Original article

Moringa Oleifera Extract Decreases Interleukin 6 Levels and Disease Activity in Rheumatoid Arthritis Patients

Nurhasan Agung Prabowo[1], Arief Nurudhin[2], Yulyani Werdiningsih[3], Dikha Dwi Putra[4], Desy Puspa Putri[5], Retno Widyastuti[6]

Abstract:

Moringa oleifera (MO) has anti-inflammatory, anti-arthritis, and immunosuppressant effects in Rheumatoid Arthritis (RA). This study aims to determine the effect of moringa oleifera extract on interleukin 6 levels and disease activity in RA. This research, with 30 patients, was divided into two groups: the intervention and the placebo. The intervention group received 40.50 mg/kg BW/day of Moringa oleifera extract for one month. IL-6 levels and SDAI scores were measured before and after the treatment. Paired t-test showed that IL-6 levels (p=0.070) and SDAI Scores (p=0.142) before and after MO administration for the control group were not significantly different (p> 0.05). Paired t-test IL-6 (p=0.001) and SDAI Scores (p=0.001) before and after giving MO to the treatment group decreased significantly (p <0.05). Independent t-test shows that the two changes, Delta-IL6 (p=0,008) and Delta-SDAI (p=0,017), are significantly different (p<0.05). Moringa Oleifera Extract decreases IL-6 Levels and SDAI Scores in RA Patients

Keywords: Moringa oleifera; Interleukin 6; SDAI Score; Rheumatoid Arthritis

Bangladesh Journal of Medical Science Vol. 22 No. 02 April'23 Page : 416-421
DOI: https://doi.org/10.3329/bjms.v22i2.65006

Introduction:

Rheumatoid arthritis (RA) is an autoimmune disease characterized by chronic and progressive systemic inflammation, in which the small joints of the hands and feet are the primary target. RA can also affect organs outside the joints, such as the skin, heart, lungs, and eyes. Complications such as cardiovascular, infection, kidney disease, malignancy, and comorbidities can increase mortality in cases of RA [(1)]. In Indonesia, the prevalence and incidence of RA cases vary between populations. The epidemiological survey results in Bandungan, Central Java, showed an AR prevalence of 0.3%. In Malang, AR prevalence for people over 40 years of age was 0.5% in municipalities and 0.6% in regencies. In the Rheumatology clinic of Cipto Mangunkusumo Hospital, Jakarta, in 2000, new cases of AR constituted 4.1% of all new cases. In the Rheumatology clinic of Hasan Sadikin Hospital, 9% of all new rheumatism cases were found in 2000-2002 [(2)]. The target for RA treatment is remission or a

1. Nurhasan Agung Prabowo,Nurhasan Agung Prabowo, Faculty of Medicine, Universitas Sebelas Maret, Ir Sutami street No 36, Kentingan, Jebres, Surakarta, Indonesia, 57126.
2. Arief Nurudhin, Faculty of Medicine, Universitas Sebelas Maret, Ir Sutami street No 36, Kentingan, Jebres, Surakarta, Indonesia, 57126.
3. Yulyani Werdiningsih,Yulyani Werdiningsih, Faculty of Medicine, Universitas Sebelas Maret, Ir Sutami street No 36, Kentingan, Jebres, Surakarta, Indonesia, 57126.
4. Dikha Dwi Putra,Dikha Dwi Putra, Faculty of Medicine, Universitas Sebelas Maret, Ir Sutami street No 36, Kentingan, Jebres, Surakarta, Indonesia, 57126.
5. Desy Puspa Putri, Faculty of Medicine, Universitas Sebelas Maret, Ir Sutami street No 36, Kentingan, Jebres, Surakarta, Indonesia, 57126.
6. Retno Widyastuti, Faculty of Medicine, Universitas Islam Sultan Agung, Kaligawe Raya Street, Terboyo Kulon, Genuk, Semarang, Jawa Tengah 50112.

Correspondence: Nurhasan Agung Prabowo, Internal Medicine Department, Faculty of Medicine, Universitas Sebelas Maret, Ir Sutami street No 36, Kentingan, Jebres, Surakarta, Indonesia, 57126, E-mail: *dr.nurhasan21@staff.uns.ac.id*

low degree of disease activity. Research in Indonesia showed RA remission using the SDAI score reached 16.7% of patients. It is, therefore, urgent to find new therapies for RA [3,4].

The Simplified Disease Activity Index (SDAI) is a more straightforward tool than the Disease Activity Score (DAS) to assess the degree of AR activity. DAS28 is not possible for every patient in outpatient rheumatology because it takes time and requires tools to calculate it. DAS28 is a tool primarily used for patients with clinical trial purposes only. Compared with DAS28, which has a complex formula and requires a calculator for evaluation, SDAI is much simpler and easier to evaluate without a calculator [5,6].

The inflammatory process plays a significant role in the pathogenesis of RA. Proinflammatory markers such as c- reactive protein (CRP), interleukin-6 (IL 6)[7], tumor necrosis factor (TNF-α), and IL-10 [8] were very high in synovial fluid and serum of RA patients. Studies have shown that inflammatory markers such as hs-CRP, IL- 6, IL-10, and TNF-α were significantly increased in RA patients compared to controls and correlated with disease activity [9] .

Moringa oleifera (MO) is native to Asia and Africa. It has been known to have many bioactive components [10,11]. The most widely used bioactive components are the leaves, which are rich in vitamins, phenols, flavonoids, and isothiocyanates [11]. Its leaves have higher bioactive components than its seeds or flowers. Moreover, it leaves is easy to find and has been researched for so many times. MO leaves extract has been shown to inhibit the production of cytokines such as Tumor Necrosis Factor Alpha (TNF-α), interleukin-6 (IL-6), and interleukin-8 (IL-8)[12].

Moringa has immunosuppressant properties by reducing the number of CD4 T-lymphocytes (T-helper lymphocytes) by cell apoptotic pathways due to excessive calcium entry into cells. In addition, Moringa oleifera will have an anti-inflammatory effect by inhibiting NF. Inhibition of nf will cause a decrease in proinflammatory cytokines IL 6, IL 1, and TNF, so tissue inflammation decreases [13] . Moringa oleifera leaf extract has shown anti-inflammatory, anti-arthritis, and immunosuppressant effects in mice with RA[14–16]. MO can suppress the production of proinflammatory cytokines such as TNF-α, IL-1, and IL-6 to relieve the inflammatory process[15,16]. This study aims to determine the effect of moringa oleifera extract on interleukin 6 levels and disease activity in rheumatoid arthritis patients.

Methods:

This study used a Randomized Controlled Trial (RCT) design with a total sample of 30 people divided into two groups: the treatment group and the control group. Each group contains 15 research objects. This research was conducted in the outpatient clinic of dr. Moewardi hospital in Surakarta, Indonesia.

The inclusion criteria used were female patients aged 18-60 who met the AR criteria according to the ACR / EULAR 2010 and VAS> 3. While the exclusion criteria used were pregnant patients, or using Methylprednisolone> 8 mg daily or using NSAIDs, or having comorbidities such as tuberculosis infection, diabetes mellitus, liver cirrhosis, malignancy, and Chronic Kidney Disease.

Moringa leaves extract was obtained from dried and macerated leaves in distilled water (100 g in 2L), then evaporated to dryness in an oven for four days at 40^{o}C. In previous studies on mice, it was found that Moringa oleifera extract had an immunosuppressive effect at doses >200 mg/kg. Researchers used a dose of 500 mg/kg in mice which were then converted to a dose for humans of 40.50 mg/kg bb/day for the treatment group, while the control group was given a placebo [12].

The treatment group was given intervention in Moringa oleifera extract, while the control group was given a placebo for one month. Each research object will be measured to know the IL-6 levels and SDAI scores before and after the intervention.

The prerequisite test used the normality test (Shapiro-Wilk) and the homogeneity test (Levene's Test of Homogeneity of Variances), then continued with analysis using the paired t-test and independent t-test. If the normality test shows that the data distribution is not normal, then the data analysis test uses the Wilcoxon and Mann-Whitney tests.

Ethical clearance

This study received ethical approval from the Health Research Ethics Committee of Moewardi Hospital with ethical approval number 1278/XI/HREC/2020.

Result:

The number of samples was 30 people divided into two groups: the treatment group and the control group. The results of the Shapiro-Wilk normality test showed that the research variables that had normal data distribution in both the control and MO treatment samples were Delta_IL6 and Delta_SDAI.

Table 1. Description and Normality Testing of IL_6 Variable Data and SDAI Score According to Sample Group and Measurement Period.

Variable	Control Group				Treatment Group			
	Description		Normality test		Description		Normality test	
	Mean	SD	S-W	Prob	Mean	SD	S-W	Prob.
IL-6_Pre	182,09	51,89	0,797	0,003*	198,30	72,23	0,898	0,089
IL-6_Post	147,99	52,23	0,963	0,751	79,87	54,68	0,834	0,011*
Delta_IL6	-34,09	67,23	0,913	0,152	-118,43	92.11	0,943	0,426
Skor SDAI_Pre	27,12	14,04	0,921	0,199	24,55	11,72	0,840	0,013
Skor SDAI_Post	22,37	11,83	0,964	0,762	9,89	10,06	0,836	0,011*
Delta_SDAI	-4,75	11,82	0,890	0,068	-14,65	9,40	0,951	0,547

Note: * Data distribution is not normal; after the sample is combined, it becomes Normal.

The IL-6_Pre variable has an abnormal data distribution in the control sample group, but the data distribution in the MO treatment sample group is normal. While the IL-6_Post variable, SDAI_Pre score, and SDAI_Post score had normal data distribution in the control sample group, the MO treatment sample group had an abnormal distribution. After testing with all data or a combination of the control sample group and the MO treatment sample group, it turns out that the three variables are normally distributed. Therefore, the independent t-test and paired t-test were used in this study.

From table 2, it is found that the independent t-test results show the IL-6 variable and SDAI score for the control group and the MO treatment group in the conditions before given MO leaves extracts were not significantly different ($p > 0.05$). This means that this analysis begins with a homogeneous IL-6 and SDAI score in the two sample groups, namely the control group and the MO treatment group.

Table 2. Comparison of IL-6 and SDAI Scores in The Control Group and The MO Treatment Group in The Conditions before Given Treatment.

Control			MO Treatment		Independent T Test	
Variable	Mean	Deviation Std	Mean	Deviation Std	Statistic Score	P value
IL-6	182,09	51,89	198,30	72,23	t = -0,706	0,486
Skor SDAI	27,12	14,04	24,55	11,72	t = 0,545	0,590

Note: ** Significant at the 1 percent degree of significance.

* Significant at the 5 percent level of significance.

Table 3. Comparison of IL-6 and SDAI Scores in The Control Group and The MO Treatment Group in Conditions after Given Treatment.

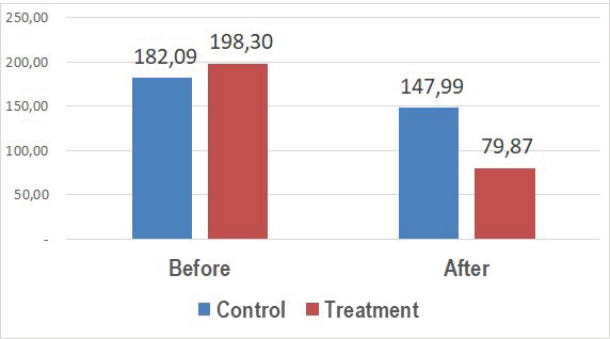
Control			MO Treatment		Independent T Test	
Variable	Mean	Deviation Std	Mean	Deviation Std	Statistic Score	P value
IL-6	147,99	62,23	79,87	54,69	t = 3,185	0,004**
Skor SDAI	22,37	11,83	9,89	10,06	t = 3,111	0,004**

Note: ** Significant at the 1 percent degree of significance.

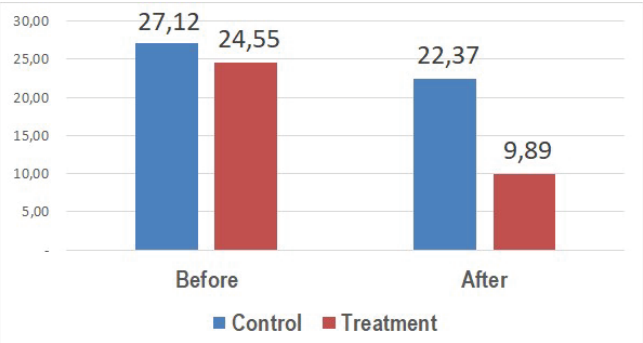
* Significant at the 5 percent level of significance.

In addition, Table 3 shows that the independent t-test results show the IL-6 variable and SDAI score for the control group and the MO treatment group in the conditions after giving MO leaves extract significantly different ($p < 0.05$). This means that the IL-6 and SDAI scores were significantly different after the treatment period in the two sample groups, namely the control group and the MO treatment group.

After the paired t-test, the results showed that the IL-6 variable and SDAI score were not significantly different before and after giving MO leaf extract to the control group ($p > 0.05$). This means that IL-6 in the control group did not change before and after given MO leaves extract treatment.

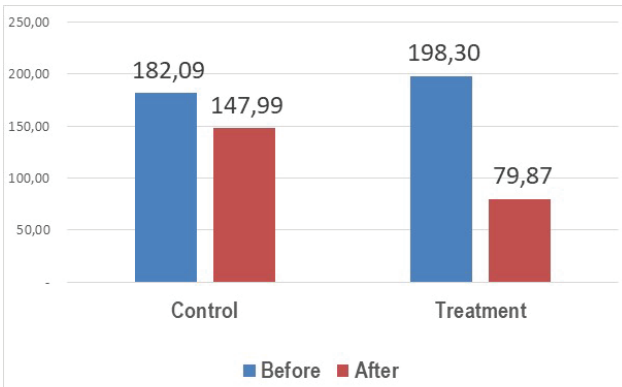


Picture 1. Comparison of IL_6 in Control Group and MO Treatment Group Before and After Treatment.

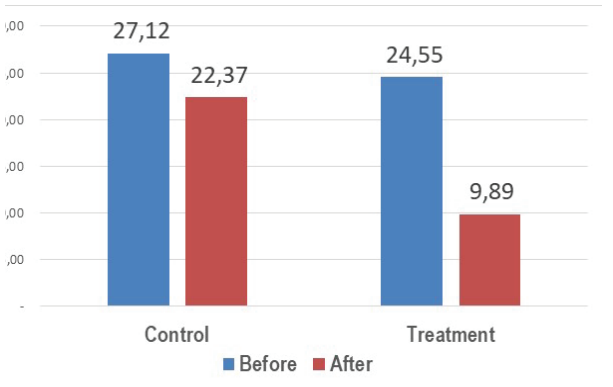


Picture 2. Comparison of SDAI Scores in Control Group and MO Treatment Group Before and After Treatment.

In addition, the paired t-test results showed that the IL-6 variable and SDAI score before and after giving MO leaf extract to the MO treatment group differed significantly ($p > 0.05$). This means that the IIL-6 and SDAI scores in the MO treatment group experienced changes before and after the MO leaves extract treatment or experienced a significant decrease after treatment was given.



Picture 3. Comparison of IL_6 Conditions Before and After Given MO Treatment to Control Group and MO Treatment Group.



Picture 4. Comparison of SDA Score Conditions Before and After Given MO Treatment in Control Group and MO Treatment Group.

The results of the paired t-test on the Delta-IL6 and Delta-SDAI variables show that it was found that the two change variables (Delta-IL6 and Delta-SDAI) differed conclusively at the 5 percent significance degree ($p < 0.05$). This condition indicates that the provision of MO leaves extract can impact reducing the IL-6 variable and SDAI score.

Discussion:

Several previous studies have shown that Moringa oleifera extract has anti-inflammatory, anti-arthritis, and immunosuppressant effects in trials with mice[14-16]. These effects are expected to suppress the inflammatory process and reduce the degree of disease activity in RA patients[14] In RA patients, there will be an increase in IL-6 levels. This increase in IL-6 levels indicates an increase in the severity of RA disease and vice versa[9,17]. Excessive ROS production will cause oxidative stress, stimulating the active transcription factor NF-κB (Nuclear factor kappa B)[18] as a marker of acute inflammation, increasing levels of oxidants expressing proinflammatory agents, including TNF-α. Acute inflammation that is

not appropriately handled, prolonged, and repeated will cause tissue necrosis [19].

In this study, M. oleifera, known to have anti-inflammatory, antioxidant, and immunomodulatory effects, was shown to lower IL 6 levels and SDAI scores in RA patients. This is in line with previous studies containing glucosinolates and isothiocyanates, has a strong effect on the production of NO (nitric oxide), can lower insulin, leptin, resistin, cholesterol, interleukin-1ß (IL-1ß), tumor necrosis factor-alpha (TNF), and glucose-6-phosphatase in diabetic rats, and based on the results of this study, it was concluded that isothiocyanate compounds might be the main bioactive ingredients that have anti-diabetic and anti-inflammatory effects. Flavonoids, like quercetin, kaempferol glucoside, and flavonoid malfat, have anti-inflammatory effects by stopping LPS macrophages from making NO. Many studies have shown that M. oleifera stops NO, VEGF, TNF, IL-2, IL-1ß, IL6, glucose-6-phosphatase, insulin, leptin, resistin, and cholesterol from doing their jobs. The most common pathway, which is thought to be the prototypical proinflammatory signaling pathway, and the parent transcription factor[13].

Studies have shown that the dose of Moringa oleifera has two effects on CD4 T lymphocytes/T helper cells: low doses stimulate the immune system, while high doses slow down the immune system. The active ingredient in Moringa leaf extract acts as an immunostimulant in the immune system, which is why the number of CD4+ T cells increases. Saponins and flavonoids are two active substances that might work as immunostimulants. Saponins and flavonoids may be able to make more of the cytokines that are needed for CD4+ T cell activity to come out. Saponins and flavonoids help regulate helper T cells by causing them to make more of the cytokine interleukin 2 (IL-2). CD4+ cells need the IL-2 cytokine to change into the Helper 2 (Th2) and Th1 T cell subsets. Moringa leaf extract can also work as an immunosuppressant in addition to being an immunostimulant. This is shown by the fact that when high doses of Moringa leaf extract were given, the number of CD4+ T cells went up less than when low doses were given. The most important effect of high doses of SLE is that they cause immunosuppression by killing lymphocytes [13].

The results of data analysis in this study indicate that the treatment group given Moringa oleifera extract showed a significant reduction in IL-6. So, it can be concluded that Moringa oleifera extract reduces or suppresses the inflammatory process. In addition, the treatment group that was given Moringa oleifera extract also showed a significant decrease in SDAI score, so it can be concluded that the administration of Moringa oleifera extract has an effect in reducing the degree of activity of RA disease. The limitation of this study is that we did not record the drug patient.

Conclusions:

Moringa oleifera extracts decrease IL-6 levels and SDAI scores in RA patients. Further research is needed regarding using Moringa oleifera extract in RA patients using different variables. It is necessary to monitor the risk of acute infection and the physical activity of the research subjects.

Conflicts Of Interest

There are no conflicts to declare.

Acknowledgments

We acknowledge research assistants, laboratory staff at the faculty of medicine, and also research and community service institutes from Universitas Sebelas Maret for their help and support for this research.

Authors' contribution

Nurhasan Agung Prabowo, Arief Nurudhin, Yulyani Werdiningsi, Dikha Dwi Putra, and Desy Puspa Putri are the researcher and data analysts and all of us prepare the manuscript

References:

1. I Nyoman Suarjana. Artritis Reumatoid. In: Buku Ajar Ilmu Penyakit Dalam. 6th ed. Jakarta: *Interna Publishing*. 2014;p. 3130-40.
2. Indonesia Reumatologi Association. Rekomendasi Perhimpunan Reumatologi Indonesia Untuk Diagnosis dan Pengelolaan Artritis Reumatoid. *Jakarta*: IRA; 2021.
3. Sun X, Li R, Cai Y, Al-Herz A, Lahiri M, Choudhury MR, et al. Clinical remission of rheumatoid arthritis in a multicenter real-world study in Asia-Pacific region. The Lancet Regional Health - *Western Pacific*. 2021 Oct;**15**:100240.
4. Prabowo NA, Adnan ZA, Nurudhin A, Werdiningsih Y, Prasetyo K. Mesenchymal Stem Cell Conditioned Medium as Good as Methyl Prednisolone in Decreasing Levels of Interleukin 10 and The Degree of Pulmonary Vasculitis in Lupus Mice. *Bangladesh J Med Sci*. 2021 Feb 1;**20**(2):426–30.
5. Salafi F, Ciapetti A. Clinical disease activity assessments in rheumatoid arthritis. *International Journal of Clinical Rheumatology*. 2013 Jun;**8**(3):347–60.
6. Sharma R, Thakare M, Thomas J, Agrawal S, Rajasekhar L, Narsimulu G. Simplified Disease Activity Index as an index of disease activity in patients with rheumatoid arthritis: a comparison with DAS28. *Indian Journal of Rheumatology*. 2009 Mar;**4**(1):11–4.
7. Prabowo NA, Setyaningrum RH, Apriningsih H. Interleukin 6 Associated with Adrenal Insufficiency in COVID-19 Patient. 2022;**4**.
8. Prabowo NA, Apriningsih H. Colchicine reduces inflammation in COVID-19 patients. IOP Conf Ser: *Earth Environ Sc*i. 2021 Jul 1;**824**(1):012087.
9. Shrivastava AK, Singh HV, Raizada A, Singh SK, Pandey A, Singh N, et al. Inflammatory markers in patients with rheumatoid arthritis. *Allergologia et Immunopathologia*. 2015 Jan;**43**(1):81–7.
10. Padayachee B, Baijnath H. An overview of the medicinal importance of Moringaceae. :9.
11. Saini RK, Manoj P, Shetty NP, Srinivasan K, Giridhar P. Relative bioavailability of folate from the traditional food plant Moringa oleifera L. as evaluated in a rat model. *J Food Sci Technol.* 2016 Jan;**53**(1):511–20.
12. Kooltheat N, Sranujit R, Chumark P, Potup P, Laytragoon-Lewin N, Usuwanthim K. An Ethyl Acetate Fraction of Moringa oleifera Lam. Inhibits Human Macrophage Cytokine Production Induced by Cigarette Smoke. *Nutrients*. 2014 Feb 18;**6**(2):697–710.
13. Nurudhin A, Widyastuti R, Prabowo NA, Adnan ZA, Werdiningsih Y. The effect of Mmoringaoleifera leaf extract on mean platelet volume and neutrophil to lymphocyte ratio in lupus. *Bangladesh J Med Sci*. 2021 Jan 1;**20**(1):68–73.
14. Fatima N, Fatima SJ. Pharmacological Screening For Anti-Arthritic Activity Of Moringa Oleifera. 2016;**9**(3):6.
15. Mahajan SG, Mali RG, Mehta AA. Protective Effect of Ethanolic Extract of Seeds of *Moringa oleifera* Lam. Against Inflammation Associated with Development of Arthritis in Rats. *Journal of Immunotoxicology.* 2007 Jan;**4**(1):39–47.
16. Fard M, Arulselvan P, Karthivashan G, Adam S, Fakurazi S. Bioactive extract from moringa oleifera inhibits the proinflammatory mediators in lipopolysaccharide-stimulated macrophages. *Phcog Mag*. 2015;**11**(44):556.
17. Orr CK, Najm A, Young F, McGarry T, Biniecka M, Fearon U, et al. The Utility and Limitations of CRP, ESR and DAS28-CRP in Appraising Disease Activity in Rheumatoid Arthritis. *Front Med*. 2018 Aug 3;**5**:185.
18. Prabowo NA, Myrtha R, Apriningsih H. Lupus Flares in COVID-19 Patients: A Case Report. 2022;3.
19. Widi Wardani AT, Purwanto B, DonoIndarto -, Wasita B, Cilmiaty AR R. Effect of Moringa Leaf Ethanol Extract on Reduced Levels of Mda, TNF-α and Description of Inflammatory Cells in Rat Sinus Mucosa Model of Acute Rhinosinusitis. *Bangladesh J Med Sci.* 2022 Feb 25;**21**(2):311–7.