***Original article:***

# The effect of *Mmoringaoleifera* leaf extract on mean platelet volume and neutrophil to lymphocyte ratio in lupus

*Arief Nurudhin[1], Retno Widyastuti[2], Nurhasan Agung Prabowo[3], Zainal Arifin Adnan[4], Yulyani Werdiningsih[5]*

**Abstract**

***Introduction*:** No definitive treatment is available for Lupus. *Moringa oleifera* is one of promising novel treatments in Lupus trough anti inflammation and immunosupresion. Mean platelet volume and neutrophil to lymphocyte ratio used to measure degree of lupus activities. ***Objective:*** Our study was aimed to identify the effect of *Moringaoleifera* extract on the Mean platelet volume and neutrophil to lymphocyte ratio in lupus. ***Methods:*** This experimental study was conducted in 30 lupus patients, located in the rheumatology clinic at Moewardi General Hospital in January-March 2019. The study group was divided into two groups: the treatment research group received 2 grams of moringa extract per day and the placebo group. The study was conducted for 4 weeks and was evaluated when the study was completed. MPV and NLR examination using a hemocytometer. Statistical analysis was performed using paired T test, independent T-test. The p value was considered significant when the $p < 0.05$. ***Results:*** The results showed a decrease in MPV (delta MPV = 4.141; r 0.656; p-0.02) and a decrease in Neurophil to lymfocyte ratio (delta NLR = 4.1391; r 0.489; p-0.04) ***Conclusion:*** The study demonstrated the effect of *Moringa oleifera* leaf extract on reduced Mean platelet volume and neutrophil to lymphocyte ratio in lupus

**Keywords:** ***Moringaoleifera*** **extract; mean platelet volume, neutrophil to lymphocyte ratio**

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## Introduction

Systemic Erimatous Lupus (SLE) is a multisystem disease caused by antibody production and deposition of complementary immune complexes which results in tissue damage.[1]

Neutrophil to lymphocyte ratio (NLR) is calculated as the absolute count of neutrophils divided by the absolute count of lymphocytes. The Mean Platelet Volume (MPV) is a platelet activation biomarker that has been recently correlated with disease activity in SLE. neutrophils will increase in inflammatory conditions, whereas the higher the degree of lupus, the more inflammation will occur. While lymphocytes will decrease in active lupus due to lymphocyte apoptosis, this is partly because there

**1.** Arief Nurudhin, Division of Rheumatology, Department of Internal Medicine, Faculty of Medicine, Sebelas Maret University, Surakarta, Indonesia and Moewardi General Hospital, Surakarta, Indonesia.
**2.** Retno Widyastuti, Division of Rheumatology, Department of Internal Medicine, Faculty of Medicine, Sebelas Maret University, Surakarta, Indonesia and. Faculty of Medicine Sultan Agung University, Semarang, Indonesia.
**3.** Nurhasan Agung Prabowo, Division of Rheumatology, Department of Internal Medicine, Faculty of Medicine, Sebelas Maret University, Surakarta, Indonesia and Moewardi General Hospital, Surakarta, Indonesiaand Sebelas Maret University Hospital, Sukoharjo, Indonesia.
**4.** Zainal Arifin Adnan, Division of Rheumatology, Department of Internal Medicine, Faculty of Medicine, Sebelas Maret University, Surakarta, Indonesia and Moewardi General Hospital, Surakarta, Indonesia and Sebelas Maret University Hospital, Sukoharjo, Indonesia.
**5.** Yulyani Werdiningsih, Division of Rheumatology, Department of Internal Medicine, Faculty of Medicine, Sebelas Maret University, Surakarta, Indonesia and Moewardi General Hospital, Surakarta, Indonesia.

**Correspondence to:** Arief Nurudhin, Division of Rheumatology, Department of Internal Medicine, Faculty of Medicine, Sebelas Maret University, Surakarta, Indonesia and Moewardi General Hospital, Surakarta, Indonesia. email : ariefnurudhin@yahoo.com

are also anti-lymphocyte antibodies in SLE. MPV reflects the degree of inflammation and the role and function and activity of platelets. NLR and MPV can be a marker of SLE disease activity and a marker of inflammation in SLE. the higher the NLR and MPV the more severe the degree of SLE disease activity and inflammation that occurs in SLE.[3-5]

The pathogenesis of the disease that is still unclear and the therapy that is given less than optimal can result in high SLE mortality. The current LES therapy is only to inhibit progression and prevent the severity of the disease. The absence of definitive cure therapy for SLE has made many research breakthroughs in the treatment of SLE. *Moringa oleifera* is one of the breakthrough therapies in SLE..*Moringa oleifera*(MO) Lam (Moringa Leaf) is a plant in the Moringaceae family, containing a variety of unique phytochemical groups that produce a spectrum of biological effects, especially anti-inflammatory.[5]

*Moringa oleifera* has two mechanisms for inhibiting Lupus. Moringa has immunosupressant properties by decreasing the number of CD4 T cells (T Helper cells)[6] with cell apoptosis pathways due to excessive calcium influx in cells (Zainal Path)[7]. In addition *Moringa oleifera* will have an anti-inflammatory effect by inhibiting nfĶβ[8]. The nfĶβ inhibition will cause a decrease in pro-inflammatory cytokines IL 6, IL 1 and TNF α so that tissue inflammation decreases[9].

Our study was aimed to identify the effect of *Moringa oleifera* extract on the Mean platelet volume and neutrophil to lymphocyte ratio in lupus.

## Methods

This research was conducted from January to July 2018 at the Moewardi Hospital in Surakarta. inclusion criteria were outpatient SLE patients. Exclusion criteria were severe flare conditions, comorbid diabetes, kidney, heart, lung and infection. Hematology examination using a hematology analyzer machine. The control group received a placebo, while the treatment group received a 2g dose of *Moringa oleifera* per day. the treatment is carried out for 28 days. at the beginning and after the treatment routine blood data was taken to provide NLR and MPV

The obtained data were expressed in mean value + standard deviation. Normality test was performed using Shapiro-Wilk test and variant homogenicity test was performed using Levene's test, FAnova test followed by Least Significant Difference (LSD) post-hoc test for data with normal and homogenous distribution and Kruskal-Wallis followed by Mann-Whitney test for data with abnormal or non-homogenous distribution; while regression analysis was carried out to identify the variable which was most affected by secretome. The significance level was at $p< 0.05$.

### Ethical clearance

Ethical clearance was sought from the Ethical Review Board from Medical Faculty of SebelasMaret University

## Results

1. **Description of the results variable**

**Table 1. Description Variable**

| Variable | Control | | MO | | p |
|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | |
| Leucocyte | 8,28 | 1,67 | 9,73 | 2,64 | 0,082 |
| Trombocyte | 314,81 | 55,67 | 312,46 | 68,67 | 0,920 |
| Eritrochyte | 4,51 | 0,61 | 4,52 | 0,41 | 0,955 |
| MCV | 84,01 | 5,86 | 81,92 | 5,01 | 0,318 |
| MCH | 27,44 | 2,78 | 26,47 | 3,06 | 0,380 |
| MCHC | 32,60 | 1,47 | 32,22 | 2,26 | 0,592 |
| RDW | 14,11 | 1,99 | 14,14 | 1,63 | 0,970 |
| MPV | 8,74 | 1,55 | 8,27 | 2,05 | 0,485 |
| PDW | 20,44 | 13,95 | 20,31 | 13,19 | 0,808 |
| Eosinofile | 0,98 | 1,23 | 2,31 | 2,38 | 0,083 |
| Basofil | 0,50 | 0,25 | 0,61 | 0,42 | 0,391 |
| Neutrphyl | 68,83 | 12,28 | 69,14 | 9,86 | 0,904 |
| Limfochyt | 22,73 | 9,81 | 21,29 | 7,91 | 0,672 |
| Monosit | 6,44 | 2,11 | 5,93 | 1,79 | 0,492 |

2. **Effect of *Moringa oleifera* (MO) on NLR and MPV in SLE patients**

**Table 2. Effect MO in SLE**

| Variable | Control | | MO | | correlation | |
|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | r | P |
| NLR | 3,23 | 1,23 | 3,11 | 1,05 | -0.489 | 0.04 |
| MPV | 8,74 | 1,55 | 8,27 | 2,05 | - 0.656 | 0.02 |

The results of the study it was found that *Moringa oleifera* will reduce the NLR in Lupus patients with the correlation coefficient is -0,489 and P=0,04 is besides that *Moringa oleifera* will also reduce the value of MPV in SLE patients with the correlation coefficient is -0,656 and the P value is 0,02

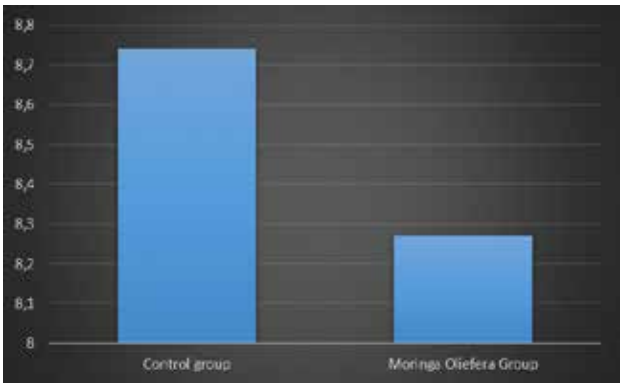


Figure 1. Effect of MO on MPV SLE

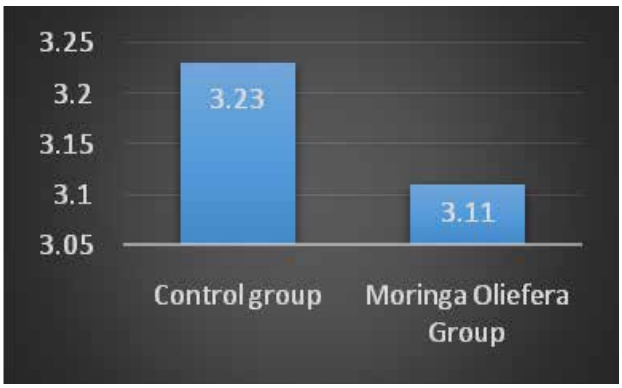


Figure 2. Effect of MO on NLR SLE

**Discussion**

In this study M. oleifera which has been known to have anti-inflammatory, antioxidant and immunomodulatory effects, has been shown to have an effect on reducing dsDNA levels in conditions of lupus nephritis and also maintaining renal histopathological conditions in lupus nephritis. This is in line with previous studies that contain glucosinolate and isothiocyanataes. has a strong inhibitory effect on NO (nitrit Oxide) production, can reduce insulin, leptin, resistin, cholesterol, interleukin-1ß (IL-1ß), tumor necrosis factor-alpha (TNFα), and glucose-6-phosphotase in diabetic mice, and is based on the findings of this study concluded that isothiocyanate compounds may be the main bioactive ingredients that have anti-diabetic activity and anti-inflammatory responses.[11]

Flavonoids, which include quercetin, kaempferol glucoside, and flavonoid malfates, show anti-inflammatory activity through inhibition of NO production in LPS macrophages.[11] Many previous studies have established the inhibitory effects of M. oleifera on NO, VEGF, TNFα, IL-2, IL-1ß, IL-6, glucose-6-phosphotase, insulin, leptin, resistin and cholesterol.[11-13]The most common pathway, which is considered a prototypical pro-inflammatory signaling pathway and the parent transcription factor, is mediated by NF-kß.[14]

NF-κB is the main transcription factor that plays an important role in controlling the inflammatory response. NF-κB activation modulates the controller signal switches the pro-inflammatory gene transcription response. Toll-like receptors (TLR) and cytokines, such as TNF and IL-1, to regulate the transcription of other pro-inflammatory genes. These NF-κB target genes are needed to activate immunity and destroy pathogens. In a state of unstimulated cells, the NF-κB protein is in the cytoplasm which is held by an inhibiting molecule called I-κB. The role of NF-κB indicates the existence of opposing functions. On the other hand, NF-κB is important for activating proinflammatory genes, which are important for improving inflammation and protection from apoptosis. Conversely, excessive activation of in vivo NF-κB will cause death, this is due to the cellular level excessive activation of NF-κB will inhibit activation of the immune response and increase sensitivity to apoptosis.[8]*Moringa oleifera* will have an anti-inflammatory effect by inhibiting nfĶβ.[9]NfĶβ barriers will cause a decrease in proinflammatory cytokines IL 6, IL 1 and TNF α so that network inflames are reduced.[9]

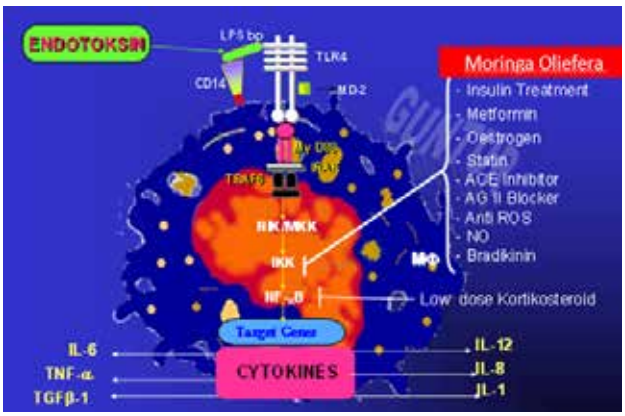


Figure 4. Effect of *Moringa oleifera* by inhibiting nfĶβ[9]

Fathir et al. Showed that the dose of *Moringa oleifera* has 2 effects on T cells CD4 T lymphocytes / T helper cells namely low doses that are immunostimulant and high doses cause immunosuppression. The increase in the number of CD4 + T cells is caused by the presence of an active substance in Moringa leaf extract which functions as an immunostimulant against the immune system. Active substances that are thought to have a role as immunostimulants are saponins and flavonoids. Saponins and flavonoids are thought to be able to induce an increase in the secretion of cytokines involved in the process of CD4 + T cell activity.[15]

Saponins and flavonoids are substances that play a role in triggering up helper T cell regulation by

stimulating increased production of interleukin 2 (IL-2) cytokines. Cytokine IL-2 is needed by CD4 + T cells to differentiate in the Heper 2 (Th2) and Th1 T cell subsets[16]. In addition to functioning as an immunostimulant Moringa leaf extract can function as an immunosuppressant. This can be seen in the administration of high doses of Moringa leaf extract causing an increase in the number of CD4 + T cells lower than the low dose administration. In LES the most important effect is high doses and causes immunosuppressants through the lymphocyte apoptosis pathway.

T lymphocytes are the main mediator of immune disease. Therefore, modification of T cell activation will be a tool for immune-chained diseases. Attakpa et al. Found that *Moringa oleifera* had an inhibitory effect on T cell proliferation. This inhibitory effect was statistically different between 200 mg / kg and 400 mg / kg but statistically did not differ between 400 mg / kg and 600 mg / kg. The inhibiting effect of *Moringa oleifera* at 400 mg / kg was not caused by cytotoxicity. Attakpa et al. Showed that administration of *Moringa oleifera* in mice will increase intracellular calcium ion levels in lymphocyte cells and will cause the death of lymphocyte cells by apoptosis.[6]

Large increases in free Ca2 + in intracellular space are very toxic[7], which results in:

1. An increase in Ca2 + activates the catabolic enzyme calpain 1 which can result in degradation of several neuronal protein structures (neurofilament peptide, tubulin and spectrin).
2. Increased Ca2 + can activate phospholipase which causes damage from cell membranes, then release of arachidonic acid which produces oxygen free radicals and the formation of superoxide enzymes, and growth factors withdrawal.
3. Increased Ca2 + with glycerol produced activates protein kinase C, this will further increase the Ca2 + influx.
4. Increased Ca2 + influx stimulates more glutamate release, resulting in neurotoxic glutamate.

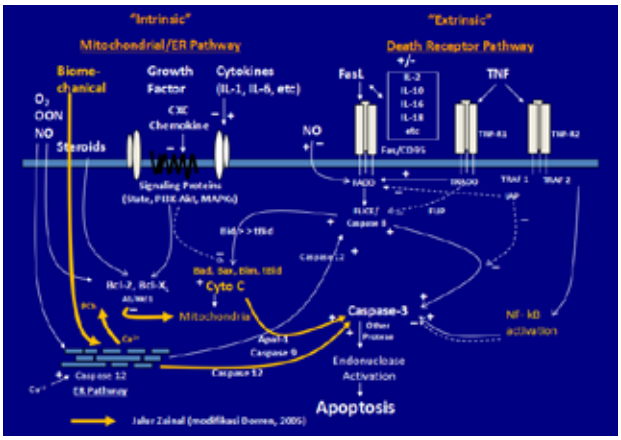


Figure 5. Intracellular calcium ions will cause cell apoptosis according to the Zainal pathway(Zainal Arifin Adnan, 2009).

High Calcium ions in cells Lymphocytes will activate apoptosis according to the Zainal pathway. When passing through the cell membrane, Ca2 + activates the phospholipase C enzyme, which is able to phosphorylate phosphotidyl inositol 1.4 biphosphate (PIP-2) to IP-3 and produce glycerol. IP-3 enters the cytosol and attaches to the surface of the endoplasmic reticulum, which results in opening the Ca2 + channel and immediately circulates Ca2 + into the cytosol, so that Ca2 + in the cytosol increases, this also activates phospholipase-A2. Phospholipase-A2 affects phosphotidylcholine to lysophosphotidil choline and arachidonic acid. Lysophosphotidylcholine will affect the fluidity of cell membranes so that Ca2 + will enter from outside the cell to the cytosol, this results in higher levels of Ca2 + accumulation in the cytosol. Excessive accumulation of Ca2 + in the cytosol will bind calcineurin protein to form a calcineurin-Ca2 + complex, which can stimulate transcription activation of Bad protein, which then affects PT pore on the open mitochondrial wall. The opening of PT Pore resulted in the release of cytochrome C from mitochondria to the cytosol then activated apaf-1. Which followed Caspase activation and subsequently apoptosis.[7]

This study is in line with Rachmawati research in Malang which states that in vitro MO has activity as an immunomodulator through its active compounds, such as saponins and flavonoids, which act immunostimulants on CD4 + (T helper cells) and CD4 + (cytotoxic T cells), as well as B220 + cell markers, together with the 2014 Fathir et al. study in the same place prove that administration of low-dose Moringa leaf extract can increase cell counts T CD4 + and CD8 + T cells in all groups of mice but high doses of Moringa leaf extract will

actually cause immunosuppression, [15,18] in this case our study utilizes the immunosuppression effect on MO because it uses a high dose of 500mg / kgBB. Tan et al in 2015, where MO was proven to be able to suppress IL 10 and IL 6 in autoreactive B cells.[16] As the pathogenesis of Lupus, these two things will help suppress the formation of antidsDNA antibodies which are specific markers of damage in LES.

In this study *M.oleifera* proved to be able to significantly reduce disease activity in mice of the lupus model, this result is inseparable from the anti-inflammatory function of MO which has been proven from several previous studies [11-13,17]. These results are also in line with several other studies on the protective effects of MO in the kidneys, Ouedrago et al. In 2011 published that MO leaf extract had a protective effect on rabbits induced with gentryin nephrotoxic substances, supporting these results Adeyemi et al., 2014 have also proved that MO can prevent nephrotoxicity in wistar rats that are induced by nickel. Both of these studies state that the protective effects of kidney in MO are based primarily on the anti-inflammatory effects possessed by MO.[19,20]

This study shows that administration of *Moringa oleifera* therapy and beyond standard therapy will reduce the value of npv and irr in lupus patients so that further research is needed that *Moringa oleifera* will be a drug for lupus patients and is safer and side effects are not so many other drugs.

## Conclusion

The study demonstrates the effect of moringa oleifera leaf extract on reduced Mean platelet volume and neutrophil to lymphocyte ratio in lupus

### Ethical clearance

Ethical clearance was sought from the Ethical Review Board from Medical Faculty of Sebelas Maret University

### Acknowledge

Authors acknowledge the contribution of all research assistants who helped in the collection of data. The authors express their profound gratitude to all participants in the study.

### Conflict of interest

The author declares that they have no conflicts of interest

### Author's contribution

Data gathering and idea owner of this study, Study design, Data gathering, Writing and submitting manuscript, Editing and approval of final draft, all events done by all the authors.All authors read and approved the final manuscript.

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