ORIGINAL ARTICLE

Effect of *Moringa oleifera* Leaf Extracts on Depression in Rheumatoid Arthritis Patients

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ABSTRACT

Introduction: Depression is a mental disorder that is increasingly common nowadays. It can emerge as morbidity in chronic diseases such as rheumatoid arthritis (RA). The leaf extract of Moringa oleifera (MO) has shown to be a complementary therapy in depression, besides its anti-inflammatory role. This study aimed to evaluate the effect of MO leaf extract on Depression in RA patients. **Methods:** This quasi-experimental study with a pretest-posttest control group design comprised 32 RA patients. The participants were divided into the control (n = 16) and intervention (n = 16) groups. The intervention group received two MO leaf extract-containing capsules b.i.d. for 28 days. The depression was evaluated by Beck Depression Inventory (BDI-II) and serum cortisol test. Statistical analyses used both paired and unpaired t-tests. **Results:** The posttest means comparison of BDI-II and serum cortisol showed a significant difference between groups (p = 0.031 and p = 0.015, respectively). The pretest-posttest difference within the control group did not show significant improvement in BDI-II and serum cortisol (p = 0.076 and p = 0.106). Meanwhile, significant BDI-II and serum cortisol improvement were found in the intervention group (p = 0.003 and p = 0.048, respectively). **Conclusion:** MO leaf extract reduces depression in RA patients based on BDI-II and serum cortisol assessment.
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Keywords: Depression, Stress, Rheumatoid arthritis, *Moringa oleifera*, Adjuvant therapy

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INTRODUCTION

Depression has emerged as a frequent mental disorder globally. Its prevalence was 27.0 % (10,943/41,344 people, 95% CI 0.24 - 0.29) across eighty-three cross-sectional studies (1). Global depression statistics also showed that approximately 121 million people have depressive symptoms (2). Depression can emerge as morbidity in several chronic autoimmune diseases such as rheumatoid arthritis (RA). RA is commonly attributed to limited daily performance and low quality of life (QoF) due to inflammation symptoms (3). Furthermore, prolonged depression may worsen the outcome of RA (4). Thus, treating depression in RA in addition to pharmacological anti-inflammatory therapy is paramount.

Several anti-depressive drugs, such as tricyclic antidepressants (TCA) and selective serotonin reuptake inhibitors (SSRI), are efficacious and safe. However, prolonged administration may cause further QoF deterioration and side effects such as headaches, fatigue, sleepiness, constipation, and dry mouth (5). Some plant-based drugs containing polyphenol and flavonoid compounds are efficacious as antidepressant drugs (6).

Moringa oleifera (MO) is a fast-growing tree of the family Moringaceae. It is widely used for food and complementary medicine in several parts of the world (7). The MO leaf extract contains flavonoids, oleic acid, streatic and palmitic acid, saponins, and vitamins A, B, and C establishing significant anti-inflammatory and antioxidant properties (8). The association between oxidative stress and depression has proven to be essential biochemical features in inflammatory-based disease pathogenesis (6). Hence, this study was performed to determine the effect of MO leaf extract on depression in RA patients.

MATERIALS AND METHODS

The MO leaves extract was provided and authenticated by Jamu Scientific Clinic of Hortus Medicus in

Tawangmangu (elevation: 1,303 m above sea level). The MO dried leaves were processed into powder, and their phytochemistry analyses were based on Formularium Ramuan Obat Tradisional Indonesia 2011. The leaf fat was dissolved by petroleum ether. A heating process then extracted them at 67°C. The final extract product was packed into size-0 hard gelatin capsules. Each capsule contained 500 mg MO leaves extract. We considered its safety dose based on a previous study by Kaur et al. (9). This present study was performed following the approval from the Health Research Ethics Committee of Dr. Moewardi General Hospital (No. 1.254/XI/HREC/2020).

Federer's formula of (n-1) (t-1) ≥ 15 was used as this sample size formula is widely used in clinical or intervention trials; n, sample size in each group; t, number of groups (t = 2) (10). Its calculation showed n ≥ 16 indicating 16 or more patients in each group. We obtained 32 RA patients from the simple random sampling method. Thus, we divided them into control (n = 16) and intervention (n = 16) group.

This quasi-experimental study with a pretest-posttest control group design was conducted in the Internal Medicine Clinic of Dr. Moewardi Hospital from 1st to 29th January 2021. The depression was evaluated by Beck Depression Inventory (BDI-II) score and serum cortisol level on the pretest (day-1) and posttest (day-29) sessions. Our psychiatric colleagues assessed all patients' BDI-II scores in a blind manner. Its score interpretation includes normal (1-10), mild mood disturbance (11-16), borderline depression (17-20), moderate depression (21-30), severe depression (31-40), and extreme depression (>40).(11) The serum cortisol was obtained from the cubital vein at 08:00 AM with a normal level of 5 - 25 µg/dL (12). We also evaluated white-blood cells (WBC), C-reactive protein (CRP), and rheumatoid factor (RF) levels, yet these parameters did not represent the depression scale.

The intervention group consumed two MO leaf extract-containing capsules b.i.d. for 28 days. Meanwhile, the control received two 100 mg sugar pills or 200 mg placebo b.i.d. for 28 days as well to avoid any possible questions by participants why the control group was differentiated from their counterpart intervention group during the study. The inclusion criteria included RA-confirmed patients older than 18, and depressed patients with BDI-II score more than 20. Patients with other metabolic diseases, infectious diseases, and WBC more than 10,000/µL indicating active inflammation were excluded. All patients received Sulfasalazine and disease-modifying antirheumatic drugs (DMARDs) as the principal therapy for their RA.

The statistical analyses used the unpaired t-test to compare the pretest and posttest score of BDI-II and serum cortisol levels between the control and intervention group. Furthermore, the paired t-test to determine the pretest-posttest score difference within each group. The mean difference is considered statistically significant if p-value ≤ 0.05.We used IBM SPSS Statistics for Windows (Version 22.0. Armonk, NY: IBM Corp) to perform statistical analyses.

RESULTS

The participants' demographic and clinical baseline profiles are presented in Table I. Most participants are female (n = 18) and have RA duration for estimated 25-36 months (n = 14). The patients received several medications due to irreversible and extraarticular manifestations of RA. Several patients received a non-steroidal anti-inflammatory drug (NSAID) (19.39 %) and

Table I: The demographic and clinical baseline profiles

Variables	n
Sex	
Male	14 (43.75 %)
Female	18 (56.25 %)
Age (years)	
21-30	12 (37.50 %)
31-40	10 (31.25 %)
41-50	6 (18.75 %)
51-60	4 (12.50 %)
Smoking history for >1 year	
Yes	16 (50.00 %)
No	16 (50.00 %)
RA duration (months)	
1-12	2 (6.25 %)
13-24	9 (28.13 %)
25-36	14 (43.75 %)
37-48	7 (21.87 %)
Body mass index	
Normal	14 (43.75 %)
Overweight	5 (15.63 %)
Obesity class I	5 (15.63 %)
Obesity class II	6 (18.75 %)
Obesity class III	2 (6.25 %)
Other medication history	
MTX	10 (20.41 %)
Leflunomide	6 (12.24 %)
Thiamine	11 (22.45 %)
Pyridoxine	10 (20.41 %)
Ramipril	4 (8.16 %)
Amlodipine	3 (6.12 %)
None	5 (10.20 %)
Irreversible and extraarticular manifestation	
Frozen shoulder syndrome	3 (9.09 %)
Xerostomia	1 (3.03 %)
Neuropathy	9 (27.27 %)
Anemia	3 (9.09 %)
Hammer toe	1 (3.03 %)
Swan neck deformity	3 (9.09 %)
Carpal tunnel syndrome	1 (3.03 %)
Periodontitis	1 (3.03 %)
None	11 (33.34 %)
RA therapy	
DMARD	32 (32.65 %)
Sulfasalazine	32 (32.65 %)
NSAID	19 (19.39 %)
Prednisone	15 (15.31 %)

MTX, methotrexate; RA, rheumatoid arthritis; DMARD, disease-modifying antirheumatic drug; NSAID; non-steroidal anti-inflammatory drugs.

prednisone (15.31 %) as additional therapy based on clinical evaluation.

The comparison of all pretest variables did not show a significant statistical difference ($p > 0.05$). However, the rheumatoid factor ($p = 0.152$, 40.562 ± 6.032 vs. 37.750 ± 4.711 of intervention and control group, respectively) was the only posttest variable that did not show a significant difference (Table II). The intervention group showed significant improvement ($p < 0.05$) in BDI-II, serum cortisol and WBC during 28 days of MO leaf extract administration. Meanwhile, WBC was the only significantly improved variable in the control group during 28 days of placebo administration (Table III).

Table II: Pretest and posttest comparison of BDI-II and serum cortisol levels between groups.

Variable	Intervention (n = 16)		Control (n = 16)		*p*-value*
	Mean	SD	Mean	SD	
Pretest					
BDI-II	43.625	9.715	48.375	9.999	0.183
Serum cortisol (µg/dL)	44.125	7.535	50.000	10.905	0.086
WBC (x10⁴/µL)	8.530	0.660	8.194	0.658	0.160
CRP (mg/L)	6.162	1.777	6.558	1.801	0.536
RF (IU/mL)	44.250	4.725	46.312	4.467	0.214
Posttest					
BDI-II	17.312	4.077	33.812	9.523	0.031
Serum cortisol (µg/dL)	25.187	6.410	40.187	5.166	0.015
WBC (x10⁴/µL)	6.013	0.724	8.326	0.330	0.001
CRP (mg/L)	4.313	1.082	8.249	0.542	0.075
RF (IU/mL)	40.562	6.032	37.750	4.711	0.152

BDI-II, Beck Depression Inventory; WBC, white blood cell; CRP, C-reactive protein; RF, rheumatoid factor; SD, standard deviation; Intervention group (n = 16) received 500 mg of MO leaf extract b.i.d; Control Group (n = 16) received 200 mg placebo of sugar pills b.i.d. Statistically significant if *p*-value ≤ 0.05.
*unpaired t-test.

Table III: Pretest and posttest comparison of BDI-II and serum cortisol levels within each group.

Variable	Pre-test		Post-test		*p*-value*
	Mean	SD	Mean	SD	
Intervention (n=16)					
BDI-II	43.625	9.715	17.312	4.077	0.003
Serum cortisol (µg/dL)	44.125	7.535	25.187	6.410	0.039
WBC (x10⁴/µL)	8.530	0.660	6.013	0.724	0.001
CRP (mg/L)	6.162	1.777	4.313	1.082	0.059
RF (IU/mL)	44.250	4.725	40.562	6.032	0.101
Control (n=16)					
BDI-II	48.375	9.999	33.812	9.523	0.076
Serum cortisol (µg/dL)	50.000	10.905	40.187	5.166	0.106
WBC (x10⁴/µL)	8.194	0.658	8.326	0.330	0.049
CRP (mg/L)	6.558	1.801	8.249	0.542	0.051
RF (IU/mL)	46.312	4.467	37.750	4.711	0.075

BDI-II, Beck Depression Inventory; WBC, white blood cell; CRP, C-reactive protein; RF, rheumatoid factor; SD, standard deviation; Intervention group (n = 16) received 500 mg of MO leaf extract b.i.d; Control Group (n = 16) received 200 mg placebo of sugar pills b.i.d. Statistically significant if *p*-value ≤ 0.05.
*unpaired t-test.

DISCUSSION

The fresh MO leaf contains vitamin A and carotenoids, which has efficacy in immune competence. Its leaf also contains 200 mg/ 100 g of vitamin C. These bioactive components possess antioxidants to protect human cells from free radicals, toxins, and pollutants (7). Polyphenol compounds, alkaloids, glucosinolates, isothiocyanates, tannins, and saponins are also prominent phytochemical properties of MO leaf for medical purposes. They exhibit anti-cancer, antiatherosclerotic, and anti-inflammatory roles (13).

The MO leaf extract possesses potent anti-inflammatory and antioxidant activity, thus indicating that it can treat brain neurochemical imbalance-induced depression in chronic diseases (14). This is well-known that elevated pro-inflammatory cytokines, including tumor necrosis factor (TNF)-α, interleukin (IL)-6, IL-10, and IL-1β, are found in depression and RA flare (15). The bioactive compounds of MO leaf extract decrease peripheral cytokines upregulation by inhibiting three pathways within brain structures. These pathways include the neuronal pathway of afferent nerves in the hypothalamic nuclei, the direct humoral pathway in the blood-brain barrier free circumventricular organs and choroid plexus, and the microglial activation pathway. Those inhibited three pathways will decrease the production of chemokines, pro-inflammatory cytokines, cortisol and proteases, which stop monocyte migration into behavior-associated brain areas (9,15).

Flavonoid is on of main constituent of MO leaf extract as several studies indicate that some minor-to-major depression can be alleviate with flavonoid (16). A preliminary animal study of chronic dose 200 mg/ day of MO leaves extract exhibited the antidepressant activity based on immobility in tail suspension test (9). This can explain that the antioxidant property of flavonoid act on neurogenesis (17). Several psychiatric disorders such as schizophrenia and depression are also linked to neurogenesis as well (18). Flavonoid helps as free radicals scavenger and inhibits free radicals' deterioration effect on cell membranes and DNA (17). Moreover, a high concentration of saponins in MO leaf extract may help as an antidepressant by the free radical-scavenging property of polyphenolic compound as neuroprotective activity (19).

The MO leaf extract could alleviate joint inflammation in RA by reducing serum levels of prostaglandin E2, CRP, and TNF-α (20). It also downregulates the NF-κB and cyclooxygenase 2. These processes significantly restored the normal joint's histopathological feature in RA. Thus, all findings showed that the MO leaf extract could reduce depression and alleviate inflammation in RA (21). Further, these beneficial downstream consequences will lead to QoF improvement, morbidity reduction, and medication adherence (22).

The BDI-II showed a reliable, valid, and culturally relevant assessment and screening instrument to evaluate depression in Indonesia. A study showed that BDI-II has 83.3% sensitivity and 86.8% specificity to diagnose a major depressive disorder, 80.9% sensitivity and 76.4% specificity to detect depressive-related disorder (23). Moreover, cortisol reaches maximum levels in the morning in three fractions such as 80% cortisol binding globulin (CBG), 10–15% is bound to albumin,

and the rest 5–10% circulates as biologically active and free cortisol (12). In severe or chronic illness, the stress imposed upon the patient causes CBG to decrease due to elevated IL-6 levels, resulting in high levels of active and free cortisol (24). Thus, depression severity could be represented by both morning cortisol levels and BDI-II assessment.

This present study has several limitations. We suspected a slight effect of smoking history, the RA duration, and several participants' baseline profiles on the results. There were also the potential effect of DMARD and sulfasalazine in our patients during the study. Hence, we suggest further research to conduct a similar study without any medication than the MO leaf extracts administration while maintaining the patient's safety during the study. Moreover, multiple center studies and randomized-controlled trials with participants of more than 32 were also suggested.

CONCLUSION

The MO leaf extract has a potential antidepressant role besides its main properties of anti-inflammatory and antioxidant. It significantly reduces depression in RA patients based on BDI-II and serum cortisol assessment. Thus, the 500 mg MO leaves extract could become potential adjuvant therapy in RA patients with depression.

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