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The effects of ethyl acetate fraction of *Moringa oleifera* leaves on kidney and liver function in sepsis rat model



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ABSTRACT

Backgrounds: Life-threatening organ dysfunction such as sepsis often happens in developing and developed countries. Ethyl acetate fraction of *Moringa oleifera* leaf (MO-EA fraction) is claimed by several studies to be able to control inflammation and oxidative stress.

Objective: This study aims explore the effect of MO-EA fraction in improving the liver and kidney function in sepsis rat model.

Materials and Methods: This study is an experimental research using sepsis rat model. The research was conducted on a total of 66 white Wistar rats aged 3-4 months, weighing 200-300 grams, randomly allocated into nine treatment groups, negative, and normal control groups. The treatment and the negative control group received LPS induction to reproduce the inflammation stress during a sepsis. The treatment groups receive various daily dose of MO-EA fraction starting of 10, 20, and 40 mg/kgBW. The measured

outcome were serum creatinine, ureum, serum glutamic oxaloacetic transaminase (SGOT), and serum glutamic pyruvic transaminase (SPGT).

Results: There are statistically significant differences of the level of ureum, creatinine, SGOT, and SPGT between various treatment group receiving various dose of MO-EA fraction and negative and normal control group within seven days after LPS induction. $\alpha=0.05$.

Conclusion: The serum level of ureum, creatinine, SGOT and SGPT in the sepsis rats administered with MO-EA were lower than the control group. The best MO-EA fraction is A2 which was 20 mg/kgBW/day given from 5 days before the LPS induction continuing to the seventh day after the LPS induction. MO-EA fraction supplementation may have a potential as a preventive therapy for endothelial stress in sepsis rat model.

Keywords: MO-EA, Sepsis, *Moringa oleifera*

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INTRODUCTION

Sepsis has caused one-third to one-half deaths in hospitalised patients.¹ The pathogenesis of sepsis includes dynamic and complex processes of cellular activation (neutrophils, monocytes and microvascular endothelial cells) as well as trigger mechanisms in the neuroendocrine system, complement activation, activation of coagulation and fibrinolytic systems resulted from an infection. The inflammation caused endothelial stress in the aortic and renal proximal tubules which resulting in multiple organ dysfunction. Multiple organ dysfunction may causes elevation in serum ureum and creatinine level, liver enzymes and histological changes of the endothelium.²

The damage to microvascular endothelial glycocalyx aggravates sepsis can be to septic shock with the release protein of heparanase, syndecan-1, sulfate, endocan and angiopoietin. Many studies have been conducted for addition of anti-inflammatory drugs to standard antimicrobial therapy. It is investigated that the administration of small doses of steroids in mice in the sepsis model is able to significantly reduce the expression of NF- κ B and caspase-3.³

Thus, these suggested that the reduction of inflammation process may prevent endothelial damages in sepsis.⁴

Moringa oleifera, a plant that is widely available in Indonesia and known as a traditional medicine, is claimed to be able to suppress inflammation.⁵ However, there is a need for further research in the effects of *Moringa* leaves in patients with sepsis and septic shock. Hence, the researchers are interested in studying the anti-inflammatory and ROS reducing properties of the MO-EA fraction in sepsis rat model. This study aims to analyse the effect of MO-EA fraction on decreasing levels of ureum, creatinine, serum glutamic oxaloacetic transaminase (SGOT), and serum glutamic pyruvic transaminase (SPGT).

MATERIALS AND METHODS

This study is an experimental research to analyze the effect of MO-EA fraction in sepsis rat model. We received the approval from Dr. Moewardi General Hospital Health Research Ethics Committee (ethical clearance letter no. 1.272/XI/HREC/2019).

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A total of 66 Wistar white male rat three to four month old with bodyweight between 170 and 200 grams were obtained from the Inter Faculty Center, Universitas Gajah Mada, Indonesia. During the study, the rats were fed with standard BR1 which doses were adjusted to the average weight of their body, but their drinks were not limited (*ad libitum*). These rats were randomly allocated to nine different treatment groups (A1, A2, A3, B1, B2, B3, C1, C2, C3), a positive control group (KP), and a negative control group (KN). Every group received several different procedures described in Table 1. MO-EA feeding was started at the earliest on Day -7.

To recreate a sepsis condition in the rats, we performed LPS induction with intraperitoneal injection of 0.25 mg lipopolysaccharide (LPS) per kilogram bodyweight on Day 0 in all of the groups except KN group.

The serum concentration of ureum, creatinine, SGOT and SPGT levels were measured on the day when the rats were sacrificed. The rats were sacrificed on the Day +7 of the LPS induction.

The data were analyzed using SPSS version 22. When the data are normally distributed, the numerical variable will be analysed using ANOVA test. Otherwise, the Mann Whitney U test will be used. The post-hoc analysis will be conducted with Least Significant Difference (LSD) test. A p-value of less than 0.05 is considered significant.

RESULTS
The Effects of Ethyl Acetate Fraction of *Moringa Oleifera* Leaves on Ureum Levels

The lowest ureum level was found in group KN (10.37±0.22 mg/dL) and the highest in group KP (41.93±1.20 mg/dL). Among the MO-EA treated groups, the lowest ureum level was found in group

A2 (12.25 ± 0.64 mg/dL), and the highest was group C1 (37.80 ± 0.38 mg/dL). Figure 1 and Table 2 depict the level of ureum in each group and the post-hoc analysis comparing ureum level in each group. There is no statistically significant difference in the ureum levels between group A1 and B2, and

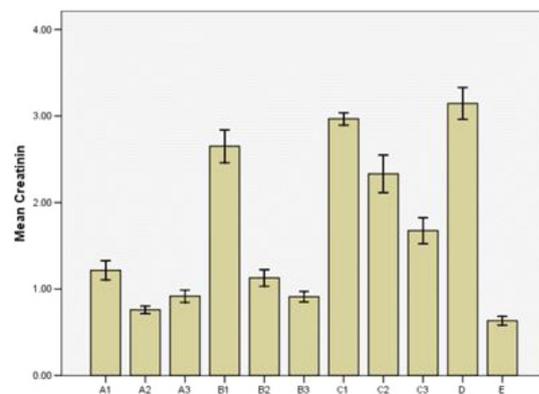


Figure 1 A bar diagram depicting the average ureum level in each group

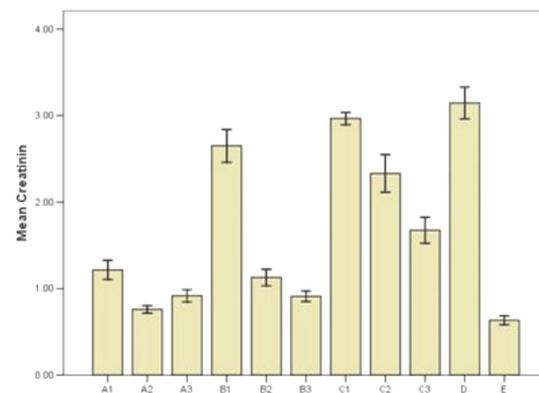


Figure 2 A bar diagram depicting the average creatinine level in each group

Table 1 The groups and the various in vivo treatment received

Group	Number of rats	LPS induction	MO-EA daily dose	MO-EA supplementation	
				Day started	Day ended
A1	6	Yes	10 mg/kgBW	Day -5	Day +7
A2	6	Yes	20 mg/kgBW	Day 0	Day +7
A3	6	Yes	40 mg/kgBW	Day +3	Day +7
B1	6	Yes	10 mg/kgBW	Day -5	Day +7
B2	6	Yes	20 mg/kgBW	Day 0	Day +7
B3	6	Yes	40 mg/kgBW	Day +3	Day +7
C1	6	Yes	10 mg/kgBW	Day -5	Day +7
C2	6	Yes	20 mg/kgBW	Day 0	Day +7
C3	6	Yes	40 mg/kgBW	Day +3	Day +7
KN	6	No	None	-	-
KP	6	Yes	None	-	-

KN: Normal Control, KP: Positive Control

Table 2 Ureum serum level in each group on Day +7 from the LPS induction

Group	Ureum level in mg/dL (mean±SD)	LSD tests
A1	17.46 ± 0.41	a,b
A2	12.25 ± 0.64	c
A3	13.99 ± 0.62	d
B1	20.83 ± 1.01	e
B2	18.20 ± 0.51	a,f
B3	16.47 ± 0.88	b,g
C1	37.80 ± 0.38	h
C2	31.55 ± 1.90	i
C3	23.07 ± 1.62	j
KP	41.93 ± 1.20	k
KN	10.37 ± 0.22	l

KN: Normal Control, KP: Positive Control

Notes: The statistically significant difference in mean±standard deviation according to LSD test is shown when the mean± standard deviation is followed by different letter. When mean± standard deviation was followed by the same letter, it means that there is no significant difference statistically.

Table 3 Creatinine serum level in each group on Day +7 from the LPS induction

Group	Creatinine level in U/L (mean±SD)	LSD tests
A1	1.22 ± 0.06	a
A2	0.76 ± 0.02	b
A3	0.92 ± 0.04	c
B1	2.65 ± 0.09	d
B2	1.13 ± 0.05	e
B3	0.91 ± 0.03	c,f
C1	2.97 ± 0.04	g
C2	2.33 ± 0.11	h
C3	1.67 ± 0.08	i
KP	3.14 ± 0.09	j
KN	0.63 ± 0.02	k

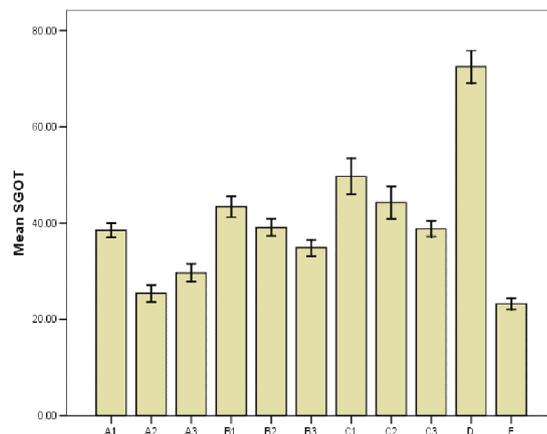
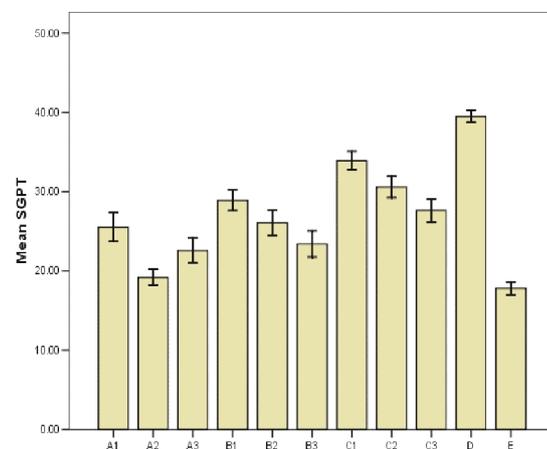
KN: Normal Control, KP: Positive Control

Notes: The statistically significant difference in mean±standard deviation according to LSD test is shown when the mean± standard deviation is followed by different letter. When mean± standard deviation was followed by the same letter, it means that there is no significant difference statistically.

A1 and B3. However, other comparisons showed statistically significant differences between each groups (p-value <0.05)

The Effects of Ethyl Acetate Fraction of *Moringa Oleifera* Leaves on Creatinine Levels

The lowest creatinine level was found in group KN (0.63±0.02 U/L) and the highest in group KP. (3.14±0.09 U/L). Among the MO-EA treated

**Figure 3** A bar diagram depicting the average SGOT level in each group**Figure 4** A bar diagram depicting the average SGPT level in each group

groups, the lowest creatinine level was found in group A2 (0.76± 0.02 U/L), and the highest was group C1 (2.97 ± 0.44 U/L). Figure 2 and Table 3 depict the level of creatinine in each group and the post-hoc analysis comparing creatinine level in each group. There is no statistically significant difference in the creatinine levels between group A3 and B3. However, others showed statistically significant differences when compared between groups (p-value <0.05)

The Effects of Ethyl Acetate Fraction of *Moringa Oleifera* Leaves on SGOT Levels

The lowest SGOT level was found in group KN (23.22±0.57 U/L) and the highest in group KP. (72.50±1.70 U/L). Among the MO-EA treated groups, the lowest SGOT level was found in group A2 (25.41±0.90 U/L), and the highest was group C1 (49.68±1.86 U/L). Figure 3 and Table 4 depict the level of SGOT in each group and the post-hoc analysis comparing creatinine level in each group. There is no significant difference in the SGOT levels

Table 4 SGOT serum level in each group on Day +7 from the LPS induction

Group	SGOT level in U/L (mean±SD)	LSD tests
A1	38.52 ± 0.73	a,b
A2	25.41 ± 0.90	c
A3	29.70 ± 0.94	d
B1	43.37 ± 1.09	e
B2	39.08 ± 0.91	a,f,g
B3	34.88 ± 0.84	h
C1	49.68 ± 1.86	i
C2	44.26 ± 1.72	j
C3	38.84 ± 0.81	b,e,f,k
KP	72.50 ± 1.70	l
KN	23.22 ± 0.57	m

KN: Normal Control, KP: Positive Control

Notes: The statistically significant difference in mean±standard deviation according to LSD test is shown when the mean± standard deviation is followed by different letter. When mean± standard deviation was followed by the same letter, it means that there is no significant difference statistically.

Table 5 SGPT serum level in each group on Day +7 from the LPS induction

Group	SGPT level in U/L (mean±SD)	LSD tests
A1	25.49 ± 0.91	a,b
A2	19.18 ± 0.51	c
A3	22.58 ± 0.80	d
B1	28.89 ± 0.67	e
B2	26.06 ± 0.79	a,f
B3	23.38 ± 0.84	g
C1	33.90 ± 0.57	h
C2	30.59 ± 0.69	i
C3	27.59 ± 0.71	j
KP	39.49 ± 0.40	k
KN	17.80 ± 0.40	l

KN: Normal Control, KP: Positive Control

Notes: The statistically significant difference in mean±standard deviation according to LSD test is shown when the mean± standard deviation is followed by different letter. When mean± standard deviation was followed by the same letter, it means that there is no significant difference statistically.

between group A1 and B3, A1 and C3, B1 and C3, B2 and C3. But, others showed significant differences between each groups (p-value <0.05).

The Effects of Ethyl Acetate Fraction of *Moringa Oleifera* Leaves on SGPT Levels

The lowest SGPT level was found in group KN (17.80±0.40 U/L) and the highest in group KP (39.49±0.40 U/L). Among the MO-EA treated

groups, the lowest SGPT level was found in group A2 (19.18±0.51 U/L), and the highest was group C1 (33.90±0.57 U/L). Figure 4 and Table 5 depict the level of SGPT in each group and the post-hoc analysis comparing creatinine level in each group. Apart from the comparison of group A1 and B2, there are significant differences in each group when compared to another (p-value <0.05).

DISCUSSION

The highest level of ureum, creatinine, SGOT and SGPT are observed in the KP group, while the lowest level are found in the KN group. It means that a condition of kidney and liver dysfunctions have been recreated successfully.⁶

Among the groups receiving MO-AE fraction, group A2 has the lowest average serum level of ureum 12.25±0.64 mg/dL, creatinine 0.76±0.02 U/L, SGOT 25.41±0.90 U/L, and SPGT 19.18±0.51 U/L. In contrast, group C1 has the highest average of serum level of ureum 37.80 ± 0.38 mg/dL, creatinine 2.9±0.04 U/L, SGOT 49.68±1.86 U/L, and SPGT 33.90 ± 0.57 U/L. Our result supports other study which proposed that MO-EA may suppress inflammation.⁶ However, there is no readily available data from other research for us to evaluate the effect of MO-EA fraction to kidney and liver function tests.

CONCLUSION

There is a decrease in the serum level of ureum, creatinine, SGOT, and SGPT in the sepsis rats administered with MO-EA. The best MO-EA fraction is a daily dose of 20 mg/kgBW given 5 days before LPS induction continuing to Day+7. We conclude that the MO-EA fraction showed a potential to be used as a preventive therapy for endothelial stress in sepsis rat model.

FUNDING DISCLOSURE

The authors self-funded the research.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest in conducting and reporting the research.

AUTHORS CONTRIBUTION

The authors contributed equally in conducting the research and writing the manuscript.

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