ORIGINAL ARTICLE

Bali Medical Journal (*Bali Med J*) 2020, Volume 9, Number 3: 721-724
P-ISSN.2089-1180, E-ISSN: 2302-2914

Published by DiscoverSys

# The effect of ethyl acetate fraction of *Moringa oleifera* leaves on neutrophil and MDA levels in the improvement of liver dysfunction in male rats with sepsis model

Riswanto[1*], Tatar Sumandjar[1], Dhani Redhono[1], Robby Kurniawan[1], Arief Rahman[1]

## ABSTRACT

**Purpose:** Sepsis is life-threatening organ dysfunction caused by the dysregulation of the host response due to infection. Sepsis is caused by an imbalance between pro-inflammatory and anti-inflammatory cytokines. *Moringa oleifera* (Mo) leaves naturally contains antiinflammation and antioxidant. We aim to investigate Mo leaves in sepsis.

**Patients and methods:** This is an experimental laboratory study with Post Test Only Control Group Design. 30 Winstar males rats were divided into four treatment groups; each consist of 6 rats. The negative group, the positive control group received an injection of LPS dose of 0.25 mg/kg BW, and the interventional group with Mo-EA dose of 10, 20, 40 mg/kg BW. The Annova and Kruskal-Wallis test was used in this study. The significant p-value<0.05. The measured outcome were neutrophil levels, malondialdehyde (MDA) levels, and Serum Glutamic Pyruvate Transaminase (SGPT) / Serum Glutamic Oxaloacetic Transaminase (SGOT).

**Results:** The neutrophil, MDA, SGOT, and SGPT levels were decreased significantly within the treated group. MO Fraction can decrease the serum of MDA, Neutrophil, and SGOT/SGPT levels in days 3 and 7 ($p<0.05$).

**Conclusion:** There was a decrease in the level of Neutrophil, MDA, SGOT, and SGPT improvement of Hepar organ dysfunction after administration of MO Extract, so it is useful to inhibit the progression of sepsis.

**Keywords:** Sepsis, *Moringa oleifera*, Neutrophil, MDA, SGOT, SGPT

**Cite this Article:** Riswanto., Sumandjar, T., Redhono, D., Kurniawan, R., Rahman, A . 2020. The effect of ethyl acetate fraction of *Moringa oleifera* leaves on neutrophil and MDA levels in the improvement of liver dysfunction in male rats with sepsis model. *Bali Medical Journal* 9(3): 721-724. DOI: 10.15562/bmj.v9i3.1850

[1]Departement of Tropical infection, Dr Moewardi Regional State Hospital/ Faculty of Medicine - Sebelas Maret University, Surakarta, Indonesia

*Correspondence: Riswanto; Department of Tropical infection, Dr. Moewardi Regional State Hospital/ Faculty of Medicine – Universitas Sebelas Maret; riswanto_interna@yahoo.com

Received: 2020-06-04
Accepted: 2020-11-05
Published: 2020-12-01

## INTRODUCTION

There are 16 countries in Asia (including Indonesia) that had conducted numerous studies in 2009 covering 150 intensive care unit (ICU), which had revealed as many as 10.9% incidents of sepsis and septic shock events with a mortality rate of 44.5%. In 2012, Cipto Mangunkusumo Hospital (RSCM) reported a 1-month observation in their ICU. It revealed 23 sepsis and septic shock patients from around 84 ICU inpatients, with a mortality rate of 47.8% and early mortality rate of 34.7%. Inpatients who had attended RSCM ward showed 10.3% of patients with sepsis as a diagnosis from all inpatients admitted into the internal medicine ward. The definition of sepsis is dynamic and changes over time since 1991, which introduced sepsis as systemic inflammatory response syndrome (SIRS) accompanied by conjectures or evidence of infection.[1] Another definition of sepsis as life-threatening organ dysfunction caused by dysregulation of host response against an infection. Organ dysfunction can be identified by acute alteration of SOFA score ≥ 2 points generated by infection. Abundant studies were focusing on sepsis pathogenesis and its particular pathophysiology state that unbalanced pro-inflammation cytokines cause sepsis, therefore precipitating "cytokine storm." At the beginning of sepsis, hyperdynamic events of the innate immune system, including neutrophils, macrophages, and complements.[2] Neutrophils, as one of the manufacturers of pro-inflammation cytokines in normal circumstances, possess a half-life of 7 to 12 hours *in vivo* and escalate more as the correspondence indulged in sepsis condition.

Debates about sepsis and septic shock pathogenesis, treatments and managements, and fundamentally their definitions still circulating IDSA (Infectious Diseases Society of America), who takes an opposing stance against SSC-3, that even with antibiotic treatments, showed 5% with positive cultures, neglecting fungal infection. Moreover, the existence of SOFA score < 2 on IV-line infection and bacterial resistance, in addition to multi-drug resistance to antibiotics, strengthen their opposition. Mantzarlis (2017) innovated using antioxidants as a potential sepsis therapy, and Guntur A adopted low-dose steroid application in the early stage of sepsis.[3]

There is still a considerable amount of debate about sepsis pathophysiology and its treatment. Thus we came up with an idea revolving around local wisdom, applying *Moringa oleifera* (kelor leaves) as sepsis treatment. Kelor leaves has been studied for its capability as an alternative medicine for asthma, diabetes mellitus, atherosclerosis, and others.[4] Interestingly, the variety of its bioactive substances comprises external antioxidants (Vitamin C and Vitamin E) and minerals of external antioxidants (Mangan, Zinc, and Copper). Therefore, the idea of researching kelor leaves fraction as an alternative sepsis therapy using sepsis rat model as a subject emerged according to this data.

## MATERIAL AND METHODS

The study was an experimental laboratory study. Subjects were divided into a control group and an intervention group. This study was conducted by comparing the outcome of the two different groups. This study used *The Post Test Only Control Group Design*. The design was picked because of its high validity and its controllability. This kind of study could minimize the occurrence of bias testing and interaction testing. This study was approved by Dr Moewardi General Hospital Health Research Ethics Committee (ethical clearance letter no. 1343/ XII/ HREC/ 2019).

The intervention of male white mice (*Rattus norwegians*) and MDA check took place in the Experimental Animal Housing PAU UGM Jogjakarta. The neutrophil testing took place in the Faculty of Veterinary Medicine UGM Jogjakarta. The interference in 3-4 months old male white mice that weighed 200-300 grams was received from Veterinary Medicine of Gajah Mada University. The mice were fed with standardized food, BR1. Male white mice (*Rattus norwegians*) were picked because they genetically resemble human genes and adapt to the environment quickly.

We calculated the minimal sample amount of this study using Federer formula:[5]

$$(t - 1)(n - 1) > 15$$

t: Quantity of group intervention (4 groups)

n: Amount of samples

Based on the formula, the minimal sample amount of each group was calculated. Each group was estimated to have at least five mice. The estimated amount of samples was 30 mice. This study was conducted in 5 intervention groups, each consisting of 6 mice, with a total sample of 30. The study used purposive sampling, which is classified as a non-probability sampling. Samples were taken based on subjects that met the criteria until the total number of targeted subjects were met.[6]

The experimental design used the posttest only control group design. The initial assessment was not carried out considering all groups came from one population, and experimental planning can be developed without any initial assessment (pretest), using only final assessment/posttest only control group design. We randomly divided animals into five intervention groups, positive control (PC), negative control (NC), and the treatment groups (A, B, C). Each group underwent different interventions. All intervention groups were injected intraperitoneally with 0.25 mg lipopolysaccharide (LPS) per kilogram body weight to induce sepsis. Negative Control Group (NC) is injected with normal saline with the same procedure as other groups and were not fed with MO fraction. The *Moringa oleifera* (MO) extraction had been done at Gadjah Mada University (UGM). Each mouse in groups A, B, and C were fed using a feeding tube at a dose 10, 20, and 40 mg/Kg body weight, respectively.

The neutrophil levels were measured using hematology analyzer sysmax. SGOT/SGPT levels were counted using spectrophotometry. Enzyme-linked immunosorbent assay (ELISA) was used to determined MDA serum concentration. The data were analyzed using SPSS version 22. The statistical analysis for numerical variables is conducted with ANOVA test if the data is normally distributed. Otherwise, the Kruskal Wallis test will be used for the non-parametric tests. Statistically significant is considered if p-value < 0.05.

## RESULTS

We used 30 white male rats weighing 200-300 grams and 3-4 months old. Rats were divided into five groups, with three groups were treatment group, and two groups were control groups. The results showed that MO extract was statistically significant ($p< 0.001$) to reduce neutrophil, MDA, and SGOT/ SGPT levels in Septic rats induced by LPS.

### The effect of MO extract on Neutrophil serum level

In this study, the neutrophil serum level was decreased in the treatment group on day 3 and 7. Positive control group, serum neutrophil levels were higher than in treatment groups A, B, and C with p-value<0.001 (Table 1).

### The Effect of MO extract to MDA serum level

There were differences in the MDA serum level between positive and negative controls. MDA levels increased significantly in positive controls compared with negative controls. MDA serum level in positive controls was higher than treatment groups A, B, and C with p-value<0.001. The reduction of MDA serum levels occurred on days 3 and 7 (Table 2).

**Table 1. The differences of Neutrophil Levels According to Various Administration of MO Extract, Day-3 and Day-7 Post LPS Induction**

| Intervention | Neutrophil Levels, Day 3 Post LPS Induction | Neutrophil Level, Day 7 After LPS Induction |
|---|---|---|
| A | 50.45 ±9.82 | 33.92 ±3.26 |
| B | 49.85 ±14.75 | 33.67 ±5.19 |
| C | 46.27 ±12.91 | 32.55 ±2.35 |
| PC | 59.25 ±11.35 | 58.25 ±11.35 |
| NC | 16.30 ±5.57 | 14.92 ±3.50 |
| p-value | p<0.001 | p<0.001 |

Note :
A = Treatment Group 1
B = Treatment Group 2
C = Treatment Group 3
PC = Positive Control
NC = Negative Control

**Table 2. The differences of MDA Levels Based on Various Administration of MO Extract, Day-3 and Day-7 Post LPS Induction**

| Intervention | MDA Level, day 3 After LPS Induction | MDA Levels, Day-7 Post LPS Induction |
|---|---|---|
| A | 105.47 ±24.40 | 121.40 ±11.14 |
| B | 83.88 ±42.86 | 119.37 ±8.15 |
| C | 100.78 ±22.43 | 119.72 ±8.46 |
| PC | 148.53 ±101.64 | 142.92 ±14.99 |
| NC | 75.25 ±4.13 | 109.43 ±1.41 |
| p-value | p=0.004 | P<0.001 |

**Table 3. The Differences of SGPT Levels Based on Various Administration of MO Extract, Day-3 and Day-7 Post LPS Induction**

| Intervention | SGPT Levels, Day-3 Post LPS Induction | SGPT Levels, Day-7 Post LPS Induction |
|---|---|---|
| A | 32.44 ±1.60 | 28.89 ±0 .67 |
| B | 29.94 ±1.78 | 26.06 ±0 .79 |
| C | 27.58 ±1.87 | 23.38 ±0 .84 |
| PC | 39.77 ±1.32 | 39.49 ±0 .40 |
| NC | 17.67± 0 .56 | 17.80 ±0 .40 |
| p-value | P<0.001 | P<0.001 |

**Table 4. Differences of SGOT Levels Based on Various Administration of MO Extract, Day-3 Post LPS Induction**

| Intervention | SGOT Levels, Day-3 Post LPS Induction | SGOT Levels, Day-7 Post LPS Induction |
|---|---|---|
| A | 50.78 ±3.47 | 43.37 ±1.09 |
| B | 47.02 ±4.58 | 39.08 ±0 .91 |
| C | 41.76 ±1.39 | 34.88 ±0 .84 |
| PC | 72.44 ±1.95 | 72.50 ±1.70 |
| NC | 23.36 ±0 .72 | 23.22 ±0.57 |
| p-value | P<0.001 | P<0.001 |

### The Effect of MO extract to SGOT/SGPT serum level

Elevated SGOT/SGPT serum level indicates damage or inflammation of the liver. Both SGOT and SGPT serum levels decreased compared to the positive control group. Lowering of SGOT and SGPT serum levels on days 3 and 7 were statistically significant (p<0.001) (Table 3 and Table 4).

## DISCUSSION

This study showed the administration of MO extract of any dose significantly lower serum level of neutrophil, MDA, and SGOT/SGPT in septic model mice after induction of LPS on day 3 and 7. There is no similar study known to the researchers evaluating the Neutrophil, MDA, and SGOT/SGPT serum levels in an animal sepsis model. Rats were chosen as subjects in this experimental study, given these following qualities: 1) High fertility rate, age of sexual maturity in the sixth to the eighth week of life; 2) Age acceleration, one year of human life equals to 9 days of rat life; 3) Cost-effective; 4) Required for the preclinical trial model; 5) Advantageous gene characteristics with similarity (ontology) as high as 80% to human genes; 6) Stable genetics, albeit mating in groups; 7) Available in almost all places with their reagents available, 8) Treated humanely.[2] In this study, we injected 0,25 milligrams per kilograms of body weight (mg/kg) into the septic rat model.

The lethal dose of LPS for rats fall in the range of 5 to 15 mg/kg, which is 1000 times greater than the lethal dose of LPS for humans. LPS bolus in the peritoneum was superior to *caecal ligation and puncture* (CLP) and *colon ascendens stent peritonitis* (CASP). LPS is a less complicated procedure and has a similar reaction to sepsis pathophysiology in humans. After the injection, LPS (gram-negative cell wall/PAMPs) will recognize by the innate immune system, PRR (TLR4). Neutrophils will then be released from the bone marrow and other reservoirs into the blood circulation and transported to the infection site. This study found that H0 neutrophils increase in all the intervention group subjects. It is consistent with preexisting theory.[7] In the occurrence of sepsis, it is expected to see elevated levels of various innate immune cells, such as neutrophils, in the hyperdynamic state in the initial phase of sepsis.[2] We found an increase in day 3 neutrophils in intervention group B2, B3, C1, C2, C3, and D. The neutrophil levels of group A1, A2, A3, and B1 increased.

In contrast, the levels decreased when compared to H0. The most considerable decrease in neutrophil levels found in group A2. This finding is consistent with the preexisting theory, which states that sepsis occurs in a hyperdynamic state.

Theoretically, various cytokines (chemokines),

such as e-selectin, Vcam, Icam, are needed to transport neutrophils to an infection site. In a normal physiologic state, immune cells (neutrophils) undergo apoptosis induced by macrophages subsequently to the resolution of an infection process. In the occurrence of sepsis, there is a neutrophil apoptosis delay. This study found that there was only a slight decrease in neutrophils on the seventh day in group D. There was a decrease in MO extract intervention groups. In a study done by Yin (2018), MO extract caused Nrf2 to release KAEP1 in an oxidative stress state, Nrf2 caused autophagy.[8] Neutrophil autophagy is useful in an infection process because it can induce organ damage. Sepsis causes elevation of reactive oxygen species (ROS) (MDA). In this study, MDA elevated on the third day. The least MDA elevation was found in group A2. It is consistent with the theory, which states that ROS increases in a hyperdynamic state when sepsis occurs. MDA is a lipid peroxidase end product which formed intracellularly. It was transported outside of the cell and easily transported into the blood. This result explains the MDA elevation on the 7$^{th}$ day. This finding is consistent with the study hypothesis which states that MO extract can reduce ROS (MDA) level.[2]

Sepsis is a life-threatening organ dysfunction caused by immune system dysregulation against infection (bacteria, fungi, virus), with >2 SOFA score.[1] Organ dysfunctions in SOFA score include the brain, lungs, liver, and kidneys. In this study, liver dysfunction was inspected through SGOT and SGPT levels. Results were obtained on the 3$^{rd}$ day when SGOT/SGPT levels were found to elevate. The most minimal SGOT/SGPT elevation was observed in groups that were given MO extract on the fifth day, particularly in group A2. There was found to be an improvement of liver dysfunction marked by decreasing SGOT/SGPT levels on the seventh day. The decrease of SGOT/SGPT levels was observed to be highest in group A2. This finding is consistent with the study hypothesis, which stated the improvement of liver dysfunction in a male septic rat model. It is also compatible with the theory which states that ROS produced by neutrophils cause organ dysfunction in the occurrence of sepsis. MO extract can reduce the levels of SGOT/SGPT is corresponding to the previous study.[9] Although this study showed that MO extract reduced hyperdynamic conditions, ROS, and liver damage in sepsis, Further research needed to determine the dosage, side effects, and safety of using MO extract, whether it is applicable in humans.

## CONCLUSION

Several doses of MO extract administration significantly reduced serum levels of neutrophils, MDA, and SGOT/SGPT in sepsis model rats on days 3 and 7. MO extract may be used as adjuvant therapy in the treatment of sepsis. However, further research is needed to investigate the safety and efficacy of human.

## ACKNOWLEDGMENTS

We would like to thank all staff for their support of this study.

## AUTHOR CONTRIBUTION

All authors have contributed to all processes in this research, including preparation, data gathering and analysis, drafting and approval for publication of this manuscript.

## DISCLOSURE

The author reports no conflicts of interest in this work.

## FUNDING AND SPONSORSHIP

There is no external funding and sponsorship.

## REFERENCES

1. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, et al. The third international consensus definition for sepsis and septic shock (sepsis-3). *JAMA*. 2016; 315(8): 801-810, doi:10.1001/jama.2016.0287
2. Doi K, Leelahavanichkul A, Yuen PST, Star RA. Animal models of sepsis and sepsis-induced kidney injury. *Journal of Clinical Investigation*. 2009; Oct 1; 119(10): 2868–2878. doi: 10.1172/JCI39421.
3. Guntur HA. Imunologi, Diagnosis dan Penatalaksanaan Sepsis. dalam: *Steroid Dosis Rendah Dalam Penatalaksanaan Sepsis*. Surakarta: UNS Press.2011
4. Gopalakrishnan L, Doriya K, Kumar DS. Moringa oleifera: a review of nutritive importance and its medicinal application. *Food Science and Human Wellness*. doi: 10.1016/j.fshw.2016.04.001.
5. Zainuddin, M. *Metodologi Penelitian dan Statistik*. Surabaya: Universitas Airlangga. 2000
6. Northeastern University. Qualitative research methods overview. Available from: http:www.ccs.neu.edu/course/is4800sp12/resources/qualmethods.pdf [Accessed 12$^{th}$ of January 2020].
7. Silvestre-Roig C, Hidalgo A, Soehnlein O. Neutrophil heterogeneity: Implications for homeostasis and pathogenesis. *Blood*. doi: 10.1182/blood-2016-01-688887.
8. Yin X, Huang Xin, Shuai Mao, Guangping Wu, Liheng Guo. The Role of Autophagy in Sepsis: Protection or Injury to Organ. *Frontiers in Physiology*. 2019; 10: 1071. doi: 10.3389/fphys.2019.10171
9. Wulan H, Nur sholeh A.A ., dan Wiganti, D. Pengaruh Pemberian Fraksi Etil Asetat daun Kelor ( Moringa oleifera Lam) terhadap histopatologi hepar , kadar SGOT dan SGPT tikus jantan gakur Wistar yang terinduksi Monosodium Glutamat. *Media Farmasi Indonesia* .2018 13. 1354-1361