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## FOSTERED ANTIARTHRITIC UPSHOT OF *MORINGA OLEIFERA* LAM. STEM BARK EXTRACT IN DIVERSELY INDUCED ARTHRITIS IN WISTAR RATS WITH PLAUSIBLE MECHANISM

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*Moringa oleifera*, CFA induced arthritis, arthritic index

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**ABSTRACT:** The present research exertion was undertaken to evaluate the antiarthritic effect of methanolic extract of *Moringa oleifera* Lam stem bark. The extract was subjected to preliminary phytochemical investigation and antiarthritic activity was evaluated with turpentine oil, formaldehyde and CFA induced arthritis. Other parameter like body weight and hematological estimation were carried out. The result indicated that the methanolic extract of *Moringa oleifera* has excellent antiarthritic activity against turpentine oil, formaldehyde and CFA induced arthritis. The all groups of animals showed increase in the body weight except arthritis control and hematological perturbations induced by CFA were maintained. The complete result indicated that *Moringa oleifera* methanolic extract possesses a potent protective effect against turpentine oil, formaldehyde and CFA induced arthritis. The consequences of the present investigation proved well that *Moringa oleifera* methanolic extract is effective in the treatment of rheumatoid arthritis and that it support the common belief prevailing in traditional medicine world wide.

**INTRODUCTION:** Rheumatoid arthritis (RA), one of the commonest autoimmune diseases, is a chronic, progressive, 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.

The consequences, morbidity and mortality have a potential socioeconomic impact<sup>1</sup>. 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 to male ratio of 3:1. The initiating factors for the inflammation, local tissue destruction and systemic responses seen in RA are unknown, and although a variety of infectious agents have been implicated, the causal agent of this disease has not been identified and therefore it had not been possible to treat in a rational manner<sup>2</sup>. Currently not even a single therapeutic agent, or combination of agents available for preventing disease progression or reversing joint destruction.

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*Moringa oleifera* 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<sup>3</sup>. *Moringa oleifera* is mostly used in the preparation of sambhar and curry in India. Different parts of the plant used in traditional medicine system for the treatment of various diseases. It believed to be miracle herb, because it can be used as food as well as medicine for numerous ailments. The stem bark of *M. oleifera* used in a number of ailments, including asthma, gout, lumbago, enlarged spleen or liver, internal deep seated inflammation and calculous affections, antiulcer, diuretic, anti-inflammatory wound healing etc.<sup>4-6</sup>.

Moreover, it has been used to inhibit arthritic conditions too. Nowadays, numerous health products of *M. oleifera* is available in the market and claimed for the effect on arthritic condition is mentioned earlier, no scientific evidence of antiarthritic activity of *M. oleifera* documented till now.

Therefore, the investigation was designed to evaluate the antiarthritic effect of stem bark from *Moringa oleifera* on turpentine oil, formaldehyde and CFA induced arthritis in rats.

## MATERIAL & METHODS:

**Plant:** The stem bark of *Moringa oleifera* Lam were collected from Herbal Garden, Department of Pharmaceutical Sciences, Sam Higginbottom Institute of Agriculture & Technology Sciences, Allahabad, India and authenticated by Dr. Imran Kajmi (Pharmacognosist) and a specimen voucher (SIP/HD/054/13) of the plant sample respectively have been deposited in the herbarium of Sidharatha Institute of Pharmacy, Dehradun, Uttarakhand, India.

**Chemicals:** CFA (Sigma Aldrich, USA), Turpentine oil, formaldehyde (Merck Lab, Mumbai), Aspirin and diethyl ether (Merck Lab, Mumbai) were used for study. Other chemicals and reagents used for the study were of analytical grade.

**Preparation of extraction:** The dried leaf powder of *Moringa oleifera* (1 kg) was exhaustively

extracted using methanol in Soxhlet apparatus for 5 days. The extracts concentrated under reduced pressure and low temperature to yield 17.5 % methanolic extract of *Moringa oleifera* stem bark. A suspension of extracts in distilled water, prepared using 0.2% sodium carboxymethyl cellulose, was used for experimental studies.

**Preliminary phytochemical investigation:** The extract subjected to preliminary phytochemical investigation employing standard procedures and tests to reveal the presence of phytoconstituents such as glycosides, saponins, tannins, fats and oils, steroids, triterpenoids, alkaloids, proteins and carbohydrates, etc.<sup>7-8</sup>.

**Animals and experimental design:** Swiss albino rats (Wistar strain, 150–200g) were used for the study. The animals were acclimatized in polyethylene plastic cages at  $25 \pm 2$  °C with free access to standard pellet diet and water *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethical Committee constituted as per the rules of the Committee for the Purpose of Control and Supervision of Experiments on Animals, India (CPCSEA). All the studies were conducted in accordance with the Animal Ethical Committee of the Institute and approved by the Sidharatha Institute of Pharmacy (1435/PO/a/11/CPCSEA).

**Acute toxicity studies:** Acute toxicity of *Moringa oleifera* was determined by OECD-425 guidelines. Swiss albino wistar rats were divided into different groups and administered with graded doses of *Moringa oleifera* and observed continuously for 6 hours detecting any change in behavior, locomotion, convulsion and motility. One group of animal treated with vehicle, served as control group<sup>9</sup>.

## Acute Model for Anti-arthritis activity:

**Turpentine oil-induced joint edema in rats:** Wistar albino rats were fasted 24 h before experimentation with free excess of water. Animals were divided into five groups (n=6). Group I served as normal control and received vehicle only, group II-IV received different doses (125, 250, 500 mg/kg, p.o) of methanolic extracts of *Moringa oleifera*, respectively, and group V received a standard drug aspirin (100 mg/kg).

Acute inflammation in joint produced by injecting 0.02 ml of turpentine oil into the synovial cavity of the left knee joint, after 30 min of drug administration diameter of the joint was monitored at hourly interval for 6 h using micrometer screw gauge<sup>10</sup>. The percentage inhibition of left paw edema was calculated by following formula

$$\% \text{ Inhibition} = \frac{(VC - VT) \times 100}{VC}$$

Where VC= Paw edema of control group, VT= paw edema of the test group.

#### Formaldehyde-induced arthritis in rats:

Formaldehyde used to induce arthritis using reported method with minor modification<sup>11</sup>. All wistar rats were divided into five groups, Group I served as normal control and received vehicle only, group II-IV received different doses (125, 250, 500 mg/kg, p.o) of methanolic extracts of *Moringa oleifera* respectively, and group V received a standard drug aspirin (100 mg/kg p.o.). On day1, 30 min after the drug administration chronic non-immunological arthritis was induced by sub plantar injection of 0.1 ml of 2% formaldehyde solution and repeated on day 3. Arthritis was assessed by measuring the mean increase in paw diameter over a period of 10 days using a micrometer screw gauge<sup>10</sup>.

The percentage inhibition of left paw edema calculated by following formula

$$\% \text{ Inhibition} = \frac{(VC - VT) \times 100}{VC}$$

Where VC= Paw edema of control group, VT= paw edema of the test group.

#### Chronic Model for Anti-arthritis activity

**CFA-induced arthritis in rats:** Experimental immunological arthritis was induced in rats with some modification in the previously documented method<sup>12</sup>. 30 Wistar rats divided into the five groups (n=6). Group I served as normal control and administered with vehicle only, group II-IV received different doses (125, 250, 500 mg/kg, p.o) of methanolic extracts of *Moringa oleifera* respectively, and group V received a standard drug aspirin (100 mg/kg p.o).

The left paw of each rat was injected subcutaneously with 0.01 ml of Complete Freund Adjuvant (CFA - 0.1ml of 0.5% w/v suspension of heat killed *Mycobacterium tuberculosis* cells in liquid paraffin) except normal control group of rats. The edema of the left and right hind paw observed at 5, 7, 10, 13, 15, 18, 21 post injection of CFA using micrometer screw gauge<sup>10</sup>. The percentage inhibition of left paw edema was calculated by following formula

$$\% \text{ Inhibition} = \frac{(VC - VT) \times 100}{VC}$$

Where VC= Paw edema of control group, VT= paw edema of the test group. The changes in body weight were recorded on regular interval. On the 21<sup>th</sup> day, blood was withdrawn through retro-orbital vein puncture of all groups by anesthetizing the animals with diethyl ether and the hematological parameters such as hemoglobin content, total WBC (white blood cells), ESR (erythrocyte sedimentation rate) and RBC (red blood cells) were evaluated.

**Arthritis assessment in CFA rats:** Determination of clinical symptoms in CFA induced arthritis was evaluated by a visual scoring system on scale 0-4, where 0: no change, 1: swelling and erythema of the limb, 2: mild swelling and erythema of the limb, 3: gross swelling and erythema of the limb, 4: gross deformity and inability of the limb. A score of the 4 limb was counted and score more than 1 exhibit the arthritis whereas a maximum score of the arthritis is 16. The frequency and day of onset of arthritis also recorded<sup>13</sup>.

**Statistical analysis:** The results are expressed as the Mean  $\pm$  SEM. The significance of the difference was evaluated by one-way ANOVA. Data were considered statistically significant if P < 0.05.

#### RESULT:

**Acute toxicity studies:** *Moringa oleifera*, in acute toxicity studies did not produce any symptom of toxicity and was observed no lethality upto dose of 2000 mg/kg body weight in rats.

Hence, the extract was considered to be safe and non-toxic for further pharmacological screening.

In turpentine, formaldehyde and CFA induced arthritis model, rats developed a chronic swelling in multiple joints with the influence of inflammatory cells, erosion of joint cartilage, bone destruction and remodeling.

**Preliminary phytochemical analysis:** The methanolic extract of *Moringa oleifera* when tested chemically was found to have presence of tannins, flavanoids, anthraquinones, and Saponins.

**Effect on turpentine oil induced arthritis:** Antiarthritic activity of *Moringa oleifera* (methanolic extract) was evaluated by the assessment made on the 6<sup>th</sup>hr. The outcomes showed that treatment of different doses (125, 250, 500 mg/kg, p.o) of methanolic extract of *Moringa oleifera* inhibited swelling in the synovial cavity at dose dependent manner. After 6 h, percentage inhibition of paw edema in wistar rats at different doses of *Moringa oleifera* methanolic extract (125, 250, 500 mg/kg, p.o) were 70.25%, 90.46%, 97.33%, respectively; while Aspirin showed 95.55% of inhibition (**Table 1**).

**Effect on formaldehyde induced arthritis:** In formaldehyde induced arthritis model of wistar rats, the assessment made on the 10<sup>th</sup> day showed that, treatment with different doses of *Moringa oleifera* (125, 250, 500 mg/kg, p.o) methanolic extract significantly reduced ( $P < 0.001$ ) swelling in the injected (left) hind paw as compared to Aspirin treated group. On the 10th day the % inhibition of paw edema exhibited by different doses of *Moringa oleifera* were 62.88%, 66.87%, 90.91% respectively; while Aspirin treated animals showed

maximum inhibition of paw edema 85.61% (**Table 2**).

**Effect on CFA- induced arthritis:** In Adjuvant induced animals, Different doses (125, 250, 500 mg/kg) of *Moringa oleifera* methanolic extract significantly ( $p < 0.001$ ) inhibited arthritic swelling by 74.65% 79.26% and 90.90% as compared to the adjuvant control respectively, whereas the Aspirin treated group showed an inhibition of 94.89% (**Table 3**).

**Effect on arthritis assessment:** The occurrence of the arthritis recorded. On the day 5 injections of arthritis, the arthritis index of the different extract of *Moringa oleifera* and aspirin was recorded.

The *Moringa oleifera* stem bark extract and aspirin exhibit the lower arthritis score as compared to an arthritis model in **Table 4**.

**Effect on body weight:** The gain in body weight on day 21<sup>st</sup> in each treatment group was observed in the rats under study (**Table 5**). The body weight of the disease control groups were significantly reduced as compared to the different doses (125, 250, 500 mg/kg) of *Moringa oleifera* methanolic extract and aspirin treated groups.

**Effect on hematological parameters:** The CFA-induced hematological perturbations, such as an increase in the ESR, WBC count and decrease in Hb, RBC count were also significantly ( $p < 0.001$ ) altered at different doses (125, 250, 500 mg/kg p.o.) of *Moringa oleifera* methanolic extract and aspirin (100 mg/kg p.o.) (**Table 6**).

**TABLE 1: EFFECT OF MORINGA OLEIFERA LEAVES EXTRACTS ON TURPENTINE OIL INDUCED RAT PAW EDEMA**

Treatment	Increase in joint diameter						% inhibition
	1h	2h	3h	4h	5h	6h	
Control	0.87±0.015	1.26±0.024	1.63±0.022	1.99±0.044	2.27±0.043	2.47±0.044	-
MO I (125mg/kg)	0.75±0.009 <sup>ns</sup>	1.03±0.026*	1.33±0.029*	1.16±0.024*	0.97±0.031*	0.74±0.022**	70.25
MO II (250mg/kg)	0.55±0.034*	0.76±0.022*	0.87±0.032*	0.56±0.034*	0.36±0.018*	0.24±0.015***	90.46
MO III (500mg/kg)	0.27±0.012*	0.45±0.010*	0.52±0.028*	0.28±0.022**	0.14±0.007***	0.07±0.009***	97.33
Aspirin (100mg/kg)	0.31±0.016*	0.53±0.011*	0.64±0.013*	0.34±0.016*	0.19±0.008**	0.11±0.007***	95.55

The data are expressed in mean ± SEM (n = number of animals in each group = 6). The comparisons were made by ANOVA followed by Dunnett's test. .ns-non-significant; MO- *Moringa oleifera*. \*P < 0.05 is considered as significant. \*\*P < 0.01 is considered as very significant. \*\*\*P < 0.001 is considered as extremely significant.

**TABLE 2: EFFECT OF MORINGA OLEIFERA LEAVES EXTRACTS ON FORMALDEHYDE INDUCED RAT PAW EDEMA**

Treatment	Increase in joint diameter					% inhibition
	Day 2	Day 4	Day 6	Day 8	Day 10	
Control	0.446±0.014	0.616±0.014	0.826±0.007	1.09±0.009	1.32±0.004	-
MO (125 mg/kg)	0.38±0.014*	0.47±0.015*	0.61±0.011*	0.54±0.007*	0.49±0.007*	62.88
MO (250 mg/kg)	0.366±0.011*	0.44±0.009*	0.54±0.007*	0.51±0.008*	0.44±0.011*	66.67
MO (500 mg/kg)	0.226±0.019*	0.294±0.014*	0.28±0.027*	0.25±0.016*	0.12±0.009*	90.91
Aspirin	0.268±0.012*	0.334±0.015*	0.34±0.012*	0.29±0.011*	0.19±0.016*	85.61

The data are expressed in mean ± SEM) (n = number of animals in each group = 6). The comparisons were made by ANOVA followed by Dunnett's test. ns-non-significant; MO- *Moringa oleifera*. \*P < 0.05 is considered as significant. \*\*P < 0.01 is considered as very significant. \*\*\*P < 0.001 is considered as extremely significant.

**TABLE 3: EFFECT OF MORINGA OLEIFERA LEAVES EXTRACTS ON CFA INDUCED RAT PAW EDEMA**

Treatment	Increase in joint diameter						% inhibition	
	5 Days	7 Days	10 Days	13 Days	15 Days	18 Days		21 Days
Normal Control	84±0.14	84.2±0.16	84.2±0.14	84.2±0.18	84.3±0.15	84.3±0.16	84.4±0.014	-
Arthritic Control	1.01±0.007	1.14±0.008	1.24±0.013	1.38±0.014	1.55±0.014	1.96±0.019	2.15±0.029	-
MO (125mg/kg)	0.95±0.004 <sup>ns</sup>	0.90±0.016*	0.82±0.015*	0.76±0.013*	0.69±0.006*	0.54±0.013*	0.54±0.013*	74.65
MO (250mg/kg)	0.88±0.007*	0.83±0.014*	0.75±0.011*	0.68±0.015*	0.61±0.015*	0.52±0.012*	0.44±0.014*	79.26
MO (500mg/kg)	0.77±0.005*	0.61±0.003*	0.49±0.013*	0.38±0.086*	0.24±0.013*	0.12±0.007*	0.11±0.014*	94.89
Aspirin (100mg/kg)	0.83±0.003*	0.69±0.009*	0.55±0.011*	0.45±0.013*	0.31±0.012*	0.20±0.013*	0.19±0.027*	90.90

The data are expressed in mean ± SEM) (n = number of animals in each group = 6). The comparisons were made by ANOVA followed by Dunnett's test. ns-non-significant; MO- *Moringa oleifera*, CFA- Complete Freund's Arthritic. \*P < 0.05 is considered as significant. \*\*P < 0.01 is considered as very significant. \*\*\*P < 0.001 is considered as extremely significant.

**TABLE 4: EFFECT OF MORINGA OLEIFERA LEAVES EXTRACTS AND STANDARD DRUG ON THE DEVELOPMENT OF CFA ARTHRITIC INDEX**

Treatment	Arthritic Index						
	Day 5	Day 7	Day 10	Day 13	Day 15	Day 18	Day 21
Arthritic Control	6.2±0.583	7±0.547	7.8±0.374	8.8±0.376	9.2±0.489	9.6±0.509	10.2±0.201
MO (125mg/kg)	5.6±0.509 <sup>ns</sup>	6.2±0.374 <sup>ns</sup>	7±0.447*	8±0.211*	8.2±0.374*	7.8±0.244*	6.6±0.547*
MO (250mg/kg)	5±0.447 <sup>ns</sup>	5.4±0.405 <sup>ns</sup>	6±0.316*	6.6±0.401*	6.8±0.374*	6.2±0.212**	5±0.316**
MO (500mg/kg)	3.8±0.678 <sup>ns</sup>	4.2±0.374*	4.4±0.509*	4.8±0.734*	4.6±0.678***	4±0.547***	2.8±0.583***
Aspirin (100mg/kg)	4±0.374 <sup>ns</sup>	4.6±0.402*	5±0.316*	5.8±0.583*	5.2±0.374***	4.4±0.244***	3.2±0.374***

The data are expressed in mean ± SEM) (n = number of animals in each group = 6). The comparisons were made by ANOVA followed by Dunnett's test. ns-non-significant; MO- *Moringa oleifera*, CFA- Complete Freund's Arthritic. \*P < 0.05 is considered as significant. \*\*P < 0.01 is considered as very significant. \*\*\*P < 0.001 is considered as extremely significant

**TABLE 5: EFFECT OF MORINGA OLEIFERA LEAVES EXTRACT ON BODY WEIGHT OF CFA INDUCED RAT PAW EDEMA**

Treatment	Weight Variance					Day 21
	Day 0	Day 5	Day 10	Day 15	Day 21	
Normal Control	151.2±0.342	156.2±1.035	164.6±1.232	173.8±2.304	181.2±2.353	30
Arthritic Control	156.8±1.212	153.3±0.932	143.4±0.432	135.1±0.235	128.4±3.954	-28.4
MO (125mg/kg)	157.2±0.374	160.7±1.234	164.4±4.323	166.4±2.431	172.2±1.435	15
MO (250mg/kg)	159.6±0.213	161.2±1.344	166.8±2.343	170±3.545	177.3±2.331	17.7
MO (500mg/kg)	158.2±1.454	161.2±2.456	167.8±3.433	173.3±1.232	182.4±0.754	24.2
Aspirin (100mg/kg)	157.4±0.765	161.8±0.456	167.4±0.345	172.4±1.434	181.2±2.043	23.8

The data are expressed in mean ± SEM (n = number of animals in each group = 6). The comparisons were made by ANOVA followed by Dunnett's test. ns-non-significant; MO- *Moringa oleifera* CFA- Complete Freund's Arthritic. \*P < 0.05 is considered as significant. \*\*P < 0.01 is considered as very significant. \*\*\*P < 0.001 is considered as extremely significant.

**TABLE 6: EFFECT ON HAEMATOLOGICAL PARAMETERS IN ADJUVANT-INDUCED ARTHRITIS IN RATS**

Haematological Parameter	Normal Control	Arthritic Control	MO I (100mg/kg)	MO II (250mg/kg)	MO III (500mg/kg)	Aspirin (100mg/kg)
Total WBC count (cells/cu.mm)	6.47±0.126	8.96±0.242	8.11±0.172*	7.71±0.056*	7.06±0.056*	7.48±0.129*
RBC count (million/cu.mm)	6.9±0.066	5.72±0.105	6.11±0.065*	6.31±0.086*	6.85±0.039	6.54±0.035*
Hb (cells/cu.mm)	13.8±0.374	11.2±0.375	12±0.316*	12.8±0.012	13.4±0.041	13.2±0.382
ESR (million/cu.mm) (gm%)	11.4±0.245	14.8±0.374	13.6±0.510*	12.6±0.401	12±0.316	12.2±0.374

The data are expressed in mean ± SEM (n = number of animals in each group = 6). The comparisons were made by ANOVA followed by Dunnett's test. ns-non-significant; MO- *Moringa oleifera*, WBC-White Blood Cell, RBC-Red Blood Cell, Hb-Hemoglobin, ESR-Erythrocytes Sedimentation Rates. \*P < 0.05 is considered as significant. \*\*P < 0.01 is considered as very significant. \*\*\*P < 0.001 is considered as extremely significant.

**DISCUSSION:** 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 medicine agent<sup>14</sup>. The anti-arthritic effect of methanolic extract of *Moringa oleifera* stem bark could be observed in acute (Turpentine oil and Formaldehyde induced arthritis) and chronic (Freund's complete adjuvant induced polyarthritis in rat) model of inflammation.

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<sup>9</sup>.

One of the reasons for wide utilization of the rat animal model method is due to the strong correlation between efficacy in the animal model and rheumatism condition in human.

Turpentine oil induced acute inflammation is due to release of mediator like histamine and serotonin in early phase; then kinin in intermediate phase and in a later phase releasing prostaglandin<sup>15</sup>.

Different doses of *Moringa oleifera* significantly inhibit the turpentine oil induced joint edema, suggest that the possible mechanism of action inhibition the different phase of inflammation.

The Antiproliferative and antiarthritic activity was evaluated by using common method inhibition of formaldehyde induced edema. The injection of formaldehyde into animal paw produced localized inflammation (releasing of histamine, serotonin and kinin) and pain<sup>16</sup>. Formaldehyde induced arthritis is biphasic in nature i.e. an untimely neurogenic element followed by a later tissue mediated response<sup>17</sup>.

Three different doses of *Moringa oleifera* significantly inhibit the proliferative global oedematous response at dose dependent manner. The dose of the *Moringa oleifera* (500 mg/kg) is more effective than the standard aspirin.

In the Adjuvant induced arthritic method, it was observed that swelling and redness developed in rats after injecting the CFA over 24 hours. It seems that bacterial peptidoglycon and muramyl dipeptide are responsible for its induction<sup>18-19</sup> and chronic inflammation reaction slowly developed next 8 – 10 days. Chronic inflammation occurs in multiple joints with the influence of inflammatory cells, erosion of joint cartilage and bone destruction. Cytokines, GM-CSF (Granulocyte-macrophage colony-stimulating factor), interferons like mediator are responsible for the chronic inflammation and pain, destruction of bone and cartilage<sup>20-21</sup>.

Another mechanism of chronic inflammation is leukocytes phagocytes forms a complex with the mediators (cytokines, GM-CSF, histamine, 5 hydroxytryptamine, bradykinin, various chemotactic factors, interferons and prostaglandin) and released the lysosomal enzymes, causing injury to cartilage and other tissue<sup>22-25</sup>. In the arthritis condition, arthritis changes the hematological parameters. Another condition of arthritis is a WBC increased in due to an IL-1B mediated rise in the respective colony stimulating factors. *Moringa oleifera* showed the effect in the arthritic rat at dose dependent manner.

The drug showing the effect on two ways – firstly it decreases the release of certain mediator like cytokines, histamine, 5-hydroxytryptamine, bradykinin, interference and prostaglandin. Secondly it may be stimulating the DTH response and increased phagocytic index and protection against chlophosphamide induced myelo-suppression by increasing total WBC count which are directly associated with the immunomodulatory activity.

As the level of WBC increases in arthritis rats, the migration of leukocytes to the inflamed area was significantly suppressed by different doses of *Moringa oleifera* stem bark (methanolic extract), which is associated with indicated that a significant decrease in the WBC count.

Another symptom of arthritic patient is common occurrence anemia<sup>26</sup>. In arthritic condition gastrointestinal blood loss occurred due to medication and in bone marrow changes in patients with inflammatory arthritis, which prevent the release of iron for incorporation into red blood cells<sup>27-28</sup>.

In the present investigation, arthritic control group rats showed reduction of RBC, Hb and increase the level of WBC and ESR (erythrocyte sedimentation rate). All of these symptoms indicated as anemia conditions. Our result suggests that different doses of *Moringa oleifera* showed significant recovery from the induced anemia with an increase in the level of RBC, Hb and decreases the level of WBC, ESR. During arthritis the body weight of the animals significantly decreases due to deficient absorption of nutrients through the internet and that treatment with the tested drug and standard drugs normalizes the process of absorption<sup>29</sup>.

The increase in the body weight in the *Moringa oleifera* and Aspirin treated groups may involve improvement of intestinal absorption of the nutrients and reduction in the distress caused by the severity of the arthritis.

**CONCLUSION:** In conclusion, *Moringa oleifera*, in a dose-dependent pattern, was effective in attenuating turpentine oil induced paw edema, formaldehyde-induced paw edema and CFA induced arthritis in rat models of acute and chronic inflammation, and therefore it could be investigated as a potential treatment for acute and chronic arthritis conditions in humans. The results obtained justify the use of the plant extract in traditional Indian medicine for the treatment of painful inflammatory and arthritic conditions.

Further work in our lab is in process to isolate, identify, characterize, and elucidate the structure of the phytoconstituents responsible for the observed pharmacological activities in this study. Explicate the exact mechanism of action of *Moringa oleifera* stem bark in curtailing the effect of arthritis.

**Conflicts of interest:** All authors have none to declare.



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