Effect of Electroacupuncture on Urea and Creatinine Levels in the Wistar Sepsis Model

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ABSTRACT

Objective: Sepsis is a life-threatening organ dysfunction caused by dysregulation of a host's response to infections. Sepsis—one of the most common contributing factors to acute kidney injuries in critically ill patients—is caused by bacterial endotoxins that lead to excessive production of proinflammatory cytokines. This condition can be treated with few side-effects by using electroacupuncture (EA) to regulate the neuro-endocrine immune system to control the production of these cytokines. A number of studies have proven that EA stimulates the vagus nerve to manage inflammatory responses through the cholinergic pathway, slowing sepsis. This study was conducted to investigate the effect of bilateral EA at ST 36 (*Zusanli*) on rats' renal function by measuring their levels of plasma urea and creatinine.

Materials and Methods: This study was a randomized, double-blinded, laboratory experimental post-test, with both subjects and laboratory investigators blinded. Twenty-eight male Wistar rats were divided randomly into 4 groups of 7 rats each: (1) a control group; (2) a sepsis group; (3) an EA+group; and (4) a sham EA+group. EA and sham EA was applied once for 30 minutes before intraperitoneal administration of live *Escherichia coli* bacteria ATCC 25922. Six hours after administration of the bacteria the rats' plasma urea and creatinine levels were measured.

Results: There was a statistically significant difference in the mean levels of urea ($P < 0.001$, 95% confidence interval (CI): 57.1–76.6) and creatinine ($P = 0.005$, 95% CI: 0.14–0.62) between the sepsis and control groups.

Conclusions: These findings suggest that EA pretreatment at ST 36 attenuated the induced inflammatory bacteria response and mitigated acute kidney injury.

Keywords: electroacupuncture, sepsis, anti-inflammation, acupuncture, renal function, acute kidney injury

INTRODUCTION

SEPSIS IS A LIFE THREATENING organ dysfunction caused by dysregulation of a host's response to infections.^{1,2} The most-common cause of sepsis is an infection of gram-negative or gram-positive bacteria. It is known that sepsis causes disruption of organ function through inflammatory mechanisms at the molecular, cellular, and organ levels.³

As of 2016, the number of patients with sepsis in the world was estimated to reach more than 30 million annually, with

deaths reaching up to 6 million patients.⁴ In 1999, from the identified 92 cases of sepsis in Dr. Cipto Mangunkusumo National Hospital (RSCM), the mortality rate reached 78.3% and, in 2000, it increased to 160 cases with a mortality rate of 84.4%.⁵ In 2013, data from RSCM's medical record showed that the mortality rate due to sepsis was 28%.⁶

Endotoxin or lipopolysaccharide bacteria is known as a potent mediator microbe in pathogenesis implication of sepsis. This induces the production of proinflammatory cytokines (i.e., tumor necrosis factor- α [TNF- α] and interleukin[IL]-6).

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This would activate an inflammatory process and damage tissue, which can cause organ dysfunction later and ultimately lead to septic shock.^{7,8} Long-term inflammation will cause breakdowns in tissue perfusion that affects other functions such as those in the liver and kidney. Clinical assessment of kidney function is performed by a laboratory analysis of urine for levels of urea and creatinine. Interdisciplinary intervention is required to prevent or even reduce septic damage in a patient.⁹

Current therapy is focused on early goals-directed therapy and antibiotics.¹⁰ Therapy with steroids to reduce inflammation is still controversial due to their lack of effectiveness as well as their side-effects.¹¹ Several studies have shown that acupuncture helps reduce inflammation through neurohumoral regulation and a neuroendocrine immune mechanism. Such therapy reduces proinflammatory cytokines that worsen sepsis.^{12,13}

Electroacupuncture (EA) is the electrical stimulation of acupuncture points through electrodes to needles inserted on the points. It is known that EA stimulation in septic rats reduces their levels of proinflammatory cytokines, such as TNF- α , IL-1, and IL-6, through anti-inflammatory vagus-nerve and catecholamine regulation. In addition, EA can also be given through pressure on an anti-inflammatory pathway (i.e., toll-like receptor 4 [TLR4]/nuclear factor kappa B [NF κ B]).^{12,13}

This research is the first to assess the effect of EA on plasma urea and creatinine in septic Wistar rats induced with living bacteria.

Theoretical Review

As previously discussed, sepsis is a life-threatening condition related to organ dysfunction caused by infection.^{1,2} Since 1992, systemic inflammation response syndrome (SIRS) was recognized and known as a form of sepsis caused by an infection. The criteria for SIRS are 2 or more symptoms of the following: a temperature $>38^{\circ}\text{C}$ or $<36^{\circ}\text{C}$; a respiratory rate >20 times/minute or a partial pressure of carbon dioxide <32 mmHg; a heart rate >90 times/minute; and a leukocyte count $>12,000/\text{mm}^3$ or $<4,000/\text{mm}^3$ or $>10\%$ immature leukocytes.¹⁴ Severe sepsis is a condition of acute organ dysfunction, hypoperfusion, or hypotension.¹ Acute kidney injury often occurs in sepsis, which leads to an increased risk of mortality. There is also a decrease in microvascular perfusion in the kidney, tubular dysfunction that affects blood clearance, and a decrease of urine production. This is due to the decrease of cardiac output, followed by an inflammation-mediated cytokine mechanism and dysregulation of immune responses.¹⁵

Endotoxin or the lipopolysaccharide (LPS) bacterium is known as a potent microbial mediator in the pathogenesis of sepsis.¹⁶ LPS will bind to protein in the plasma called lipopolysaccharide-binding protein (LBP), which then will bind with CD14 molecules found on the cell surfaces and in

plasma. LPS can also be identified directly by MD-2, a component of TLR4. LPS binds to TLR4 on the membranes of immune cells, and these receptors are activated through the dependent pathway of MyD88, which subsequently activates NF κ B and gene mitogen-activated protein kinase (MAPK). This gene releases proinflammatory cytokines, including IL-8, IL-6, IL-1 β , IL-1, IL-12, and IFN- γ .^{17,18}

In sepsis, severe inflammation occurs and interferes with mitochondrial function in various cells. Excessive production of nitric oxide (NO), carbon monoxide, hydrogen sulfide, and various reactive oxygen species inhibits the respiratory chain directly and causes damage to mitochondrial proteins and various other structures, such as lipid membranes.¹⁹

Urea is an endproduct of catabolism of proteins and amino acids produced by the liver and distributed through intracellular and extracellular fluids to the blood for later filtration by the glomerulus. Urea level is one of the parameters used to detect acute kidney failure. Creatinine is the result of the breakdown of muscle creatine phosphate and is used to measure glomerular filtration ability and to monitor the course of kidney disease.²⁰

EA provides electrical stimulation at an acupuncture point through electrode placement on the inserted needle at the acupuncture point. After the needle is inserted, sometimes, there will be a sensation often described as a feeling of heaviness, tingling, and aching on the acupuncture point. Electrical stimulation flows through a pair of needles that are placed at the acupuncture point for 20–30 minutes.²¹

One advantage of EA, compared to manual acupuncture, is that EA can provide a stable and standardized stimulus. Another advantage is that EA can provide specific stimuli according to the desired frequency and effects and can also provide strong stimulation with a minimum possibility of damaging the surrounding tissue, which can occur in manual acupuncture if excessive stimulation is given.²²

Data from previous studies have indicated that EA stimulates neuronal activation at the acupuncture point, the signal is passed onto the spinal cord afferent nerve and then to a higher level (the brain), and triggers the autonomic nervous system to regulate the inflammatory response in the target organ. Stimulation of the vagus nerve activates the nicotinic receptor of acetylcholine on target cells to regulate the inflammatory response. Stimulation of EA at acupuncture points is known to activate neurons in the nucleus tractus solitarius (NTS). Areas of the brainstem that receive stimuli from the somatic nerve and input from the vagus afferent can be seen from immunostaining c-Fos and recording the electrophysiologic activity.²² EA stimulation increases neuron activity in the NTS. Together, these 2 stimuli will, in turn, stimulate efferent activity, producing anti-inflammatory effects.^{23,24}

Hu et al.²⁵ showed that EA at ST 36 (*Zusanli*) had a protective effect against organ damage by decreasing proinflammatory cytokines; decreasing creatinine, alanine aminotransferase (ALT), TNF- α , NO, and myeloperoxidase

activity; and reducing organ damage and dysfunction in rats with sepsis; this research supported the preventive effect of EA against multiple organ dysfunction syndrome. Zhao et al.²⁶ found that EA can reduce serum TNF- α and IL-6 levels, and regulate NF κ B expression in mice with endotoxemia through LPS induction.

MATERIALS AND METHODS

This study was a randomized, double-blinded, laboratory experimental post test conducted in December 2018. The study was conducted at the Experimental Animal Laboratory, of the Biomedical Research Center and Basic Health Technology, Research and Development Agency, at the Ministry of Health of the Republic of Indonesia.

The population for this study was 28 male Wistar rats that met the inclusion criteria. The inclusion criteria for the population were 8–12 weeks' old male *Rattus norvegicus* Wistar rats weighing 250–350 g. The rats had to be active with good appetites (active movement, with hair that was not dull or falling out, and not bald).^{12,27,28} Exclusion criteria were inflammation and infection in the area of the acupuncture point³⁰ and signs of systemic inflammation.^{14,27,28}

The bacteria—*Escherichia coli* ATCC—were obtained from the Microbiology Department, Faculty of Medicine and Health, Atma Jaya Catholic University of Indonesia. One mL of bacteria with a concentration of 2×10^{10} colony-forming units (cfu)/mL in saline was given via an intraperitoneal (IP) injection to each rat in the sepsis groups of the 4 groups that the rats were divided into.

The sample size was estimated using the calculation formula for experimental studies based on Federer²⁹ and taking dropouts into account, resulting in a total sample size of 28 rats. The rats were randomly allocated using a computer-based random-number table into 4 groups: (1) a control group (not given any treatment); (2) a sepsis or negative control group (given live bacterial injection); (3) an EA + group (EA for 30 minutes before bacterial injection); and (4) a sham EA + group (sham EA for 30 minutes before bacterial injection).

The 4 groups were given the same anesthetic agent using intramuscular injection of ketamine (0.075 mg/g body weight) and xylazine (0.02 mg/g body weight). In the control group, the rats did not receive any treatment. In the sepsis group, the rats were given injection of 1 mL of *Escherichia coli* ATCC 25922 live bacteria 2×10^{10} cfu/mL IP.

In the EA + group, after asepsis and antisepsis, acupuncture needles were inserted into ST 36 bilaterally and stimulated at 2 Hz for 30 minutes. After 30 minutes, the rats were given an IP injection of 1 mL of the bacteria. AWQ-104L was used as electrostimulation device. In the sham EA + group, after asepsis and antisepsis, an adhesive pad was placed on the acupuncture point and the needle punctured the pad without penetrating the skin and electrical

stimulation was not turned on. After 30 minutes, the rats were given an IP injection of 1 mL of the bacteria.

Six hours after the bacterial injection, a blood-sample test was performed and an examination of each rat's urea and creatinine levels was also performed. Then, the rats in all of the groups were euthanized, and certain tissues and all organs were stored for further research. Experimental animals are euthanized by taking as much blood as possible from the heart (exsanguination). The flow of the research can be seen in Figure 1.

The data from the tests were then entered into a table and processed by statistical testing using SPSS 20.0.

Note that this research was approved by the Medical Research Ethics Committee of FKUI-RSCM Jakarta No: 1292/UN2.F1/ETIK/2018. In conducting this research, researchers applied the 3R principles in the research protocol described as Replacement, Reduction, and Refinement.³⁰

RESULTS

This study was a randomized, double-blinded, laboratory experimental post test conducted on 28 rats that met the study's inclusion criteria. All rats were randomly allocated into the 4 groups. Each group had 7 rats in it and there were no dropouts, so a total of 28 rats completed the study.

All of the basic characteristics of the rats in the four groups were tested for normality of the data using the Saphiro–Wilk test; the results were $P > 0.05$ for each characteristic. To assess the mean differences among the 4 groups, a one-way analysis of variance (ANOVA) was performed. The normality test showed the basic characteristics data. The mean ages were as follows: control group, 9.9 ± 1.2 weeks; sepsis group, 9.43 ± 0.9 weeks; EA + group, 10 ± 0.8 weeks, and sham EA +, 9.4 ± 1.2 weeks. The mean differences of these 4 groups were not statistically different ($P = 0.676$). For body weight, the mean weights were as follows: control group, 290.15 ± 27.3 g; sepsis group, 285.86 ± 18.9 g; EA + group, 284 ± 15.43 g; and sham EA + group, 288.71 ± 23.8 g. There were no significant differences among the groups with respect to body weight ($P = 0.952$).

The mean differences in the temperatures and heart rates (HRs) among the 4 groups were not statistically significant ($P = 0.349$).

To summarize, the mean differences in age, weight, temperature, and HRs in the 4 groups were not statistically significant differences. Hence, the mean differences in these characteristics in the 4 groups were not statistically significant ($P = 0.785$). Table 1 shows these basic characteristics; it was concluded that the groups were comparable because there were no statistically significant mean differences in these characteristics.

A test of homogeneity variances showed a P -value < 0.05 ; therefore, an ANOVA test with *post-hoc* Tamhane testing was chosen.

TABLE 1. BASELINE CHARACTERISTICS OF THE MALE WISTAR RATS

Characteristics	Groups				P-value
	Control (n = 7)	Sepsis (n = 7)	EA + sepsis (n = 7)	Sham EA+ sepsis (n = 7)	
Age in wks (Mean $\pm$ SD)	9.9 $\pm$ 1.2	9.43 $\pm$ 0.9	10 $\pm$ 0.8	9.4 $\pm$ 1.2	0.676*
Body weighting (Mean $\pm$ SD)	290.15 $\pm$ 27.3	285.86 $\pm$ 18.9	284 $\pm$ 15.43	288.71 $\pm$ 23.8	0.952*
Temperature in $^{\circ}$ C (Mean $\pm$ SD)	35.53 $\pm$ 0.08	35.56 $\pm$ 0.09	35.6 $\pm$ 0.21	35.6 $\pm$ 0.07	0.349*
Heart rate in bpm (Mean $\pm$ SD)	352.86 $\pm$ 21.4	348.6 $\pm$ 24.1	352.86 $\pm$ 21.4	360 $\pm$ 16.3	0.785*

*One-way analysis of variance (ANOVA) statistical test.

Note: Based on the data shown here, the current authors concluded that there were no statistically significant differences ($P > 0.05$) in the basic characteristics of the rats among the 4 groups; therefore, the 4 groups were comparable. The test of homogeneity variances shows a P -value < 0.05 ; therefore, an ANOVA test with *post-hoc* Tamhane testing was chosen.

EA, electroacupuncture; wks, weeks; bpm, beats per minute; SD, standard deviation.

The one-way ANOVA statistical test results shown in Table 2 revealed a P -value of < 0.001 for both kidney injury markers—urea and creatinine. From these data, the current authors concluded that there were at least 2 groups who had significant differences in both urea and creatinine. Therefore, *post-hoc* analysis was performed to investigate which groups had significant differences.

Table 3 shows the results from the *post-hoc* Tamhane's test for urea levels in all 4 groups. This article only reviews some of the important groups. The urea levels between the control group and the sepsis group had a mean difference of 67.2 mg/dL with a P -value < 0.001 . The sepsis group and the EA + group had a mean difference of 48.9 mg/dL with a P -value < 0.001 . The sepsis group and the sham EA + sepsis group had a mean difference of 7.4 mg/dL with a P -value of 0.15. Meanwhile, the EA + group and the sham EA + sepsis group had a mean difference of 41.5 mg/dL with a P -value < 0.001 . The EA + sepsis group had shown a significant difference, compared with the sham EA + sepsis group with respect to urea levels. These results indicated that EA might

have protective effect against sepsis that is indicated by the differences in urea levels in this Wistar sepsis model.

The *post-hoc* Tamhane's test for creatinine levels in all groups is shown in Table 4. The creatinine levels between the control group and the sepsis group had a mean difference of 0.38 mg/dL with a P -value < 0.005 . The sepsis group and the EA + sepsis group had a mean difference of 0.34 mg/dL with a P -value < 0.007 . The sepsis group and the sham EA + sepsis group had a mean difference of 0.07 mg/dL with a P -value of 0.964. Meanwhile, the EA + sepsis group and the sham EA + sepsis group had a mean difference of 0.27 mg/dL with a P -value < 0.009 . The EA + sepsis group had a significant difference, compared with the sham EA + sepsis group with respect to creatinine levels.

Both urea and creatinine levels in the group who received EA + sepsis were significantly lowered compared to the sham EA + sepsis group. Therefore, EA might have produced a protective effect against kidney injury as indicated by the differences in urea and creatinine levels in this Wistar sepsis model.

TABLE 2. MEAN DIFFERENCES IN UREA AND CREATININE LEVELS IN ALL GROUPS

Characteristics	Groups				P-value*
	Control (n = 7)	Sepsis (n = 7)	EA + sepsis (n = 7)	Sham EA + sepsis (n = 7)	
Urea (mg/dL)	32.56 $\pm$ 4.3	99.57 $\pm$ 6.5	50.68 $\pm$ 7.3	92.19 $\pm$ 3.6	< 0.001
Creatinine (mg/dL)	0.28 $\pm$ 0.02	0.67 $\pm$ 0.17	0.33 $\pm$ 0.05	0.60 $\pm$ 0.14	< 0.001

*One-way analysis of variance (ANOVA) statistical test.

Note: The one-way ANOVA statistical test showed a P -value of < 0.001 for both kidney-injury markers urea and creatinine. From these data the current authors concluded that there were at least 2 groups that had significant differences in both urea and creatinine. Therefore, *post-hoc* analysis was performed to determine which groups had significant differences.

EA, electroacupuncture.

TABLE 3. *Post-Hoc* ANALYSIS OF MEAN DIFFERENCES IN BLOOD UREA LEVELS IN ALL GROUPS

Groups	Mean difference (mg/dL)	95% confidence interval		P-value*
		Minimum	Maximum	
Control vs. sepsis	67.2	57.1	76.6	< 0.001*
Control vs. EA + sepsis	18.1	7.6	28.7	< 0.001*
Control vs. sham	59.6	52.9	66.4	< 0.001*
EA + sepsis				
Sepsis vs. EA + sepsis	48.9	37.2	60.6	< 0.001*
Sepsis vs. sham	7.4	1.9	16.8	0.15*
EA + sepsis				
EA + sepsis vs. Sham	41.5	31.1	51.9	< 0.001*
EA + sepsis				

**Post-hoc* Tamhane's test. Number of rats per group=7.

Note: This table shows the results from the *post-hoc* Tamhane's test for urea levels in all groups. The EA + sepsis group had shown a significant difference, compared with the sham EA + sepsis group in urea levels. From these results, the current authors suggested that that EA might have a protective effect against kidney damage due to sepsis.

EA, electroacupuncture.

TABLE 4. *Post-Hoc* ANALYSIS OF MEAN DIFFERENCES IN BLOOD CREATININE LEVELS IN ALL GROUPS

Groups	Mean difference (mg/dL)	95% confidence interval		P-value*
		Minimum	Maximum	
Control vs. sepsis	0.38	0.14	0.62	0.005*
Control vs. EA + sepsis	0.04	0.03	0.11	0.52*
Control vs. sham	0.31	0.11	0.52	0.006*
EA + sepsis				
Sepsis vs. EA + sepsis	0.34	0.11	0.58	0.007*
Sepsis vs. sham	0.07	0.19	0.33	0.964*
EA + sepsis				
EA + sepsis vs. sham	0.27	0.07	0.48	0.009*
EA + sepsis				

**Post-hoc* Tamhane's test. Number of rats per group=7.

Note: The *post-hoc* Tamhane's test for creatinine level in all groups is shown in this table. The EA + sepsis group had shown a significant difference, compared with the sham EA + sepsis group in creatinine levels. Both urea and creatinine levels in the groups who received EA were significantly lower, compared to the sham EA + sepsis group. Therefore, EA might have a protective effect against kidney injury.

EA, electroacupuncture.

DISCUSSION

This study was a randomized, double-blinded laboratory post-test performed on 28 rats who met the study's inclusion criteria. The rats and the laboratory assessors did not know the allocation of the rats into the 4 groups. All rats remained until the end of the study.

This is the first study in Indonesia to use EA modalities in experimental animals with sepsis to measure blood urea and creatinine level as markers of acute kidney injury in sepsis. Sepsis induction was performed by IP injection of live *E. coli* ATCC 25922 2×10^{10} cfu/mL, which was known from previous studies to induce an inflammatory process leading to sepsis.^{31,32}

The use of EA in this study had an advantage because the stimulation given to the acupuncture points was controlled precisely. The 2-Hz frequency is mediated by μ and δ opioid receptors that are known to stimulate the release of endogenous β -endorphin in a manner similar to that of manual-acupuncture stimulation.²¹

Several sepsis studies in experimental animals have shown promising results and might resemble human immune responses to sepsis. The difference is that experimental animals, such as mice or rats, need 1000 to 10,000 times higher doses of bacteria than a human would need to induce symptoms of systemic inflammation and release of inflammatory cytokines into the circulation as responses to

sepsis. Several reasons for the research conducted on rats are the ability to minimize the diversity of research subjects, research variables can be controlled, samples can be obtained maximally for research purposes, and research can be performed in high-risk conditions.^{33,34}

Live bacterial injection will trigger the systemic inflammation responses that involve responding to the LPS in the cell walls of gram-negative bacteria. When bacteria are inoculated into a rat intravenously (IV) or IP, this will trigger the systemic inflammatory response to LPS that resembles the responses of patients with sepsis.^{33,34}

The male Wistar rats, ages 10–12 weeks, weighing 250–350 g, as per to previous studies.^{12,27} The ages of the rats in the current study ranged from 8 to 12 weeks as these are the ages of adult rats, and their body weights ranged from 250 to 300 g. These criteria were met so the expected responses would resemble that of human adults. Male rats were chosen to reduce the risk of bias due to hormonal influences in female rats. It is important to analyze the basic characteristics of temperature and HR because these 2 parameters are included in SIRS and sepsis diagnostic criteria.¹⁴ The ANOVA test was performed to analyze 4 four basic characteristics and the 4 groups did not have statistically significant differences in age ($P=0.676$), body weight ($P=0.952$), temperature ($P=0.349$), and HR ($P=0.785$). From these data, it can be concluded that the 4 groups were comparable.

FIG. 1. Twenty-eight male Wistar rats who met the study's inclusion criteria were randomly divided into 4 groups, (1) a control group; (2) a sepsis group; (3) an electroacupuncture (EA) + sepsis group (EA for 30 minutes before bacterial injection); and (4) a sham EA + sepsis group (sham EA for 30 minutes before injection of bacteria). Six hours after the bacterial injection, a blood-sample test was performed and examinations of urea and creatinine levels were also performed. The data were then entered into a table and processed by a statistical analysis.

In sepsis, there is an inflammatory process triggered by LPS from the bacterial wall. LPS binds to TLR4 on the surfaces of the membrane of immune cells, and activation of this receptor is mediated by the dependent MyD88 pathway, which, in turn, will activate NF κ B and MAPK and cause the release of proinflammatory cytokines. Activation of the MAPK pathway will induce activator protein-1 transcription factors known to have important roles in the expression of proinflammatory cytokines.^{17,18}

The hyper-inflammation condition in sepsis can damage various organs' functions. The condition of acute kidney injury often occurs in sepsis and certainly increases the mortality rate. Decreased microvascular perfusion in the kidneys and tubular dysfunction can affect blood clearance and decrease urine production. This problem is also due to the decrease in cardiac output accompanied by inflammation mediated by the mechanism of cytokines and body immunity.^{35,36} According to these mechanisms, and results

of previous studies,²⁸ the researchers in this study chose urea and creatinine parameters to be assessed, because both of these levels can give an idea of kidney functionality.

Previous research by Huang et al.,²⁸ included 120 rats randomly divided into 10 groups. Treatment with manual acupuncture at ST 36 lasted for 30 minutes, and twirling stimulation was given with a frequency of 60 cycles for 1 minute, followed by a 1-minute rest. Treatment was divided into 2 groups: before and after LPS-induction groups. LPS injection was given at a dose of 20 mg/kg body weight. In the after group, acupuncture was given 3 hours after induction, and, 6 hours, later all 10 groups were terminated. This previous study assessed blood concentrations of urea, creatinine, aspartate aminotransferase, ALT, and total bilirubin to kidney and liver damage. Histopathologic analysis and tests of levels of myeloperoxidase, NO, and inducible NO synthase (iNOS) mRNA were also performed. In addition, HR monitoring was also performed by measuring mean arterial

pressure. The researchers in that study concluded that the pretreatment of ST 36 had a protective effect on the kidneys and liver, while there was no effect of post-treatment acupuncture.²⁸

The current study had better results of kidney-function improvement in the EA+sepsis group. There were significant decreases in urea ($P < 0.001$) and creatinine ($P = 0.007$) levels, compared to the non-EA groups. This study is in line with Huang et al.,²⁸ in which acupuncture treatment before sepsis induction had a protective effect against kidney injury associated with sepsis, as measured by blood urea and creatinine levels. However, the current study differed from Huang et al., because EA was used at ST 36 and IP live *E. coli* were used for sepsis induction.

Gu et al.²⁷ discovered that EA therapy at PC 6 (*Neiguan*) and ST 36 had anti-inflammatory effects, hence EA produced a renal-protective effect. That study used 60 male Wistar rats who were given a 5 mg/kg IV LPS injection. A frequency of 2 Hz with an intensity of 1 mA were used and lasted for 30 minutes. EA therapy was given 20 minutes before the LPS injection. Two hours after sepsis induction, the rats were terminated, and an evaluation of the protective effects on the rats' kidneys was performed by assessing urea, creatinine, and renal histopathology.

The current study showed that EA using 1 point—ST 36—has the same effect as Gu et al.'s study for the renal-protective markers—urea and creatinine. There was a difference in the method of sepsis induction, as the current study used live bacteria, while Gu et al.'s study used LPS.

In veterinary medicine, a clear definition of sepsis has not been standardized. Therefore, sepsis refers to the clinical diagnosis based on the signs of inflammation. Sepsis is a condition when the animal has an infection accompanied by signs and symptoms of toxicity (pathologic temperature changes in the forms of hyperthermia or hypothermia, anorexia, decreased motor activity, lethargy, chills, and piloerection) that occurred rapidly with severe progression in one study.³⁷ Clinical progression with disruption of the cardiovascular system, increased inflammatory cytokine levels, and death within 12–24 hours after LPS injection occurred in another study.³⁸ Several animal studies adapted the SIRS criteria for sepsis diagnosis.^{31,39}

In this current study, in addition to assessing kidney function for inflammation, systemic inflammatory parameters, according to the SIRS criteria for temperature, HR, and leukocyte count, were also assessed. In assessing the mean temperatures in the 4 groups, it was noted that there was an increase of temperature in the sepsis group, the EA+sepsis group, and the sham EA group, compared with the control group (who did not receive bacterial injection). The mean temperatures were as follows: control group, 35.7°C; sepsis group, 37°C; the EA+sepsis group, 36.1°C, and the sham EA+sepsis group, 37.1°C. It was observed that EA+sepsis group had a lower mean temperature and that was even close to the temperature in the control group, compared with the sepsis and the sham EA+sepsis group.

In line with the decrease in temperature, decreased HR can also be observed. In the mean HR assessment of the 4 groups, it was noted that there was an increased HR in the sepsis group, the EA+sepsis group, and the sham EA+sepsis group, compared with the control group. The mean HRs were as follows: control group, 351 beats per minute (bpm); sepsis group, 428 bpm; EA+sepsis group, 364 bpm; and the sham EA+sepsis group, 434 bpm. It was observed that the EA+sepsis group had the lowest HR among the other groups, compared to the sepsis group.

The inflammatory markers often used in studies are IL-6, IL-1 β , TNF- α , C-reactive protein, and leukocyte count. In this current study, leukocyte counts were performed. The mean count in the sepsis group was 2879/ μ L while in EA+sepsis group the count was 6806/ μ L. One of the criteria in sepsis conditions is a decrease in leukocyte count of $>4000/\mu$ L; this occurred in both the sepsis and sham EA+sepsis groups. Meanwhile, the average number of leukocytes in the EA+sepsis group was almost similar to that of the control group.

The acupuncture point used in this study was bilateral ST 36. Huang et al.²⁸ and Gu et al.²⁷ showed that acupuncture at ST 36 can reduce levels of inflammatory cytokines, such as TNF- α , IL-1 β , iNOS, and NF κ B. ST 36 also has been reported to increase adrenocorticoid hormone, which is known to inhibit the release of NF κ B, an important transcription factor for various proinflammatory cytokines. Stimulation of ST 36, in accordance with its dermatomes of the L-2–L-5 segments, will stimulate the segmental parasympathetic and central nervous systems, provide stimulus to NTS, and activate the vagus nerve which has anti-inflammatory effect through the cholinergic pathway by binding acetylcholine with the $\alpha 7$ nAChR receptors, expressed in tissue macrophages. This interaction will inhibit the release of inflammatory cytokines by regulating NF κ B.^{40,41}

Acute kidney injury from sepsis contributes to 70% of deaths. Bacterial LPS is known to be the main initiator of inflammation and hemodynamic alteration in sepsis and is closely related to acute renal impairment. A study by Yu et al. found that the endotoxin produced by bacteria can cause an increase in TNF- α and free radicals, both of which are thought to be the main causes of acute kidney injury in sepsis conditions. It has been proven that stimulation of the vagus nerve with EA can modulate systemic inflammatory responses to endotoxin, inhibit the synthesis of TNF- α , and prevent the development of shock. In addition, EA is known to have renal-protective effects through increasing activity of antioxidant enzymes and decreasing the production of lipid peroxide.⁴¹

The results of this current study showed a statistically significant difference in the mean urea levels between the sepsis group and the EA+sepsis group ($P < 0.001$), and between the EA+sepsis group and the sham EA+sepsis group ($P < 0.001$). There was also a statistically significant differences in the mean creatinine levels between the sepsis group

and the EA+ sepsis group ($P=0.007$), between EA+sepsis group and the sham EA+sepsis group ($P=0.009$), and between the sepsis group and the sham EA+sepsis group ($P=0.964$). In addition to the leukocyte counts, the EA+sepsis group had a better leukocyte count, compared with the sepsis group and the sham EA+sepsis group. Based on the data obtained in this study, it can be said that EA has a renal-protective effect through an anti-inflammatory mechanism, so EA can prevent further kidney damage that can cause death.

The results of this study are expected to be used as reference data for further research in both experimental animals and humans. In sepsis, it is known that the release of various inflammatory cytokines can cause multiorgan damage, which can eventually cause death. EA therapy can be given before a patient develops a septic condition, so EA is expected to have protective effects on various organs, especially kidney organs, as reflected by the improvements of urea and creatinine levels.

One of the limitations of this study was that it did not examine other inflammatory biomarkers and did not assess other organ functions, such as the heart, lungs, intestines, and liver. Grading can also be done gradually, adjusted with the monitoring time. These limitations were none other than due to the limited funds and time of the study.

CONCLUSIONS

EA has a renal-protective effect that can be seen from decreased urea ($P<0.001$) and creatinine ($P=0.007$), compared with groups of septic rats who did not receive EA therapy.

Further research is needed, using more-sensitive biomarker inflammatory examinations (such as IL-6, TNF- α , IL-1 β , IL-10, iNOS, and NF κ B), and performing examinations on the function and histopathology of other organs related to sepsis (such as the liver, heart, and intestines). Follow-up studies can also be developed using other sepsis-induction methods that resembles the condition of sepsis in humans more, such as cecal ligation and puncture.

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