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Research Article

PHARMACOLOGICAL SCREENING FOR ANTI-ARTHRITIC ACTIVITY OF *MORINGA OLEIFERA*

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ABSTRACT

Objective: The present study was performed to investigate the anti-arthritic activity of ethanolic and aqueous leaf extracts of *Moringa oleifera* (MO) against formaldehyde-induced arthritis in laboratory rats.

Methods: Arthritis was induced in albino Wistar rats by administration of 0.1 ml formaldehyde (2% v/v, sc) into subplantar region of the right hind paw. Diclofenac sodium (10 mg/kg, i.p.) was used as the standard drug. The ethanolic leaf extracts of MO at doses of 250 and 500 mg/kg and aqueous leaf extract at 500 mg/kg body weight p.o were administered for 10 days. At the end of the study period, changes in paw edema volume, paw thickness, arthritis score, and C-reactive protein (CRP) levels were recorded along with histopathology of knee joints in all groups were studied.

Results: There was a significant reduction recorded in paw edema volume, paw thickness, arthritis score, and CRP levels on treatment with diclofenac sodium, ethanolic and aqueous extracts of MO. When compared between the two extracts, aqueous extract in the dose of 500 mg/kg body weight was found to be more potent.

Conclusion: The results demonstrated significant anti-arthritic activity of ethanolic and aqueous leaf extracts of MO at a dose of 500 mg/kg body weight.

Keywords: *Moringa oleifera*, Formaldehyde, Arthritis, C-reactive protein.

INTRODUCTION

Traditional and folklore medicines play an important role in healthcare. There exist a plethora of knowledge and benefits of herbal drugs in our ancient literature of ayurvedic and unani medicine. According to the World Health Organization (WHO), 80% of the population in developed countries relies on traditional medicine for their primary healthcare. Despite considerable progress in therapies using expensive synthetic drugs, the search for herbal remedies is growing which can be accounted for the effectiveness, minimal side effects in clinical experience, and relatively low cost of the herbal drugs. Herbal drugs or their extracts are prescribed widely, even when their biologically active compounds are unknown. Exploration of the chemical constituents of plants and pharmacological screening would provide the basis for developing new lead molecules in strategic favor of Natural product Drug Discovery. This shows the need for planned activity guided pharmacological evaluation of herbal drugs [1].

Rheumatoid arthritis (RA) is an autoimmune disease in which there is joint inflammation, synovial proliferation, and destruction of articular cartilage [2]. It is one of the most common autoimmune diseases and is a chronic, progressive, and systemic inflammatory disorder affecting the synovial joints and typically producing symmetrical arthritis that leads to joint damage, which has accountability for the deformity and disability [3]. The consequences of this, morbidity, and mortality have a potential socioeconomic impact. It is the most common cause of physical disability in developed countries, and the prevalence ranges between 0.3% and 1.50% with a female:male ratio of 3:1 [2]. There is no known cure for RA but several drugs such as anti-inflammatory and disease modifying anti-rheumatoid drugs (DMARDs) are used in mono or combination therapies to inhibit the disease process [3,4]. However, prolonged use of these drugs is associated with deleterious side effects such as gastric ulceration, hemorrhage, anemia, and kidney dysfunction [5-7]. Thus, in recent times, researchers have been directed towards the use of biologics and plant-derived drugs in the treatment of RA [8].

Although conventional treatment options for this condition have improved in terms of effectiveness, the use of non-steroidal anti-inflammatory drugs (NSAIDs) such as etoricoxib, DMARDs such as methotrexate, sulfasalazine, leflunomide, hydroxychloroquine, and corticosteroids such as prednisolone, methylprednisolone have all been associated with adverse effects [3]. Because of this reason, many patients and practitioners are seeking alternative approaches for providing an effective cure for the treatment of disease and to overcome the serious drawbacks such as gastrointestinal bleeding and bone loss. Hence, there is an urgent need to find safer compounds for the management of RA [9]. Thus, revival with herbal and other complementary therapies in the management of chronic diseases (RA and other inflammatory disorders) is well documented [10-12].

Moringa oleifera (MO) Lam. (Drumstick tree) a plant in a family of Moringaceae, is widely cultivated in India, used as food and active ingredient of the food preparation, medication and oil manufacturing. Almost all parts of the plant have been utilized in traditional medical practices. It is believed to be a miracle herb because it can be used as food as well as medicine for numerous ailments. The leaves and young buds of the plant are used as a vegetable and can be rubbed on the temples for relieving headache while the root and root bark are regarded as antiscorbutic and can be externally used as counter-irritants. The juice of leaves mixed with honey is used for the treatment of eye diseases. The leaves of the plants have also been reported for its antitumor, hypotensive, antioxidant, radio-protective, anti-inflammatory, and diuretic properties [1]. The leaves of MO used in a number of ailments, including internal deep-seated inflammation and calculous infections, etc. In view of the above, the present work was planned to investigate the anti-arthritic potential of an ethanolic, and aqueous extract of leaves of MO on different parameters using formaldehyde-induced arthritis model in albino Wistar rats.

METHODS

Plant collection and extraction

The leaves of MO were collected and identified by Dr. K. Madhava Chetty, Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh, India. A voucher specimen No.951 has been deposited in the department.

Extraction process

The leaves were thoroughly washed under running tap water so as to remove any type of contamination. Then, washed leaves were air dried in the shade, powdered in the grinder and sieved. The dried powder (300 g) was loaded onto a Soxhlet evaporator and extracted with circulating 70% ethanol at 80°C by the hot Soxhlet extraction method for 72 hrs. Later for concentrating, the obtained extract was flash evaporated at 50°C and preserved in an airtight glass container at 4-8°C until the time of use for the study. The concentration of the extract produced 84 g (28% W/W) yield.

The plant-derived aqueous extract tested in this study was prepared in our laboratory by mixing 100 g dried and powdered leaves of MO with 1000 mL boiling water for 5 minutes. The mixture was then filtered twice through a sterile filter paper into a sterile tube. The aqueous extract stock solution (100 mg/mL) was freshly prepared for each set of experiments and stored at 4°C for up to 5 days [13].

Chemicals

Ethanol and formaldehyde (of Qualigens Fine Chemicals from Crescent Chemicals, Hyderabad), diclofenac sodium injection IP (Apollo Pharmacy, Hyderabad, India) were used for the study. Other chemicals and reagents used for the study were of analytical grade taken from the institute laboratory.

Phytochemical screening

The leave extract of MO was subjected to qualitative analysis for the various phytoconstituents: Alkaloids with Dragendorff's reagent, flavonoids with alkaline reagent (sodium hydroxide) and lead acetate, tannins with 1% gelatin solution, phenolic compounds with ferric chloride solution, glycosides with legal test and Froth test for saponins [14].

Experimental animals

Healthy adult rats of Albino Wistar strain weighing between 150 g and 250 g were used for the experiments. All the animals were obtained from the Animal House of Shadan Institute of Medical Sciences, Peerancheru Hyderabad, Telangana, India. All the protocols of animal experiments were approved by the Institutional Animal Ethics Committee of the institute and study was carried out in accordance to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals. The animals were housed in clean cages provided with husk bedding and maintained at 24°C ± 2°C under 12 hrs light/dark cycle and were fed ad libitum with standard pellet diet and had free access to water. The animals were given standard pellet diet supplied by Pranav Agro Industries Ltd. Sangli. Before their use, they were allowed 1 week for acclimatization in the laboratory area.

Acute oral toxicity study

Healthy adult Albino Wistar rats (150-250 g) were subjected to acute oral toxicity studies - fixed dose procedure as per the Organization for Economic Co-operation and Development (OECD) guidelines 2001 (AOT-420) with ethanolic and aqueous leaf extracts of MO. Animals were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hrs, with special attention given during the first 4 hrs, and daily thereafter, for a total of 5 days. The changes in skin, fur, eyes, mucous membranes, respiratory, circulatory, autonomic, central nervous system, somatomotor activity, and behavior pattern were noted (OECD guidelines, 2001) [15].

Formaldehyde-induced arthritis

Animals were divided into six groups of six animal each (n=6) and the baseline paw thickness measured by a micrometer screw gauge and paw volume by plethysmograph on the day 0 of the experiment. Group 1 received the vehicle (1 ml/kg, saline p.o.) and served as the normal control. Group 2 served as the negative (arthritic) control and did not receive anything. Group 3 received the standard drug diclofenac sodium (10 mg/kg, i.p.) [16], Groups 4 and 5 received ethanolic extracts of MO in doses of (250 and 500 mg/kg body weight p.o), respectively, and Group 6 received aqueous extracts of MO in dose of 500 mg/kg body weight p.o. 30 minutes after oral administration of vehicle/drugs, arthritis was induced by subplantar administration of 0.1 ml formaldehyde (2% v/v) into the right hind paw of all the animals [16]. This was designated as day 1. Vehicle/drug treatment was continued for the duration of 9 more days. Formaldehyde (0.1 ml 2% v/v) was again injected into the same paw on the third day [16]. The increase in paw thickness and paw volume was measured on days 0, 2, 4, 6, 8 and 10, 30 minutes after administration of the respective vehicle/drug treatment. The body weight changes were recorded every day by digital balance. Arthritis was assessed by measuring the mean increase in paw thickness and edema volume over a period of 10-day.

The percentage inhibition of right paw edema was calculated by the following formula:

$$\% \text{ Inhibition} = (V_C - V_T) \times 100/V_C$$

Where, V_C = Paw edema volume of control group, V_T = Paw edema volume of the test group.

Assessment of arthritis

The progression of formaldehyde-induced arthritis was evaluated by measuring the following parameters on 0, 2, 4, 6, 8, and 10th day after formaldehyde injection.

Paw volume

The swelling in the hind paw from the ankle was measured periodically on the days mentioned above using plethysmograph. The edema component of arthritis was estimated by calculating the difference between day 0 paw volumes and paw volume at various time points [16,17].

Paw thickness

The edema component of arthritis was estimated by calculating the difference between day 0 paw thicknesses and paw thicknesses at various time points using screw gauge [18].

Arthritis score

Rats were scored for arthritis (arthritis index) daily by a set visual criterion [18,19].

The following scoring system was used:

No change = 0

Swelling and erythema = 1

Mild swelling = 2

Gross swelling = 3

Gross swelling and deformity = 4

Biochemical estimations

Blood was collected by retro-orbital puncture under the influence of ether anesthesia for biochemical estimation of C-reactive protein (CRP) in plain tubes on the 10th day of the study. The serum was separated, and samples were analyzed for CRP by enzyme-linked immunosorbent assay kit for CRP [19,20].

Histopathological examination

For histopathology, on the 10th day at the end of the experiment, all animals were anesthetized under light ether anesthesia and sacrificed by cervical decapitation. Then, the right hind (arthritis induced) limb was removed just distal to the knee, washed with saline and stored in 10% formalin. The fixed tissues were then decalcified and slides of

sagittal slices through the hind paw stained with hematoxylin and eosin and viewed under 4*(×10) magnifications (Scanner view) at Vijaya Diagnostic Centre, Hyderabad. Slides were reviewed for the evaluation of soft tissue swelling, bone demineralization, pannus formation, cartilage erosion, and joint space narrowing.

Statistical analysis

The results were expressed as means±standard error of mean. The data were analyzed by one-way ANOVA followed by Tukey's multiple comparison tests. The level of significance was set at $p<0.05$. All statistical tests were carried out using Prism 6.0 (Graph Pad, San Diego CA, USA) statistical software.

RESULTS

Results of preliminary phytochemical analysis

The ethanolic and aqueous extracts of MO, when tested chemically, were found to show the presence of alkaloids, tannins, flavonoids, glycosides, and saponins.

Results of acute toxicity study

There was neither change in behavioral pattern or any sign of toxicity during the observations up to 5 days for mortality. The extracts were safe up to a maximum dose of 2000 mg/kg. Hence, the extract was considered to be safe and non-toxic for further pharmacological screening. The evaluation was carried out at doses of 250 and 500 mg kg of ethanolic extracts and 500 mg/kg of the aqueous extract.

Results of anti-arthritic activity

Effect on paw edema volume in Freund's incomplete adjuvant (FIA) rats

Rats injected with formaldehyde showed a significant increase in paw edema volume when compared to the normal rats and standard. Ethanolic extract of MO at the dose of 500 mg/kg and aqueous extract of MO at the dose of 500 mg/kg showed a significant reduction in rats paw edema volume when compared with the arthritic rats (Table 1).

Effect on paw thickness in FIA rats

An increase in paw thickness was seen in all animals throughout the observation period. Although all drug-treated groups showed a decrease in paw thickness as compared to the control, the difference was significant on all observation days only in Group 3 (diclofenac sodium), Group 5 (500 mg/kg of ethanolic extract of MO), and Group 6 (500 mg kg of aqueous extract of MO) (Table 2).

Effect on arthritis score in FIA rats

The arthritis score in arthritic paw was increased significantly in all the animals in 6 days after induction of arthritis. However, a significant reduction in arthritis score was seen in Group 3 - diclofenac sodium 10 mg/kg, Group 5 - 500 mg/kg of an ethanolic extract of MO, and Group 6 - 500 mg/kg of aqueous extract of MO on the day 10 of the study. Further, a non-significant decrease in arthritis score was found in Group 4 - 250 mg/kg ethanolic extract compared to normal control, on the day 10 of the study (Table 3).

Effect on serum CRP levels in FIA in rats

The CRP level in serum samples were found to decrease significantly in Group 3 - diclofenac sodium (7.47 mg/mL), Group 5 - 500 mg/kg of ethanolic extract of MO (8.06 mg/mL), and Group 6 - 500 mg kg of aqueous extract of MO (7.65 mg/mL) compared to Group 2 formaldehyde-induced arthritic rats (8.77 mg/mL) (Table 4).

Results of histopathological analysis

Rats in the normal control group showed normal cartilage and a normal synovium (Fig. 1a). The arthritic rats in formalin control group showed soft tissue swelling, thinning of cartilage plates, bone erosion and alterations in the bone structure of knee joints (Fig. 1b). The diclofenac sodium treated rats showed a reduction in inflammation but the cartilage showed degenerative changes and hypertrophied tissue (Fig. 1c). The ethanolic extract of MO (250 mg/kg) treated rats showed thinning of cartilage and bone erosion (Fig. 1d). The ethanolic extract of MO (500 mg/kg) treated rats showed hypertrophied tissue; however, pathology was maintained better than the ethanolic extract 250 mg kg treated group (Fig. 1e). Rats treated with aqueous extract of MO (500 mg/kg) showed regeneration of cartilage and new bone formation with mild changes in the hypertrophied synovial lining, which proves the anti-arthritic potential of the aqueous extract of MO (Fig. 1f).

DISCUSSION

In the present study, the anti-arthritic activity of ethanolic and aqueous extracts of MO was evaluated using formaldehyde-induced arthritis.

Table 1: Mean changes in paw edema volume and percent inhibition in formaldehyde-induced arthritis in rats

Group n=6	Day 2	Day 4	Day 6	Day 8	Day 10	% inhibition
Normal control	0	0.16±0.166	0.16±0.166	0	0.166±0.166	-
Arthritic control	3.66±0.33$^{@}$	4.66±0.843$^{@}$	4.33±0.49$^{@}$	6.5±0.718$^{@}$	7.33±0.21$^{@}$	-
Standard diclofenac sodium 10 mg/kg	2.33±0.210***	3.66±0.42***	2.66±0.49***	2.0±0.57***	1.0±0.36***	86.36
MOET 250 mg/kg	3.00±0.258***	3.00±0.44**	2.00±0.44**	2.167±0.30**	2.16±0.47***	70.44
MOET 500 mg/kg	3.16±1.66***	3.00±0.36**	2.33±0.210***	1.833±0.40***	1.33±0.33***	81.82
MOAE 500 mg/kg	2.83±0.3***	2.83±0.30**	1.16±0.16***	1.167±0.30***	0.83±0.16***	88.64

The data are expressed in mean±SEM. MOEE: *Moringa oleifera* ethanolic extract, MOAE: *Moringa oleifera* aqueous extract. The data were analyzed using ANOVA followed by Tukey's multiple comparison tests. **p<0.01, ***p<0.001 compared to normal and arthritis control, $^{@}$p<0.001 compared to normal control and standard, FIA: Freund's incomplete adjuvant, SEM: Standard error of mean

Table 2: Mean changes in paw thickness and percent inhibition in formaldehyde-induced arthritis in rats

Group n=6	Day 2	Day 4	Day 6	Day 8	Day 10	% inhibition
Normal control	0.11±0.06	0.30±0.14	0.34±0.16	0.25±0.09	0.17±0.09	74.87
Arthritic control	2.90±0.11$^{@}$	2.78±0.11$^{@}$	3.19±0.30$^{@}$	3.58±0.24$^{@}$	3.41±0.29$^{@}$	-
Standard diclofenac sodium 10 mg/kg	2.22±0.39***	1.72±0.35**	1.15±0.17***	0.75±0.25***	0.41±0.17***	87.98
MOET 250 mg/kg	2.18±0.28NS	2.46±0.40NS	2.26±0.27*	1.49±0.23**	1.11±0.36**	68.64
MOET 500 mg/kg	3.24±0.18**	2.29±0.218**	1.74±0.18**	1.58±0.23**	1.07±0.25***	73.18
MOAE 500 mg/kg	2.40±0.43***	1.43±0.25*	0.91±0.16***	0.61±0.20***	0.24±0.09***	82.73

The data are expressed in mean±SEM. MOEE: *Moringa oleifera* ethanolic extract, MOAE: *Moringa oleifera* aqueous extract, N: Non-significant. The data were analyzed using ANOVA followed by Tukey's multiple comparison tests. *p<0.05, **p<0.01, ***p<0.001 compared to normal and arthritis control, $^{@}$p<0.001 compared to normal control and standard

Table 3: Means of arthritis score in formaldehyde-induced arthritis in rats

Group n=6	Day 2	Day 4	Day 6	Day 8	Day 10
Normal control	0	0	0	0	0
Arthritis control	1.66±0.42	3.0±0.2	2.83±0.16	3±0.12	3.50±0.22
Standard diclofenac sodium 10 mg/kg	1.66±0.21**	2.33±0.210***	2.16±0.166***	1.66±0.33***,$	0.66±0.42@
MOET 250 mg/kg	1.66±0.42**	2.50±0.22***	2.50±0.23***	2.16±0.16***	1.33±0.42$
MOET 500 mg/kg	1.51±0.22**	2.16±0.166***,$	2.33±0.21***	2.16±0.166***	1.00±0.44NS,#
MOAE 500 mg/kg	1.45±0.1**	2.83±0.16***	2.50±0.22***	2.00±0.44***	0.66±0.42@

The data are expressed in mean±SEM. MOEE: *Moringa oleifera* ethanolic extract, MOAE: *Moringa oleifera* aqueous extract. The comparisons were made by ANOVA followed by Tukey's multiple comparison tests. **p<0.01, ***p<0.001, NS: Non-significant compared to Normal control, $p<0.01, #p<0.001, @p<0.0001 compared to arthritis control, SEM: Standard error of mean

Table 4: Effect of treatments on CPR levels in formaldehyde-induced arthritis in rats

Group n=6	Day 10
Normal control	7.18±0.18$
Arthritic control	8.77±0.04**
Standard diclofenac sodium 10 mg/kg	7.47±0.24$
MOET 250 mg/kg	9.54±0.08NS
MOET 500 mg/kg	8.06±0.05*
MOAE 500 mg/kg	7.65±0.13@

The data are expressed in mean±SEM. MOEE: *Moringa oleifera* ethanolic extract, MOAE: *Moringa oleifera* aqueous extract, The comparisons were made by ANOVA followed by Tukey's multiple comparison tests. *p<0.05 compared to normal control and arthritis Control, **p<0.01 compared to normal control, @p<0.05 compared to arthritis control, $p<0.01 compared to arthritis control. NS: Non-significant compared to arthritis and normal control, CRP: C-reactive protein, SEM: Standard error of mean

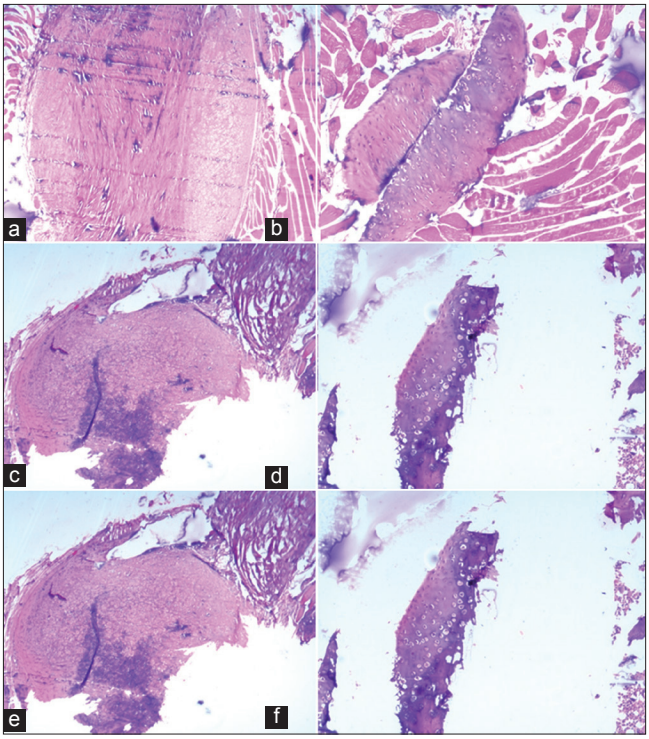

Fig. 1: Histopathological changes in knee joints of experimental animals. Method of staining: Stained with hematoxylin and eosin. Magnification: 4*(×10). (a) Normal cartilage and synovium. (b) Soft tissue swelling, thinning of cartilage plates, bone erosion, and alterations in bone structure seen in arthritic rats. (c) Reduction in inflammation but the cartilage showed degenerative changes and hypertrophied tissue in the standard drug treated group. (d) Thinning of cartilage and bone erosion in 250 mg/kg of ethanolic extract of *Moringa oleifera* (MO) treated rats. (e) Hypertrophied tissue and bone erosion in 500 mg/kg of ethanolic extract of MO treated rats. (f) Regeneration of cartilage and new bone formation with mild changes in the hypertrophied synovial lining in 500 mg/kg of aqueous extract of MO treated rats

RA is considered as a chronic systemic autoimmune disease with the main characteristic of chronic joint inflammation that ultimately leads to joint destruction. It affects approximately 1% of the world's population and can lead to severe disability.

Screening of anti-arthritic activity using formaldehyde-induced arthritis in rats is considered as a modern, scientific, internationally approved standard experimental procedure. Formaldehyde injection elicits localized inflammation and pain in the early phase subsequently followed by a phase of tissue mediated response [21]. This late phase produces proliferative joint inflammation leading to articular changes similar to those seen in RA [22]. Therefore, formaldehyde-induced arthritis in Wistar strain albino rats is the most commonly used experimental models for preclinical screening of NSAIDs, DMARDs, and plant extracts for anti-arthritic effect [23]. The choice of the animal strain has been found to be very important for the performance of this test. Wistar-albino rats have been proven to be very suitable in contrast to other substrains [24]. In the present study, we have observed induction of RA in rats, on formaldehyde administration.

NSAID's are widely used clinically for RA. However, despite their great number, their therapeutic efficacy seems to be hampered by the presence of a number of undesired and often serious side effects. Selective cyclooxygenase-2 (COX-2) inhibitors make an alternative approach to arthritic treatment with reduced gastrointestinal side effects, but on long-term treatment leads to serious cardiovascular and thrombotic side effects. However, a series of new biological monoclonal antibodies (anti-Tumor necrosis factors (TNF), anti-IL-1Ra anti-CD 20, anti-IL-2, IL-4) were preferred for RA, but these are highly expensive [25]. Thus, there arises the need to look for some new and safe anti-rheumatic drugs.

Alternative medicine for the treatment of various diseases is getting increasing popularity day by day. Because it shows fewer side effects as compared to other system of medicine, many medicinal plants have proven effects on arthritic symptoms as compared to that of conventional medicines [26]. Arthritis is a chronic inflammatory disease which affects several joints of the body like cartilage, synovium, tendon, and muscle. Mostly researcher has claimed that inhibition of adjuvant – induced arthritis in rats, is the suitable test procedure to screen anti-arthritic activity. The rats develop chronic swelling in multiple joint with the influence of inflammatory cells, attrition of joint cartilage and bone damage. It closely resembles with human arthritis disease [27].

For hundreds of years, traditional healers have prescribed different parts of MO for the treatment of skin diseases, respiratory illnesses, ear and dental infections, hypertension, and inflammation. It has remarkable potential which could be harnessed for medicinal and nutritional purposes. In recent times, several studies have been

able to demonstrate its medicinal significance. In our study, 10 days of treatment with MO and diclofenac sodium showed a significant reduction in rat paw edema volume and paw thickness. The ethanolic and aqueous leaf extracts of MO demonstrated significant anti-arthritic activity at a dose of 500 mg/Kg p.o.

The injection of formaldehyde into rat paw produced localized inflammation and pain which is biphasic in nature, i.e., an early neurogenic component followed by a later tissue mediated response [28]. The development of edema in the paw of the rat after injection of formaldehyde (0.1 ml, 2% w/v) is due to the release of histamine, serotonin, and the prostaglandin (PG) like substances at the site of injection. Both histamine and PG are the key mediators in inflammatory hyperalgesia that is mediated through the activation of local pain receptors and nerve terminals producing hypersensitivity in the area of injury.

Determination of paw edema is according to the grapevine simple, susceptible, and rapid procedure to evaluate the degree of inflammation and assess the therapeutic effects of drugs [29]. The formaldehyde-induced arthritic rats developed a chronic swelling in multiple joints with the influence of inflammatory cells, erosion of joint cartilage, and bone destruction and remodeling which have close similarity to human RA. These inflammatory changes eventually result in the complete destruction of joint stability and mobility in the arthritic rats [30].

Diclofenac sodium which is used as a standard drug is an NSAID, which acts by inhibition of PGs synthesis by blocking COX enzymes responsible for inflammation [30]. In the present study, we have recorded significant reduction in all the parameters used for studying arthritic activity. Similarly, inhibition of paw edema volume and paw thickness observed in formaldehyde models may be due to the possible mechanism of MO extracts to inhibit histamine, serotonin, and the PG, which are responsible for inflammation. Treatment with ethanolic and aqueous extracts of MO 500 mg/kg orally in this model suggests significant improvement in arthritis-induced rats and was comparable to the standard drug diclofenac sodium.

Another potential marker for increased risk of RA and inflammation can be CRP since CRP is a sensitive marker of systemic inflammation and is elevated in RA condition. It is an acute-phase protein and has been identified as an important biomarker for various inflammatory, degenerative, and neoplastic diseases [31]. Elevated levels of CRP have been found in the blood during almost all diseases associated with active inflammation or tissue destruction, particularly in RA patients [32]. Sustained increase in serum CRP levels suggests a lasting production and stimulation of acute-phase proteins during disease progression. Thus, in the present study, we have investigated the association of CRP and RA. It was observed that 10 days of treatment with ethanolic and aqueous leaves extract of MO showed a significant reduction in CRP levels compared to control group.

An inflammatory reaction, increased cellularity of synovial tissue and joint damage, is the pathological hallmarks of RA [4]. Persistent inflammation produces swollen joints with severe synovitis, decreased nociceptive threshold, and massive sub-synovial infiltration of mononuclear cells, which along with angiogenesis leads to pannus formation. Expansion of the pannus induces bone erosion and cartilage thinning, leading to loss of joint function [5]. In our study, we have observed regeneration of cartilage and new bone formation with mild changes in the hypertrophied synovial lining in 500 mg/kg of aqueous and ethanolic extracts of MO treated groups compared to control which proved their anti-arthritic potential.

Phytochemical investigations and review of the literature reveal the presence of alkaloids, tannins, flavonoids, glycosides, reducing sugars, and saponins. These components may exert its anti-inflammatory activity by inhibiting the 5-lipoxygenase pathway, along with the COX-2 pathway, which is very important in producing and maintaining inflammation. Saponins and alkaloids are known to inhibit articular swelling, decrease arthritic index, and regulate down the content of IL-IB and TNF-α in the inflammatory tissues of arthritic rats. Flavonoids are often used for their antioxidant effect against free radicals. There are also strong indications that they have antiviral, anti-inflammatory, and anti-hypertensive properties [33]. Therefore, it can be proposed that the anti-inflammatory and anti-arthritic activity of Moringa leaf extracts could be due to combined effect of flavonoids, saponins, and alkaloids, which are the major chemical constituents of the ethanolic and aqueous extracts of the leaves. The presence of these compounds in the extract may explain the possible mechanism for anti-arthritic properties of this plant and can strongly support the anti-arthritic potential of the MO plant and its use in traditional alternative and complementary medicine.

CONCLUSION

Results of the present study contribute toward validating the traditional use of MO formulation in the treatment of RA and inflammation. When compared between the two extracts, aqueous extract was found to be potent than the ethanolic. However, it may not be possible to depict the exact pathophysiology and progression in this debilitating disease using animal models. Therefore, further investigational studies in human subjects are warranted to elucidate the exact mechanism of anti-arthritic activity of this formulation.

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