharya's research,[45] ROS is produced after bacteria are phagocytized intracellularly. However, excessive ROS production causes damage to the cells and tissue, therefore SOD production was increased to suppress ROS production and inhibit the apoptosis pathway through the BAX and BCL-2 protein inhibition. In this study, the SOD level was increased on 200 mg/kgBW and 400 mg/kgBW dose in nanoparticle form indicating that

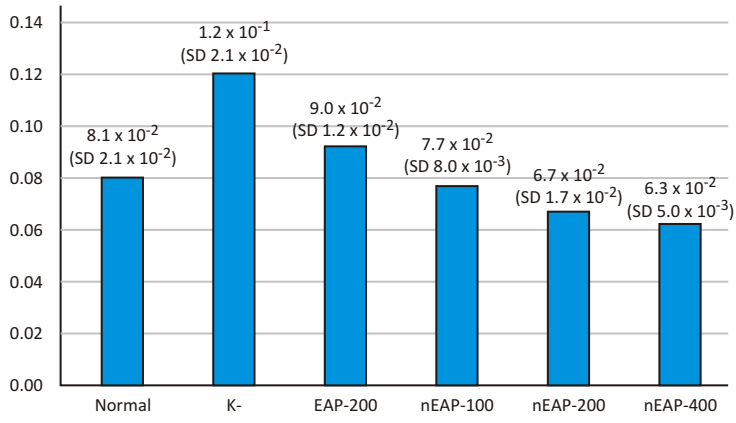

Figure 4. Malondialdehyde Plasma Level (Unit/mL)
(N (Normal), K- (Negative Control), EAP-200 (*A. paniculata* Extract 200 mg/kgBW), nEAP-100 (100 mg/kgBW Nanoparticle of *A. paniculata* Extract), nEAP-200 (200 mg/kgBW Nanoparticle of *A. paniculata* Extract), and nEAP-400 (400 mg/kgBW Nanoparticle of *A. paniculata* Extract))

TABLE 4
Statistical Analysis of MDA Plasma Level

	N	K-	EAP-200	nEAP-100	nEAP-200	nEAP-400
N	–	<0.001*	0.228	0.745	0.187	0.076
K-	<0.001*	–	0.008*	<0.001*	<0.001*	<0.001*
EAP-200	0.228	0.008*	–	0.131	0.016*	0.005*
nEAP-100	0.745	<0.001*	0.131	–	0.313	0.141
nEAP-200	0.187	<0.001*	0.016*	0.313	–	0.626
nEAP-400	0.076	<0.001*	0.005*	0.141	0.626	–

(N (Normal), K- (Negative Control), EAP-200 (*A. paniculata* Extract 200 mg/kgBW), nEAP-100 (100 mg/kgBW Nanoparticle of *A. paniculata* Extract), nEAP-200 (200 mg/kgBW Nanoparticle of *A. paniculata* Extract), and nEAP-400 (400 mg/kgBW Nanoparticle of *A. paniculata* Extract); *different results are statistically significant)

ROS production also increased during bacterial infection. This finding indirectly implied that there has been an increase in phagocytosis and intracellular bacterial killing activity, or other words, the clearance of *L. monocytogenes* bacteria after treatment with *A. paniculata* caused by antibacterial effect and also the immunostimulation effect of *A. paniculata*. Another study is needed to prove that the phagocytosis and intracellular killing are increased by *A. paniculata*. Previous study of Venkata *et al.*,[46] stated that nanoparticle of *A. paniculata* extract at a dose of 80 mg/kgBW increase SOD level in the brains of Rats induced with pentylenetetrazole (PTZ). Research by Kumari *et al.*,[47] showed that Cerium Oxide Nanomaterial of *A. paniculata* extract (cAP-CNP) that contains andrographolid and flavonoid can increase SOD activities until 43.03% by in vitro method through Nitrobule Tetrazolium (NBT) reduction.

Another finding in this study is the *A. paniculata* both in crude extract and nanoparticle also has an antioxidant effect by significantly reduces MDA level. Dose of 200 mg/kgBW and 400 mg/kgBW in nanoparticle form has reducing MDA level better than dose of 200 mg/kgBW in crude extract form and also the effect dose of 200 mg/kg BW in nanoparticle form was equivalent to reduce MDA level in dose of 400 mg/kgBW, implying that, dose of 200 mg/kgBW in nanoparticle form is the most optimal dose for lowering MDA level in the blood. MDA is the end product of peroxidation of unsaturated fatty acids which is used as an indicator of free radicals (ROS) accumulation in the process of intracellular bacterial killing.[48] A high MDA level indicates an increase of ROS content, whereas a low MDA level indicates that the ROS level is also low. MDA, which tends to be low while antioxidant level (SOD) are high, indicates that there is a balance in the cell to minimize cell damage and prevent cell death due to oxidative stress.[8] According to Ibrahimi *et al.*,[49] the flavonoids and alkaloids in EAP trigger SOD production in the body,

suppress the metabolism of toxic substances, and decrease lipid peroxidation (MDA). The study of Venkata *et al*.,[46] also stated that nanoparticle of *A. paniculata* extract at a dose of 80 mg/kgBW decreased MDA level in the brains of pentylenetetrazole-induced rats (PTZ).

This study proved that nanoparticle of *A. paniculata* extract is able to clearance bacterial in liver and blood indirectly through increasing phagocytosis and intracellular killing and also proved antioxidant effects of *A. paniculata* extract by showing the increase of SOD level and decrease of MDA level. The results of this study proved that the nanoparticle form is better than the crude extract form. The weakness of this study is the interval of research doses are wide. Through this study, authors hoped that nanoparticle of *A. paniculata* extract can be develope as supporting therapies for listeria infection.

CONCLUSION

Nanoparticle of *A. paniculata* at various doses and form, increases bacteria clearance and SOD level, and reduce MDA level in rat infected with *L. monocytogenes*. Dose of 200 mg/kgBW in nanoparticle form was the most optimum dose to increases bacteria clearance and SOD level, and reduce MDA level in rat infected with *L. monocytogenes*.

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REFERENCES

1. Mpundu P, Mbewe AR, Muma JB, Mwasinga W, Mukumbuta N, Munyeme M. A Global Perspective of Antibiotic-Resistant Listeria monocytogenes Prevalence in Assorted Ready to Eat Foods: a Systematic Review. *Veterinary World*. 2021;14(8):2219–2229. https://doi.org/10.14202/vetworld.2021.2219-2229
2. Park M, Horn L, Lappi V, Boxrud D, Hedberg C, Jeon B. Antimicrobial Synergy between Aminoglycosides and Licorice Extract in Listeria monocytogenes. *Pathogens*. 2022;11(4):1–9. https://doi.org/10.3390/pathogens11040440
3. Sibanda T, Buys EM. Listeria monocytogenes Pathogenesis: The Role of Stress Adaptation. *Microorganisms*. 2022;10(8):1–26. https://doi.org/10.3390/microorganisms10081522
4. de Alcântara AI, Mancebo DB, Souza TL, Nunes SL, Vitor LFJ. Anti-inflammatory Activity of Caffeine (1,3,7-trimethylxanthine) after Experimental Challenge with Virulent Listeria monocytogenes in Swiss mice. *International Immunopharmacology*. 2021;100:1–7. https://doi.org/10.1016/j.intimp.2021.108090
5. Xia X, Chen Y, Xu J, Yu C, Chen W. SRC-3 Deficiency Protects Host from Listeria monocytogenes Infection through Increasing ROS Production and Decreasing Lymphocyte Apoptosis. *International Immunopharmacology*. 2021;96:1–10. https://doi.org/10.1016/j.intimp.2021.107625
6. Osek J, Wieczorek K. Listeria monocytogenes--How This Pathogen Uses Its Virulence Mechanisms to Infect the Hosts. *Pathogens*. 2022;11(12):1–25. https://doi.org/10.3390/pathogens11121491
7. Hochnadel I, Hoenicke L, Petriv N, *et al*. Safety and Efficacy of Prophylactic and Therapeutic Vaccine Based on live-attenuated Listeria monocytogenes in Hepatobiliary Cancers. *Oncogene*. 2022;41(14):2039–2053. https://doi.org/10.1038/s41388-022-02222-z
8. Al-Bayati HHK, Alwan MJ. Study on Some Side Effect of Accompaniment of The Listeriosis During Pregnancy. *Biochem Cell Arch*. 2020;20(2):4693–4700. https://connectjournals.com/03896.2020.20.4693
9. Febrina L, Helmi, Rijai L. Profil Kadar Malondialdehida, Glukosa dan Kolesterol pada Tikus Putih yang Terpapar Asap Rokok. *J Trop Pharm Chem* . 2016;3(4):277–282. doi:https://doi.org/10.25026/jtpc.v3i4.115
10. Zhang J, Zheng P, Li J, *et al*. Curcumin-Mediated Sono-Photodynamic Treatment Inactivates Listeria monocytogenes via ROS-Induced Physical Disruption and Oxidative Damage. *Foods*. 2022;11(6):1–11. https://doi.org/10.3390/foods11060808
11. Cesinger MR, Schwardt NH, Halsey CR, Thomason MK, Reniere ML. Investigating the Roles of Listeria monocytogenes Peroxidases in Growth and Virulence. *American Society for Microbiology*. 2021;9(1):1–14. https://doi.org/10.1128/Spectrum
12. Ogundola AF, Akhigbe RE, Saka WA, *et al*. Contraceptive Potential of Andrographis paniculata is via Androgen Suppression and not Induction of Oxidative Stress in Male Wistar Rats. *Tissue and Cell*. 2021;73:1–10. https://doi.org/10.1016/j.tice.2021.101632
13. Zhang Y, Zhang J, Duan L. The Role of Microbiota-Mitochondria Crosstalk in Pathogenesis and Therapy of Intestinal Diseases. *Pharmacological Research*. 2022;186:1–7. https://doi.org/10.1016/j.phrs.2022.106530
14. Badawy B, Gwida M, Sadat A, *et al*. Prevalence and Antimicrobial Resistance of Virulent Listeria monocytogenes and Cronobacter sakazakii in Dairy Cattle, the Environment, and Dried Milk with the In Vitro Application of Natural Alternative Control. *Antibiotics*. 2022;11(8):1–18. https://doi.org/10.3390/antibiotics11081087
15. Mehta S, Sharma AK, Singh RK. Pharmacological Activities and Molecular Mechanisms of Pure and Crude Extract of Andrographis paniculata: An update. *Phytomedicine Plus*. 2021;1(4):1–19. https://doi.org/10.1016/j.phyplu.2021.100085
16. Li N, Xu D, Huang RH, *et al*. A New Source of Diterpene Lactones From Andrographis paniculata (Burm. f.) Nees--Two Endophytic Fungi of Colletotrichum sp. With Antibacterial and Antioxidant Activities. *Frontiers in Microbiology*. 2022;13:1–16.https://doi.org/10.3389/fmicb.2022.819770
17. Bhaisare S, Pathak S, Ajankar V V. Physiological Activities of the King of Bitters (Andrographis paniculata): A Review. *Cureus*. 2023;15(8):1–6. https://doi.org/10.7759/cureus.43515
18. Rajeshkumar S, Santhoshkumar J, Jule LT, Ramaswamy K. Phytosynthesis of Titanium Dioxide Nanoparticles Using King of Bitter Andrographis paniculata and Its Embryonic Toxicology Evaluation and Biomedical Potential. Bioinorganic Chemistry and Applications. 2021;2021:1-11. https://doi.org/10.1155/2021/6267634
19. Dappula SS, Kandrakonda YR, Shaik JB, *et al*. Biosynthesis of Zinc Oxide Nanoparticles using Aqueous Extract of Andrographis alata: Characterization, Optimization and Assessment of Their Antibacterial, Antioxidant, Antidiabetic and Anti-Alzheimer's properties. *Journal of Molecular Structure*.

2023;12731:1–15. https://doi.org/10.1016/j.molstruc.2022.134264
20. Anantharaman S, Rego R, Muthakka M, Anties T, Krishna H. Andrographis paniculata-mediated Synthesis of Silver Nanoparticles: Antimicrobial Properties and Computational Studies. *SN Applied Sciences*. 2020;2(9):1–14. https://doi.org/10.1007/s42452-020-03394-7
21. Amin R, Apriliandy SM, Purwanita P, Irsan SM. Efficacy of Sambiloto Extracts, Andrographis paniculate, (Burm. F) in Inhibiting Diabetic Retinopathy Progression: An in Vivo Study. *Reports of Biochemistry & Molecular Biology*. 2022;11(3):1–8. www.RBMB.net
22. Sandhiya V, Ubaidulla U. A Review on Herbal Drug Loaded into Pharmaceutical Carrier Techniques and its Evaluation Process. *Future Journal of Pharmaceutical Sciences*. 2020;6(1):1–16.https://doi.org/10.1186/s43094-020-00050-0
23. Muthulakshmi V, Balaji M, Sundrarajan M. Biomedical Applications of Ionic Liquid Mediated Samarium Oxide Nanoparticles by Andrographis Paniculata Leaves Extract. *Materials Chemistry and Physics*. 2020;242:1–8. https://doi.org/10.1016/j.matchemphys.2019.122483
24. Huong QTT, Nam NTH, Duy BT, *et al*. Structurally Natural Chitosan Films Decorated with Andrographis Paniculata Extract and Selenium Nanoparticles: Properties and Strawberry Preservation. *Food Bioscience*. 2023;53:1–11. https://doi.org/10.1016/j.fbio.2023.102647
25. Alkhuriji AF, Majrashi NA, Alomar S, *et al*. The Beneficial Effect of Eco-Friendly Green Nanoparticles Using Garcinia mangostana Peel Extract Against Pathogenicity of Listeria monocytogenes in Female BALB/c Mice. *Animals*. 2020;10(573):1–14. https://doi.org/10.3390/ani10040573
26. Meiyasa F, Sri B, Jenie L, Nuraida L, Tarigan N. The Ability of Lactobacillus plantarum BSL in Reducing The Tissue Damage of Liver and Spleen in Rats Infected by Listeria monocytogenes ATCC 7644. *Agrisaintifika (Jurnal Pertanian)*. 2019;3(2):88–94. doi:https://doi.org/10.32585/ags.v3i2.542
27. Wardani G, Mahmiah, Sudjarwo SA. Immunostimulatory Activity of Chitosan Nanoparticles on Wistar Albino Rats. *Pharmacognosy Journal*. 2018;10(5):892–898. https://doi.org/10.5530/pj.2018.5.150
28. Nitihapsari YG, Sari OP, Ferine M. Pengaruh Ekstrak Jintan Hitam (Nigella sativa) Terhadap TNF-a Mencit Balb-C yang Diinfeksi MRSA. In: *Pengembangan Sumber Daya Perdesaan Dan Kearifan Lokal Berkelanjutan IX*. Prosiding Seminar Nasional dan Call for Papers; 2019:139–146.
29. Rompas WF, Wewengkang DS, Mpila DA. Antibacterial Activity Test of Lamellodysidea herbacea Sponge Extract from The Waters of Poopoh Village, Minahasa Regency (Uji Aktivitas Antibakteri Ekstrak Spons Lamellodysidea herbacea dari Perairan Desa Poopoh Kabupaten Minahasa). *PHARMACON*. 2023;12(3):295–301.
30. Mutiarahmi CN, Hartady T, Lesmana R. Use of Mice as Experimental Animals in Laboratories That Refer to The Principles of Animal Welfare: a Literature Review. *Indonesia Medicus Veterinus*. 2021;10(1):134–145. https://doi.org/10.19087/imv.2020.10.1.134
31. Liu W, Liang L, Zhang Q, *et al*. Effects of Andrographolide on Renal Tubulointersticial Injury And Fibrosis. Evidence of its Mechanism of Action. *Phytomedicine*. 2021;91(153650):1–11. https://doi.org/10.1016/j.phymed.2021.153650
32. Mohammed IM, Surchee SMA, Yousif WT. Tikrit Journal for Agricultural Sciences In vivo, Antibacterial Effect of Green and Black Tea Extracts on Infected Liver and Kidney of Rats. *Journal for Agricultural Sciences*. 2019;19(4):60–75. http://tujas.tu.edu.iq
33. Husna N, Kusdarwati R, Ulkhaq MF. Bacterial Viability of Edwardsiella tarda from Silver Rasbora (Rasbora argyrotaenia) after Infection with Immmersion Methods. In: IOP Conference Series: Earth and Environmental Science. Vol 1036. *Institute of Physics;* 2022:1–5. https://doi.org/10.1088/1755-1315/1036/1/012005
34. Fakoya S, Aderoboye O, Olusola S. Phytobiotics Effects of Pawpaw (Carica papaya) Leaves and Fluted Pumpkin (Telferia ocidentalis) Leaves Extracts against Certain Aquatic Pathogens. *Medicinal & Aromatic Plants*. 2019;8(1):1–7. https://doi.org/10.35248/2167-0412.19.8.328
35. Yin HB, Chen CH, Colorado-Suarez S, Patel J. Biocontrol of *Listeria monocytogenes* and *Salmonella enterica* on Fresh Strawberries with Lactic Acid Bacteria During Refrigerated Storage. *Foodborne Pathogens and Disease*. 2022;19(5):324–331. https://doi.org/10.1089/fpd.2021.0091
36. Premanath R, Lakshmi DN. Antibacterial, Antifungal and Antioxidant Activities of Andrographis paniculata Nees. Leaves. *International Journal of Pharmaceutical Sciences and Research*. 2011;2(8):2091–2099. www.ijpsr.com
37. Yu T, Jiang X, Xu X, Jiang C, Kang R, Jiang X. Andrographolide Inhibits Biofilm and Virulence in Listeria monocytogenes as a Quorum-Sensing Inhibitor. *Molecules*. 2022;27(10):1–13. https://doi.org/10.3390/molecules27103234
38. Guo X, Zhang LY, Wu SC, *et al*. Andrographolide interferes quorum sensing to reduce cell damage caused by avian pathogenic Escherichia coli. *Veterinary Microbiology*. 2014;174(3–4):496–503. https://doi.org/10.1016/j.vetmic.2014.09.021
39. Ma L, Liu X, Liang H, *et al*. Effects of 14-alpha-lipoyl andrographolide on quorum sensing in Pseudomonas aeruginosa. *Antimicrobial Agents and Chemotherapy*. 2012;56(12):6088–6094. https://doi.org/10.1128/AAC.01119-12
40. Liang J, Huang X, Ma G. Antimicrobial Activities and Mechanisms of Extract and Components of Herbs in East Asia. *RSC Advances*. 2022;12(45):29197–29213. https://doi.org/10.1039/d2ra02389j
41. Sinha SN, Paul D. Phytosynthesis of silver nanoparticles using andrographis paniculata leaf extract and evaluation of their antibacterial activities. *Spectroscopy Letters*. 2015;48(8):600–604. https://doi.org/10.1080/00387010.2014.938756
42. Paramasivam V, Paulpandian P, Venkatachalam K, *et al*. Cytotoxicity and Antimicrobial efficiency of gold (Au) nanoparticles formulated by green approach using Andrographis paniculata leaf extract. *Journal of King Saud University – Science*. 2023;35(5):102687. https://doi.org/10.1016/j.jksus.2023.102687
43. Veldman LBM, Belt-Van Zoen E, Baars EW. Mechanistic Evidence of Andrographis paniculata (Burm. f.) Wall. ex Nees, Pelargonium sidoides DC., Echinacea Species and a Combination of Hedera helix L., Primula veris L./Primula elatior L. and Thymus vulgaris L./Thymus zygis L. in the Treatment of Acut. *Pharmaceuticals*. 2023;16(9). https://doi.org/10.3390/ph16091206
44. Li H, Liang D, Huang J, *et al*. The Bactericidal Efficacy and The Mechanism of Action of Slightly Acidic Electrolyzed Water on Listeria monocytogenes' Survival. *Foods*. 2021;10(11):1–13. https://doi.org/10.3390/foods10112671
45. Bhattacharya A. *Andrographis Paniculata Aqueous Extract Attenuates Lps-Induced Andrographis Paniculata Aqueous Extract Attenuates LPS*. National Institute of Pharmaceutical Education and Research (NIPER), Kolkata,; 2022. https://doi.org/10.13140/RG.2.2.22162.12488

46. Baru Venkata R, Prasanth DSNBK, Pasala PK, *et al*. Utilizing Andrographis paniculata leaves and roots by effective usage of the bioactive andrographolide and its nanodelivery: investigation of antikindling and antioxidant activities through in silico and in vivo studies. *Frontiers in Nutrition*. 2023;10(May):1–13. https://doi.org/10.3389/fnut.2023.1185236
47. Kumari S, Manohar Aeshala L, Kumar A, Singh S. Augmenting Antioxidative Properties of Cerium Oxide Nanomaterial with Andrographis paniculata Mediated Synthesis and Investigating its Biomedical Potentials. https://doi.org/10.37819/nanofab.009.1807
48. Jaguezeski AM, Souza CF, Perin G, *et al*. Changes in Cardiac and Hepatic Energetic Metabolism in Gerbils Infected by Listeria monocytogenes. *Microbial Pathogenesis*. 2020;138(June 2019). https://doi.org/10.1016/j.micpath.2019.103786
49. Ibrahimi ZAAR Al, Alsalame HAAA, Jawad ZN. Estimation the protective role of Andrographis paniculata aqueous extracts on nephrotoxicity in male rabbits treated with butylated hydroxytoluene (BHT). *International journal of health sciences*. Published online August 9, 2022:1775–1789. https://doi.org/10.53730/ijhs.v6ns8.11638