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Original article

Antiarthritic, anti-inflammatory activity of *Moringa peregrina* seed oil and leaves in Freund's complete adjuvant-induced arthritis in rats

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ABSTRACT

Rheumatoid Arthritis (RA) is the most frequent form of autoimmune arthritis, with a global prevalence of 0.3%–1% according to the World Health Organization. Objective: This study aimed to investigate the effect of *Moringa peregrina* leaves and seed oil on RA and other inflammations in male Wistar albino rats. Method: We equally divided 25 rats into five groups. One was the nonarthritic (control) group, which comprised randomly selected rats. The four other groups were injected with 0.1 ml of Complete Freund's Adjuvant with 10 mg/ml concentration of heat-killed *Mycobacterium tuberculosis* once intradermally in the right paw. These four groups were randomly categorized as follows: Arth group, arthritic group; ML group, administered with 0.5 g of *Moringa* leaves; MO group, administered with 1 ml of *Moringa* seed oil; and MLO group, administered with combined *Moringa* leaves and *Moringa* seed oil. In the biochemical measurements of the blood, we included the rheumatoid factor (RF), proinflammatory cytokines (IL-1α, IL-12P70, IL-17A, IL-6, IL-1β, and TNF-α), and anti-inflammatory cytokines (IL-4, Il-13, and IL10). Results: The Arth group had higher RF levels than the nonarthritic group; after treatment, which referred to the MO, MLO, and ML groups, the RF level decreased. Moreover, the Arth group had higher proinflammatory cytokines (IL-6, IL-1β, and TNF-α) than the nonarthritic group. After treatment, the IL-6 and IL-1β levels in the treatment groups declined. Furthermore, the TNF-α level significantly decreased in all treatment groups. Conversely, the treatment groups had significantly higher ($p \leq 0.05$) anti-inflammatory cytokine levels (IL-4, IL-10) than the Arth group. Both the IL-4 and IL-10 levels significantly (both: $p \leq 0.05$) increased in the MO group followed by the ML group and the MLO group. In conclusion, the *M. peregrina* leaves and seed oil can positively enhance anti-inflammatory activities. Moreover, the *M. peregrina* oil has significant antiarthritic and anti-inflammatory effects. However, further studies are needed to clarify the effective components of *M. peregrina* leaves and oil responsible for the antiarthritic and anti-inflammatory activities.

1. Introduction

Rheumatoid Arthritis (RA) is the most frequent type of autoimmune arthritis, affecting millions of people worldwide. RA can affect people at any age, although the occurrence is substantially higher among people over 40 years of age, with females being 2 to 3 times more prone to RA than males (Chen et al., 2019). It affects approximately 0.5%–1.0% of adults in the developed world. The World Health Organization (WHO) statistics reported that the global prevalence of RA varies between 0.3% and 1%. However, the exact prevalence of RA in Saudi Arabia remains uncertain (Almoallim and Alharbi, 2014).

RA results from the interactions between hereditary (genetic) and environmental factors, such as psychological stress, other medical illnesses, and cigarette smoking (Yarwood et al., 2016). It is mainly characterized by swelling, pain, and decreased joint function; in late stages, joint stiffness and bone deformity occurs (Guo et al., 2018). Other distinct symptoms include joint inflammation, synovial breakdown, articular cartilage destrcution, and motor disabilities (Gao et al., 2019). In the synovial membrane,

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inflammatory cell production is stimulated by proinflammatory modulators responsible for bone damage (Guo et al., 2018).

Currently, nonsteroidal anti-inflammatory drugs are used to reduce clinical signs such as pain and joint swelling and unfortunately, 30% of patients do not respond to such treatment. Furthermore, long-term administration of these drugs can cause severe gastrointestinal tract side effects (Gao et al., 2019). Therefore, more studies on using alternative therapies, such as herbal remedies, are needed.

Some plants have several medicinal properties, thereby used for treating inflammations, liver disease, cancers, hematological, and hepatic and renal function disorders (Nafiu et al., 2019). Lately, *Moringa peregrina* gained considerable attention because of its traditional, nutritional, medical uses. The Moringaceae family consists of 13 species, but the most famous is *Moringa oleifera*, which is native in India and grows in the southern Himalayan valleys and in tropical and subtropical regions. Another species of the Moringaceae family is *M. peregrina*, which grows in Egypt and Sudan. *M. peregrina* is also common in Saudi Arabia, especially in Al-Madinah, Al-Wajh, and Tihama regions (Gopalakrishnan et al., 2016). This type of *Moringa* plant is the fastest-growing deciduous tree among the *Moringa* species, reaching up to 3–10 m, and it has a grayish green bark adapted to high aridity. Its leaves are 30–40 cm long, obovate, and deciduous (Olson et al., 2016).

M. peregrina seed oil is used for cooking, moisturizing the skin, preventing sun strikes, and treating abdominal pain and other illnesses. In Saudi Arabia, it is locally called as Al Ban or Al Yeser tree (Abusuwar and Abohassan, 2017). Traditionally, young *M. peregrina* leaves are used for treating wounds, while the bark juice is used for treating fever, headache, constipation, back and muscle pains, and burns (Senthilkumar et al., 2018). Interestingly, *M. peregrina* leaves are recently reported as rich sources of potassium, calcium, phosphorus, iron, A and C vitamins, and essential amino acids compared with the fruit sources of these nutrients (Asghari et al., 2015). The leaves also contain antioxidants such as phenols and flavonoids, which consist of 32 compounds in various alcohol extracts of the leaves. It is also rich in alkaloids, tannins, phenolics, saponins, and steroids. These compounds have potential antioxidant, anticarcinogenic, antimicrobial, and immune-boosting effects (Kilany et al., 2020). Meanwhile, research indicated that the antioxidant activity is higher in the leaves than in the other parts of the plant (Mabrouki et al., 2020).

Complete Freund's adjuvant (CFA) can generate RA in certain animals and display results similar to human RA because it promotes sudden local inflammatory responses, including arthritis (Bihani et al., 2014). Additionally, the CFA-generated RA animal model was used to predict novel compounds as anti-RA, allowing the utilization of a fewer number of laboratory animals as compared with other arthritis-induced models (Tuncel et al., 2016).

Hence, the present study aimed to first explore the possible therapeutic potential of *M. peregrina* oil or *M. peregrina* dried leaves or a combination of both as a dietary supplement in rat models experimentally induced with RA.

2. Materials and methods

2.1. Preparation of samples (leaves and oil)

2.1.1. Leaves

The leaves of *M. peregrina* were obtained from Station Research and Agricultural Experiments South Riyadh logic Dirab (Riyadh, KSA). They were dried at room temperature (25 °C) for 48 h and then reshaped into capsules. Total fat extraction and fatty acid analysis were performed at IDAC Laboratory in Al-Kharj, Saudi Arabia. Total fat was extracted according to the EN-ISO 6492 method (2018), while the fatty acid analysis was measured according to the AOAC 996.01 method (Bligh and Dyer, 1959).

2.1.2. Oil

The oil extracted from *M. peregrina* seeds was obtained from Al-Namouthajiah Alowla Farm in Al-Madinah. It was methylated to obtain fatty acid methyl esters according to the method suggested by (Bligh and Dyer, 1959) and then separated and identified by gas chromatography. Briefly, we used a gas chromatograph equipped with a capillary split/splitless injection and flame ionization detector. The capillary column was (30 m × 0.25 mm, internal diameter) was composed of 0.2 m nonbonded 90% biscyanopropyl and 10% polysiloxane. System settings were as follows: injector temperature of 250 °C, detector temperature of 275 °C with gas flows of 34 ml/min for USP hydrogen and 300 ml/min for air breathing quality with a split ratio of 100:1. The carrier gas was helium, with a linear velocity of 21 cm/s at 175 °C. The temperature was initially 120 °C held for 4 min and then increased to 5 °C/min until reaching the final temperature of 230 °C held for 5 min.

2.2. Animal model

We used 25 adult male Wistar albino rats, which weighed 160–190 g and aged 5–6 weeks. These rats were obtained and housed at the Experimental Surgery and Animal Lab, College of Medicine in King Saud University in cooperation with Prince Naif Health Research Center. These animals were housed at 24 °C, with relative humidity (50% ± 5%) and a 12-hour light/dark cycle in polypropylene cages. In addition, they were acclimatized to the laboratory environment for 7 days before the experiment began.

2.3. Animal diet

Standard Diet (4RF21 certificate) was obtained from (Mucedola, s.r.l, Settimo Milanese, Milano, Italy). Each animal was fed with 25 g/day, with free access to drinking water, throughout the experimental period of 30 days.

2.4. Induced arthritis in animals

Experimental arthritis was induced according to the modified method of Pearson and Wood (1963). We anesthetized 20 out of the 25 animals through CO_2 inhalation and injected each one of them with 0.1 ml of CFA (Chondrex, Inc., Washington, USA) containing 10 mg/ml concentration of heat-killed *Mycobacterium tuberculosis* once intradermally in the right hind paw (Groups B to E). This time of adjuvant injection was represented as day 0.

The experiment was conducted at the Experimental Surgery and Animal Lab, College of Medicine in King Saud University for 30 days in addition to a week of acclimation and preparation. All of the 25 animals were equally divided randomly according to weight into five different groups (5 rats each) as follows:

Group A (Control): Nonarthritic rats (control negative) administered with standard diet

Group B (Arth): Arthritic rats (control positive) administered with standard diet

Group C (ML): Arthritic rats administered with 0.5 g of dried *Moringa* leaves + standard diet

Group D (MO): Arthritic rats administered with 1 ml of *Moringa* oil + standard diet

Group E (MLO): Arthritic rats administered with (0.5 g of dried *Moringa* leaves + 1 ml of *Moringa* oil) + standard diet

Each rat was given 25 g of standard diet daily, except for the animals in group C, who were given 20 g of the diet because they avoided consuming the leaves during the preparation week and only started consuming leaves when the food intake was slightly

decreased. The remaining amount of food and leaves were measured daily to calculate the intake of food and leaves. At the end of the experiment, the rats were fasted for 12 h and then anesthetized by sevoflurane (Liu et al., 2015). Blood was withdrawn via the orbital sinus of the eye, and the animals were euthanized by inhaling excess drops of sevoflurane. Blood samples were collected using heparin plasma tubes. These tubes were placed immediately in a centrifuge at a speed of 1000 × *g* for 15 min. Finally, the plasma was pulled using a micropipette and then stored at Eppendorf tubes in a freezer at −30 °C for assays.

2.5. Measurement of growth indicators

2.5.1. Weight gain

The initial (W_i) and final (W_f) weights were recorded at the beginning and end of the experimental period, respectively. The weight gain (W_g) was calculated as the difference between W_f and W_i, as shown in the following equation:

$$W_g(g) = W_f - W_i$$

2.5.2. Total food intake

The amount of daily consumed food was determined by recording the amounts of daily given and remaining food. The total food intake (TFI) was calculated as the summation of daily consumed food according to the following equation:

$$\mathrm{TFI} = \sum_{i=1}^{n=30} dailyconsuemdfoodintake(\mathrm{g})$$

2.5.3. Food efficiency

Foor efficiency (FE) was calculated as ratio of W_g and TFI, as shown below:

$$\mathrm{FE} = \frac{W_g}{\mathrm{TFI}}(ratio)$$

2.5.4. Relative mass change

The relative mass change (RMC) variable represents the ratio of W_g and W_i and was calculated using the following equation:

$$\mathrm{RMC} = \frac{W_g}{W_i}(ratio)$$

2.5.5. Biochemical measurements of the blood

The biochemical measurements of the blood were conducted in Prince Mutaib bin Abdullah Chair for Biomarkers of Osteoporosis, College of Sciences, King Saud University and King Faisal Specialist Hospital Research Center in Riyadh.

2.6. Measurement of the inflammatory indicators in the blood

2.6.1. Rheumatoid factor (RF) level

The RF level, expressed as IU\ml, was measured in plasma by enzyme-linked immunosorbent assay (ELISA) using Rat Rheumatoid Factor ELISA Kit (Cusabio Biotech Company, USA) (Ingegnoli et al., 2013).

2.6.2. Anti-inflammatory cytokines

Three cytokines (IL-4, IL-13, and IL-10) were measured in plasma by using custom multiplex immunoassays, which were based on Luminex™ xMAP™ technology from Thermo Fisher Scientific Company, (Waltham, USA) (Zhang and An, 2007).

2.6.3. Proinflammatory cytokines

Six cytokines (IL-1α, IL-1β, IL-6, IL-12P70, IL-17A, and TNF-α) were measured in plasma by using Custom multiplex immunoassays, which were based on Luminex™ xMAP™ technology from Thermo Fisher Scientific Company (Waltham, MA USA) (Chen et al., 2013). The cytokine levels are expressed as pg\ml.

2.7. Statistical analysis

The results are described as means ± SD. Statistical data were examined using a one-way analysis of variance (ANOVA), with the statistical significance assumed at $p \leq 0.05$. If the total p value was statistically significant ($p \leq 0.05$), the study groups were further compared using Tukey's post-hoc test. All statistical data were analyzed using Statistical Package for the Social Sciences software (SPSS version 22. Inc., Chicago, IL, USA).

3. Results

Table 1 presents the effect of different treatments on growth indicators in all rat groups. All growth indicators in the Arth group significantly changed ($p \leq 0.05$) compared with those in the control group. However, the growth indicators of rats in the ML group, which were treated with *Moringa* leaves, were significantly reduced ($p \leq 0.05$) compared with those in the Arth group. Meanwhile, the body weight of rats in the MO group, which received *Moringa* seed oil, significantly increased ($p \leq 0.05$). However, the body weight of rats in the MLO group, which received both the *Moringa* leaf and oil extracts, was the highest among all groups. Daily food consumption and total food intake were not significantly different between the control and Arth groups but were significantly different between the ML and MO groups and both the control and Arth groups.

Table 2 shows the effects of all treatments on the RF in all experimental groups. The RF value in the Arth group significantly increased ($p \leq 0.05$) as compared with all other groups. However, using either *Moringa* leaves (ML group) or *Moringa* seed oil (MO group) or a combination of both (MLO group) attenuated the effect of CFA, leading to near-normal RF values.

Table 3 summarizes the effect of different treatments on anti-inflammatory cytokines (IL-4, IL-13, and IL-10). The anti-inflammatory cytokines (IL-4, IL-10) significantly increased ($p \leq 0.05$) in all treatment groups (ML, MO, and MLO groups) compared with those in the Arth group. The levels of IL-4 and IL-10 were significantly elevated ($p \leq 0.05$) in the MO group, followed by the ML group and then the MLO group.

Table 4 lists the effects of different treatments on proinflammatory cytokines. As confirmed by the CFA-induced arthritis model, the proinflammatory cytokines IL-6, IL-1β, and TNF-α significantly increased ($p \leq 0.05$) in the Arth group compared with those in the control group. Conversely, the IL-6 level significantly decreased ($p \leq 0.05$) in all treatment groups, but the lowest level was observed in the MO group, followed by the MLO group and then the ML group. The IL-1β level also significantly decreased ($p \leq 0.05$), with the lowest level in the MLO group, followed by the MO group and then the ML group. Likewise, the TNF-α level significantly ($p \leq 0.05$) decreased, with the lowest level in the MO, followed by the MLO and ML groups sequentially.

4. Discussion

RA is an inflammatory rheumatic ailment that causes synovial inflammation and destruction of joint bones (Guo et al., 2018). The adjuvant arthritis rat model, which is an immune inflammatory model, was used in this study because its pathogenesis and

Table 1
Effect of *M. peregrina* Leaf and Oil Intake on Growth Indicators.

Groups	Initial weight	Final weight	Weight gain	Daily food consumption	Total food intake	Food efficiency ratio	Relative mass change
Control (n = 5)	172 ± 10.75^{b}	323.4 ± 14.84^{ab}	151.4 ± 9.81^{ab}	24.83 ± 0.26^{c}	746.8 ± 4.09^{c}	0.20 ± 0.01^{a}	0.88 ± 0.07^{ab}
Arth (n = 5)	216.4 ± 10.99^{c}	370.2 ± 23.03^{c}	153.8 ± 0.85^{abc}	24.9 ± 0.17^{c}	747 ± 5.19^{c}	0.21 ± 0.03^{a}	0.71 ± 0.11^{a}
ML (n = 5)	142.4 ± 6.54^{a}	280.8 ± 19.89^{a}	138.4 ± 14.98^{a}	19.83 ± 0.20^{a}	595.2 ± 6.14^{a}	0.23 ± 0.02^{ab}	0.97 ± 0.08^{b}
MO (n = 5)	171.6 ± 6.19^{b}	356 ± 28.97^{bc}	84.4 ± 23.35^{bc}	23.25 ± 1.59^{b}	699.4 ± 44.73^{b}	0.26 ± 0.02^{b}	1.07 ± 0.10^{b}
MLO (n = 5)	186.8 ± 2.95^{b}	379 ± 26.85^{c}	192.2 ± 28.45^{c}	24.46 ± 0.66^{bc}	733.8 ± 19.94^{bc}	0.26 ± 0.03^{b}	1.03 ± 0.16^{b}

Data are represented as mean ± standard deviation. Model, ANOVA. $p \leq 0.05$ means significant. Superscript ^{ab} indicates significant differences among various groups, as indicated by ANOVA followed by Duncan's multiple range tests. **Control**: Nonarthritic rats (control negative) given with standard diet, **Arth**: Arthritic rats (control positive) given with standard diet, **ML**: Arthritic rats given with 0.5 g of dried *Moringa* leaves + standard diet, **MO**: Arthritic rats given with 1 ml of *Moringa* oil + standard diet, **MLO**: Arthritic rats supplemented with (0.5 g of dried *Moringa* leaves + 1 ml of *Moringa* oil) + standard diet.

Table 2
Effect of *M. peregrina* Leaf and Oil Intake on Rheumatoid Factor.

Groups	RF (IU/ml)
Control (n = 5)	188.88 ± 79.62^{a}
Arth (n = 5)	337.81 ± 30.75^{b}
ML (n = 5)	220.34 ± 37.16^{a}
MO (n = 5)	187.85 ± 45.60^{a}
MLO (n = 5)	191.12 ± 38.11^{a}

Data are represented as mean ± standard deviation. Model, ANOVA. $p \leq 0.05$ means significant. Superscript ^{ab} indicates significant differences among various groups, as indicated by ANOVA followed by Duncan's multiple range tests. **Control**: Nonarthritic rats (control negative) given with standard diet, **Arth**: Arthritic rats (control positive) given with standard diet, **ML**: Arthritic rats given with 0.5 g of dried *Moringa* leaves + standard diet, **MO**: Arthritic rats given with 1 ml of *Moringa* oil + standard diet, **MLO**: Arthritic rats supplemented with (0.5 g of dried *Moringa* leaves + 1 ml of *Moringa* oil) + standard diet. **RF**: rheumatoid factor.

Table 3
Effect of *M. peregrina* Leaf and Oil Intake on Anti-inflammatory Cytokines.

Groups	Anti-inflammatory cytokine markers		
	IL-4 (pg/ml)	IL-13 (pg/ml)	IL-10 (pg/ml)
Control (n = 5)	81.80 ± 0.65^{a}	41.87 ± 0.72^{a}	4.04 ± 2.00^{a}
Arth (n = 5)	84.62 ± 1.58^{ab}	40.71 ± 0.44^{a}	6.65 ± 0.95^{ab}
ML (n = 5)	88.9 ± 3.88^{b}	41.91 ± 0.48^{a}	10.50 ± 1.32^{c}
MO (n = 5)	89.19 ± 2.96^{b}	42.02 ± 1.28^{a}	11.05 ± 2.09^{c}
MLO (n = 5)	87.45 ± 1.94^{b}	41.91 ± 0.74^{a}	9.37 ± 2.59^{bc}

Data are represented as mean ± standard deviation. Model, ANOVA. $p \leq 0.05$ means significant. Superscript^{ab} indicates significant differences among various groups, as indicated by ANOVA followed by Duncan's multiple range tests. **Control**: Nonarthritic rats (control negative) given with standard diet, **Arth**: Arthritic rats (control positive) given with standard diet, **ML**: Arthritic rats given with 0.5 g of dried *Moringa* leaves + standard diet, **MO**: Arthritic rats given with 1 ml of *Moringa* oil + standard diet, **MLO** Arthritic rats supplemented with (0.5 g of dried *Moringa* leaves + 1 ml of *Moringa* oil) + standard diet. **IL**: Interleukin.

pathological manifestations are similar to those of human RA (Li et al., 2007). Currently, only few studies, regardless of whether local or global, were focused on the nutritional value and therapeutic effects of *M. peregrina* tree (Senthilkumar et al., 2018).

The leaves and seed oil of *M. peregrina* possess nutritional and medicinal values, owing to their high percentage of antioxidants and unsaturated fatty acids. The current study used *M. Peregrina* leaves and seed oil as dietary supplements to investigate their reducing effects on CFA. The study results revealed that a slight increase in body weight was induced in the Arth group. Meanwhile, dietary supplementation with *M. peregrina* led to body weight reduction in all arthritic rat groups, consistent with the results of previous studies (Gopalakrishnan et al., 2016; Kilany et al., 2020). These previous studies concluded that Moringaceae leaves can reduce body weight because of their fiber content (7.98 g/100 g DW). which keeps a healthy digestive system, adjusts the cholesterol level adjustment, and reduces serum glucose levels. Furthermore, supplementing Moringaceae leaves could adjust serum lipid profile, thereby preventing obesity in animal models. Additionally, saponins and tannins correlate with decreased energy needs for protein and lipid biosynthesis, causing a reduction in body lipid and growth performance (Dongmeza et al., 2006). Moringaceae family leaves are rich in saponins that inhibit cholesterol absorption by binding to bile acids, consequently lowering bile acids' enterohepatic circulation; it also increases fecal excretion of cholesterol, resulting in the reduction of plasma cholesterol levels. In addition, the hydroalcoholic extract of *M. peregrina* leaves reduces blood lipid levels in hyperlipidemic rats (Rouhi-Boroujeni et al., 2015). The current study demonstrated that *M. peregrina* seed oil supplementation induced an increase in body weight as compared with all other supplementations, possibly because of its increased content of protein, amino acids, sterols, and polyunsaturated fatty acids. The fat content in *M. peregrina* seeds is 55.7%, which may also account for the increased body weight gain (Al-Dabbas et al., 2010).

In the present study, the RF levels were higher in the Arth group than in the control group, consistent with a previous work (Yau and Holmdahl, 2016), which showed that RF is a predisposing factor in the development of RA. However, this effect was counteracted after dietary supplementation, as seen in the MO, MLO, and ML groups. The leaves' active constituents including alkaloids, tannins, phenolics, saponins, and steroids account for the leaves' ability to reduce RF (Sung et al., 2019).

The process of inflammation is generally tightly regulated, including the mediators that initiate and maintain inflammation and those mediators that end the process. Therefore, in chronic inflammation, an imbalance between the two mediators leads to uncontrolled inflammation, resulting in cellular damage. Thus, in RA, this damage is manifested by cartilage and bone obliteration (Aravinthan et al., 2020). As seen in the study results, inflammation was the most pronounced manifestation of CFA-induced arthritis, which included the significant alteration in the anti-inflammatory cytokines (IL-4 and IL-10); however, the IL-13 did not significantly change compared with that in the control group. Similarly, the proinflammatory cytokines (IL-1α, IL-1β, IL-6, IL-12P70, IL-17A, TNF-α) significantly increased as compared with those in the control rat group. Proinflammatory cytokines released during arthritis advancement by chondrocytes and synovial cells can also induce chondrocyte apoptosis. Freund adjuvant-induced arthritis is the most generally applied chronic research model correlated with an immune-mediated inflammatory reaction, given that rats are exceptional in developing polyarthritis after CFA treatment; thus, they are often used for study purposes (Chen et al., 2013).

Inflammation involves multiple processes, including initiation of inflammatory cells, excretion of proinflammatory cytokines, and release of various inflammatory mediators, resulting in

Table 4
Effect of *M. peregrina* Leaf and Oil Intake on Proinflammatory Cytokines.

Groups	Proinflammatory cytokine markers					
	IL-1α (pg/ml)	IL-1β (pg/ml)	IL-6 (pg/ml)	IL-12p70 (pg/ml)	IL-17A (pg/ml)	TNF-α (pg/ml)
Control (n = 5)	85.97 ± 6.01[a]	1.17 ± 0.24[b]	1187.68 ± 30.32[a]	302.71 ± 37.46[a]	132.90 ± 2.73[a]	0.97 ± 0.29[b]
Arth (n = 5)	86.95 ± 3.27[a]	2.27 ± 0.16[c]	1249.79 ± 11.49[b]	312.52 ± 30.55[a]	136.49 ± 4.83[a]	1.78 ± 0.10[c]
ML (n = 5)	86.78 ± 4.59[a]	1 ± 0.21[ab]	1208.13 ± 7.68[a]	288.98 ± 13.79[a]	133.42 ± 4.42[a]	1.23 ± 0.10[b]
MO (n = 5)	85.46 ± 3.45[a]	0.70 ± 0.54[ab]	1180.97 ± 20.08[a]	318.406 ± 25.90[a]	131.194 ± 8.65[a]	0.55 ± 0.04[a]
MLO (n = 5)	84.78 ± 3.8[a]	0.47 ± 0.08[a]	1199.55 ± 18.42[a]	319.71 ± 36.51[a]	131.54 ± 8.63[a]	1.18 ± 0.13[b]

Data are represented as mean ± standard deviation. Model, ANOVA. $p \leq 0.05$ means significant. Superscript[ab] indicates significant differences among various groups, as indicated by ANOVA followed by Duncan's multiple range tests. **Control**: Nonarthritic rats (control negative) given with standard diet, **Arth**: Arthritic rats (control positive) given with standard diet, **ML**: Arthritic rats given with 0.5 g of dried *Moringa* leaves + standard diet, **MO**: Arthritic rats given with 1 ml of *Moringa* oil + standard diet, **MLO**: Arthritic rats supplemented with (0.5 g of dried *Moringa* leaves + 1 ml of *Moringa* oil) + standard diet. **IL**: Interleukin, **TNF-α**: Tumor necrosis factor alpha.

inflammatory symptoms such as redness, swelling, fever, and pain (Guo et al., 2018). Arthritis is a pathological condition linked to the increase in certain proinflammatory mediators, such as IL-1, IL-6, and TNF-α (Kany et al., 2019), which are essential mediators in the initiation and development of arthritis (Di Paola et al., 2018). Moreover, the synovial fluid contains high amounts of inflammatory cells that release proinflammatory cytokines, such as TNF-α, and interleukins (Ou et al., 2019).

The release of anti-inflammatory and proinflammatory cytokines worsens the synovial status in various ways; the secretion of IL-1β and TNF-α plays an important role in destroying both the articular cartilage and bone. IL-1β is involved in the synovial inflammatory response and synovial fibrosis in arthritis (Chen et al., 2019). Meanwhile, elevation of the IL-1β levels induces vasospasm formation and bone cartilage destruction (Liu et al., 2020). IL-6 causes an extreme proliferation of synovial cells, promote pannus formation, and articular cartilage destruction (Kim et al., 2013). The reduction of IL-10 levels elevates mediated proinflammatory cytokines in vivo, causing an arthritic inflammatory response (Wong et al., 2000).

Our data are in line with previous study (Siouti and Andreakos, 2019), which revealed that the levels of anti-inflammatory and proinflammatory cytokines were higher in arthritic rat models than in the control group. The study findings also agreed with those reported by Aiyalu et al. (2017), which stated that the rise in TNF-α, IL-1β and IL-1α serum levels is a characteristic feature of arthritis.

In the current study, we found that treatment with *M. peregrina*, either as leaves or seed oil, decreased the studied inflammatory parameters. Consistent with our study, the study of (Saleem et al., 2020) showed that *M. peregrina* leaves have potential anti-inflammatory, antioxidant, and antiarthritic effects, which exposed that the leaf extracts of *Moringa* species contain terpenoids, have antioxidant, anticarcinogenic, and antimicrobial potentials, and could improve the immune system.

Although *M. peregrina* seeds are widely used in traditional medicine, only few scientific studies investigated their therapeutic effectiveness and mechanism of action as it contain 42%–54% of oil, which is characterized by high levels of unsaturated fatty acids, and approximately 70% of the oil is oleic fatty acid (Al-Owaisi et al., 2014). It also contains a small percentage of linoleic and linolenic acids, which play an important role in inflammation as precursors of a potent group of inflammation modulators called eicosanoids (Al Juhaimi et al., 2017). investigated the fatty acid composition of *M. peregrina* seed oil along with two other *Moringa* species; around 12 fatty acids were identified in the seed oil, with linolenic acid as the highest (32.53%), followed by palmitic acid (17.6%), oleic acid (7.14%), and arachidic (4.85%) acids. Similarly, (Abd Rani et al., 2018) reported that *Moringa* seed oils counteracted the antioxidant enzymes, subsequently reducing the levels of the proinflammatory cytokines TNF-a and IL-6 (Elsayed et al., 2016) suggested that the essential oils isolated from *M. peregrina* have cytotoxic properties against cancer cells. In the same context, flavonoids present in *M. peregrina* seed oil cause the oil's antitumor and anti-inflammatory effects (Gopalakrishnan et al., 2016). Similar to our study, a study by Elsayed et al. (2016) proposed that *M. peregrina* seeds can help prevent inflammatory diseases by inhibiting the inflammatory mediators. Furthermore, (Mabrouki et al., 2020) proposed that oil extracted from the seeds of Moringaceae has an antioxidant potential and decreases the MDA level, thereby inhibiting the production of inflammatory cytokines. Hence, the seed oil of *M. peregrina* provides a significant source of antiproliferative compounds necessary for disease prevention and health promotion (Senthilkumar et al., 2018).

The earlier indicators considered using Moringaceae family for different inflammations in many studies (Saleem et al., 2020). Thes apparent anti-inflammatory outcomes attained by Moringaceae family treatment (either leaves or seeds) can likely be attributed to the presence of flavonoids and antioxidants in its extracts. This plant has a rich source of antioxidants, including flavonoids and polyphenolic compounds (Senthilkumar et al., 2018). Flavonoids are responsible for preventing osteoporosis by causing an increase in bone mineral density. However, treatment with Moringaceae family leaves or seeds act upon the formation of IL-6 and TNF-α, which are critical cytokines that mitigate the development of RA in rats.

5. Conclusion

The present study explored for the first time the efficiency of dietary supplementation of either *M. peregrina* leaves or seed oil or a combination of both as an anti-inflammatory and antiarthritic medication because of the presence of phytochemicals and unsaturated fatty acids. Further research is needed to support its common traditional beliefs and uses.

Authors contributions

D.M.A. Designed and organized the study. A.A. contributed to the conduct of the study and performed the experiments, S.A. analyzed the data. G.S and M.M.H. drafted the manuscript and critiqued the output for intellectual content. G.S, D.M.A, A.A and M. M.H authors discussed the results and commented on the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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