Potential of plant stress hormone methyl Jasmonate against lipopolysaccharide attenuated oxidative stress and arthritis in experimental animals

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Abstract

Introduction: Methyl jasmonate is a plant stress hormone indicated in the treatment of various cancers. The purpose of the current study is to investigate the anti-arthritic and antioxidant activity in experimental animals. **Materials and Methods:** Methyl jasmonate was screened in two doses (20 mg/kg and 40 mg/kg) against lipopolysaccharide (LPS) induced arthritis in rats. The assessment of arthritis such as paw thickness, paw volume, and body weight was assessed on 0th, 7th, 14th, 21st, and 28th days. The percent change in paw thickness, body weight, and arthritis index was calculated on 7th, 14th, 21st, and 28th days. The estimation of serum marker enzymes such as alkaline phosphatase, alanine transaminase, and aspartate aminotransferase was carried out on 14th and 28th days. The antioxidant enzyme such as superoxide dismutase, catalase, GST, glutathione peroxidase, and lipid peroxidation activity in articular cartilage tissues was carried out on the 28th day. White blood cell (WBC), red blood cell (RBC), and erythrocyte sedimentation rate were determined on the 28th day. Free radical scavenging activity was carried out using 2,2-diphenyl-1-picrylhydrazyl (DPPH) method and alpha-tocopherol used as a standard for comparison. After completion of the treatment period, tarsal-metatarsal joints were subjected to histopathology. **Results:** MJ significantly reduces arthritis which was evident from the assessment of arthritis such as paw volume, paw thickness, arthritis index, and body weight. Significant reduction in lipid peroxidation and serum marker enzymes was observed in MJ-treated rats as compared to the disease control group. Significant antioxidant activity was exhibited by MJ-treated group. Dose-dependent DPPH radical scavenging was observed which assess free radical scavenging potential of MJ. Erythrocyte sedimentation rate and WBC count are significantly reduced whereas RBC count was compensated similarly to control compared to the diseased group. **Conclusion:** Methyl jasmonate exhibits significant anti-arthritic and antioxidant activity through a reduction in paw volume, thickness, serum marker enzyme, ESR, WBC count, increase in RBC count, and gain in body weight. The reduction in inflammation and cell damage may be due to antioxidant, free radical scavenging, and antilipid peroxidative effect which is supported by histopathological studies.

Keywords: Methyl jasmonate, lipopolysaccharide, oxidative stress, arthritis, phyto-hormones

INTRODUCTION

Plant stress hormones are responsible for activating the plant's cellular response to diverse stress situations, including cell death.[1] These hormones are natural bioregulators and act as a prominent intracellular mediator in intracellular signaling and defense in response to various wear and tear injuries or environmental stresses, such as ultraviolet radiation, osmotic shock, and heat. Several classes of plant stress hormones have been identified among them, salicylic acid and its derivative aspirin are extensively studied as potential anticancer therapeutics and chemopreventive agents.[2,3] Methyl jasmonate is a jasmonic acid derivative exhibits anticancer activity in prostate cancer,[4] malignant skin lesions,[5] hepatocellular carcinoma,[6]

human bladder cancer,[7] human neuroblastoma,[8] human lung cancer[9] and leukemia, oral squamous carcinoma,[10] endometrial cancer,[11] cervical cancer,[12] and colon carcinoma cells.[13] Other than anticancer activity methyl jasmonate is screened for analgesic,[14] antianxiety,[15] anti-inflammatory action,[16] and antimalarial action.[17] Anticancer activity of MJ is mainly exhibited through inhibition of LOX 5 which is generated in arachidonic pathways and important mediator in cancerous cells.[13] Suppression of antiapoptotic proteins of the Bcl-2 family exposed to radiation and increase in cytotoxicity PC-3 cells arrests cell cycle, inhibiting cell growth and proliferation, causes cell death through the intrinsic/extrinsic proapoptotic, p53-independent apoptotic, and nonapoptotic (necrosis) pathways.[8,9]

Arthritis is the most common joint disease and is among the most frequent health problems for middle-aged and older people. It is characterized by articular cartilage destruction and synovial inflammation, subchondral bone sclerosis, and osteophyte formation.[18] Articular cartilage is a connective tissue that serves as a "cushion" between the bones of the joints. In normal articular cartilage, the collagen network of the extracellular matrix is important for the biological function of cartilage.[19] Pain is the most important symptom of osteoarthritis and is the reason why individuals seek medical treatment. Pain not only contributes to functional limitations and reduced quality of life but is also the leading cause of impaired mobility in the elderly population.[20] The primary treatment given in arthritis is to reduce the pain and inflammation, and the first choice of drugs is nonsteroidal anti-inflammatory drugs (NSAIDs); however, NSAIDs ameliorate the clinical symptoms without inhibiting the progression of the disease.[21] The NSAID's are associated with several side effects when prescribed for a prolonged period of time; therefore, it is essential to find out the new chemical entities with fewer side effects and potentially effective agents to cure OA. The normal structure and function of articular cartilage are the result of a precisely balanced interaction between anabolic and catabolic processes. When this process is altered, a series of changes occur in the morphological and biomechanical characteristics of cartilage.[22] The reactive oxygen species (ROS) play an important role in homeostasis and degradation of joint cartilage and several studied advocates that the in pathological conditions, oxygen tension in synovial fluid. In response to partial oxygen pressure (pO_2) variations, mechanical stress, immunomodulatory and inflammatory mediators, and chondrocytes produced abnormal levels of ROS that are generally produced by immune cells to assume host defense.[23] Exposure to lipopolysaccharide (LPS) stimulates cellular inflammatory responses and increases the abundance of ROS releases pro-inflammatory factors such as tumor necrosis factor-alpha (TNF-α), prostaglandin E2, and cytokines that promote inflammatory responses.[24] The present study was aimed to investigate the potential of plant phytohormone methyl Jasmonate against LPS induced cartilage damage and oxidative stress.

MATERIALS AND METHODS

Preparation of Drug Solutions

Methyl jasmonate was dissolved in 95% ethanol and the solution was further diluted with distilled water. The final concentration of ethanol in the solution used for the study did not exceed 1%. The doses of MJ used in the study were selected based on the results obtained from preliminary investigations. MJ was given to experimental animals in a dose of (20–40 mg/kg) intraperitoneal injection.[14,15] LPS was procured from Sigma laboratories and prepared in PBS buffer, and sub-plantar injection is given in a dose of 1 mg/kg under diethyl ether anesthesia.[25] Indomethacin is given in a dose of 30 mg/kg orally.[26]

Experimental Animals

Adult healthy male Wistar rats, weighing between 150 and 200 g were procured from the approved animal supplier. The animals were placed in large and spacious hygienically maintained cages during the course of the experimental period. The animals were adapted for new environment for 15 days. 12 h dark and light cycle was maintained at room temperature of 25 ± 1°C and relative humidity 45–55%. The animal has free access to standard rat feed and water *ad libitum*. The experiments were conducted as per the guidelines of NIH and CPCSEA; almost care was taken during experimentation. The Institutional Animal Ethical clearance was obtained before the commencement of animal experimentation (Ref. No. JSPM/CCOPR/IAEC/2017-18/001).

Experimental Treatment Protocol

30 animals were divided randomly into 5 groups comprising 6 animals in each group. The intraplantar injection was given under diethyl ether anesthesia, and the anesthetized animals were kept in proper observation to make sure that breathing was regular and slow.

The experimental setup is as follow:
- Control: Served as control and receive vehicle p.o.
- LPS-treated: Is a disease control, receives LPS 1 mg/kg on the 0th day.
- Standard: Standard drug indomethacin (3 mg/kg) + LPS 1 mg/kg on the 0th day.
- MJ-1 treated: MJ (low concentration 20 mg/kg) + LPS 1 mg/kg on the 0th day.
- MJ-2 treated: MJ (high concentration 40 mg/kg) + LPS 1 mg/kg on the 0th day.

At the end of a treatment protocol that is on the 28th day, all the animals were weighed, and blood samples were collected by puncturing the retro-orbital plexus under mild ether anesthesia. Around 1 mL of blood is withdrawn from each animal. Blood was collected in Eppendrof's tubes and kept aside for clotting. After clotting, blood samples were

centrifuged at 3000 rpm for 15 min by Remi Micro Centrifuge manufactured to separate serum. Serum was collected and assessed for biochemical parameters. On the same day, all the animals were sacrificed by cervical dislocation and knees were separated for histopathological studies. Measurement of ESR was carried out by reported Westergren method. The mixture consists of 2 mL of blood mixed with 3% sodium citrate. The blood is drawn into a Westergren-Katz tube to the 200 mm mark. The tubes were placed in a rack in a vertical position for 1 h at room temperature, at which time the distance from the lowest point of the surface meniscus to the upper limit of the red cell sediment is measured. The distance of fall of erythrocytes expressed as millimeters in 1 h, is the ESR.[26]

Preparation of Tissue Homogenate

Animals were sacrificed on the 28th day by cervical dislocation. The joints from each group of animals were removed and cut into small pieces and homogenized with 5 vol of 50 mM Tris-HCl buffer, pH 7.4 containing 0.1 M NaCl, and 0.1% Triton X-100 and 1 vol of fine glass powder using a mortar and pestle. The homogenate was kept for sonication for 2 min. The homogenate was centrifuged at 3000 g for 5 min, using homogenizer and the resulting aliquots of this homogenate were used for estimation of lipid peroxide, glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), and GR enzyme activity.[27]

Evaluation of Severity of Arthritis - Arthritis Assessments

Paw volume

The change in paw volume of all the animals (right hind paw) was noted on days 0th, 7th, 14th, and 21st and on the 28th day, using mercury plethysmograph. The initial mercury level in the tube was recorded. The hind paw was immersed in the tube; an increase in the level of mercury in the tube was noted. Difference between the initial and final reading gives the change in paw volume.[26]

Paw Thickness

Ipsilateral thickness of the paw was measured on 0th, 7th, 14th, 21st, and 28th days using a Vernier caliper. Percent change in paw thickness was calculated on 7th, 14th, 21st, and 28th days.[28]

Arthritic index

Arthritic index was determined on 7th, 14th, 21st, and on 28th days using the following formula.[29]

Arthritic index (%) = Hind paw vol. (Final) – hind paw vol. (Initial)/Hind paw vol. (initial) ×100

Body weight

The weights of all the animals were noted on days 0th, 7th, 14th, and 21st and on day 28th at 9.00 am. Percent change in body weight was calculated on days 7th, 14th, 21st, and 28th.[30,31]

Estimation of Markers Enzymes in Serum

The serum was separated using micropipettes and estimation of marker enzymes that are alanine transaminase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) which were carried out on 14th and 28th-day using commercial kits Preccugent by autoanalyzer, Pinnacle biotechnologies Ltd. The procedure is carried out according to manufacturer guidelines.

Free Radical Scavenging Activity using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Method

DPPH scavenging activity was measured by the spectrophotometric method. A stock solution of 25 mg of DPPH (200 µM) was prepared in 100 mL of ethanol, 0.05 mL of test compounds dissolved in ethanol were added at different concentrations (50, 100, 250, and 500 µg/mL). An equal amount of ethanol was added to the control; the reaction was allowed to be completed in the dark for about 20 min. After 20 min, the decrease in absorbance of test mixtures was read at 517 nm. The percentage inhibition was calculated and expressed as percent scavenging of DPPH radical.[30,31]

DPPH %= absorbance (control) - absorbance (sample)/absorbance (control) ×100

Estimation of Reduced GSH

GSH was measured in the groups following the method described; an aliquot of articular tissue homogenate supernatant (0.4 mL) was added to dark polyethylene tube containing 1.6 mL of 0.4M Tris-EDTA buffer, pH 8.9. Tube content was vigorously mixed, and 40 uL of 10 mM dithiobisnitrobenzoic acid in methanol was added. The samples were mixed again, and the absorbance was read at 412 nm after 5 min. At least a duplicate was made for each sample. The values of unknown samples were drawn from a standard curve plotted by assaying different known concentrations of GSH. The amount of GSH was expressed as umol/g of protein.[26,32]

Measurement of SOD Activity

Total SOD activity was measured by determining the ability to inhibit the auto-oxidation of pyrogallol. The rate of auto-oxidation was determined by measuring increases in the absorbance at 420 nm. Reaction mixture containing 0.2 mM pyrogallol in 50 mM Tris-cacodylic acid buffer (pH 8.5) and

1 mM diethylenetriaminepenta acetic acid was incubated for the 90 s at 25°C. One unit of SOD activity is defined as the amount of the enzyme required to inhibit the rate of pyrogallol auto-oxidation by 50%.[33]

Measurement of TBARS

Rats were sacrificed on day 28 by excess ether. The thiobarbituric acid-reactive substance was measured as a marker of lipid peroxidation. The homogenized tissue was added to 1.5 mL of 8.1% sodium dodecyl sulfate, 1.5 mL of 20% acetate buffer (pH 3.5), and 1.5 mL of 0.8% thiobarbituric acid (TBA) solution. The mixture was heated at 95°C for 1 h. After cooling, 5 mL of n-butanol–pyridine (14:1) was added for extraction, and the absorbance of n-butanol–pyridine layer at 532 nm (Shimadzu UV-visible 1700) was measured for determination of TBA reactive substance.[33]

Measurement of GSH Peroxidase (GPx)

The reagents of H_2O_2, 1 mM GSH, 0.2 mM NADPH, and the homogenized articular cartilage were added to 0.1M Tris-HCl buffer solution (pH 7.2) and reacted at 25°C for 5 min. NADPH consumed by the reduction of the oxidized form of GSH was determined by measuring absorbance at 340 nm, and GPx activity was calculated. Enzyme activity is quoted as the units of NADPH oxidized nmol/1 mg protein/min.[30,33]

Measurement of CAT

CAT activity in the joint tissues was assayed according to the method described earlier using H_2O_2 as substrate. The reaction mixture consisted of phosphate buffer (0.01 M and pH 7.0), distilled water and 10% homogenate (prepared in 0.1 M phosphate buffer). The reaction was started by adding H_2O_2 (0.2 M), incubated at 37C for 1 min and the reaction was stopped by addition of dichromate: acetic acid reagent (1:3). The tubes were kept in a boiling water bath for 15 min and centrifuged for 10 min at 1500 g. The rate of decomposition of H_2O_2 was followed at 240nm. The enzyme activity was expressed as mmol H_2O_2 consumed/min/mg protein using a molar.[32]

RESULTS

Effect of Methyl Jasmonate on Severity of Arthritis

Paw volume

At day 0 that is at the beginning of the experimental treatment protocol, there was no significant difference found in rat hind paw volume in all the groups of animals. A significant increase in paw volume was observed in LPS injected groups on 7th, 14th, 21st, and 28th days as compared to control group rats. The standard and MJ-2 treated group and showed a significant reduction in hind paw volume ($P < 0.001$) at 7th, 14th, 21st, and 28th day. The MJ-1 treated group did not show any significant difference in reduction of hind paw volume on the 28th day [Figure 1].

Paw thickness

At 0 day, no significant difference was noted in paw thickness of all the rats. A significant increase ($P < 0.001$) in paw thickness was reported on 7th, 14th, 21st, and 28th days in LPS-treated rats as compared to control rats paw thickness. Significant reduction in paw thickness was reported in standard, MJ-1 and MJ-2 treated rats ($P < 0.001$). The reduction in paw thickness in MJ-treated rats was dose-dependent [Table 1].
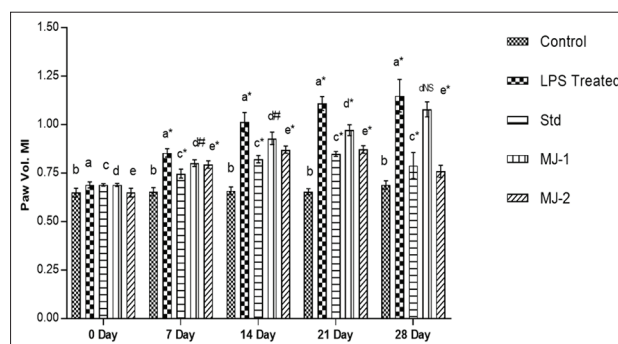
Figure 1: Effect of methyl jasmonate paw volume in LPS-induced arthritis. Values are expressed as mean ± SD, n=6, #is $P < 0.05$, $is $P < 0.01$, *$P < 0.001$, b(control group) versusa(LPS-treated group), a(LPS-treated group) versusc(LPS+std.), a(LPS-treated group) versusd(LPS+MJ-1), a(LPS-treated group) versus e(LPS+MJ-2)

Table 1: Effect of MJ on rats right paw thickness (mm) in LPS-induced arthritis					
Group	0 day	7 days	14 days	21 days	28 days
Control	3.54±0.0063[b]	3.54±0.0063[b]	3.54±0.0063[b]	3.55±0.0141[b]	3.54±0.0063[b]
LPS-treated	3.51±0.0054[a]	5.88±0.0258[a*]	6.43±0.0852[a*]	6.89±0.0651[a*]	7.16±0.0294[a*]
Std	3.52±0.0063[ac]	4.9±0.0644[ac*]	4.18±0.0640[ac*]	4.03±0.0708[ac*]	3.59±0.0320[ac*]
MJ-1	3.53±0.0075[ad]	5.18±0.0407[ac*]	4.82±0.0408[ac*]	4.54±0.2457[ad*]	4.53±0.0356[ad*]
MJ-2	3.53±0.0063[ae]	4.92±0.0547[ae*]	4.25±0.0326[ae*]	4.21±0.0767[ae*]	3.91±0.0528[ae*]

Values are expressed as mean±SD, *n*=6, #is *P*<0.05, $is *P*<0.01, *P<0.001, b(control group) versus a(LPS-treated group), a(LPS-treated group) versus c(LPS+Std.), a(LPS-treated group) versus d(LPS+MJ-1), a(LPS-treated group) versus
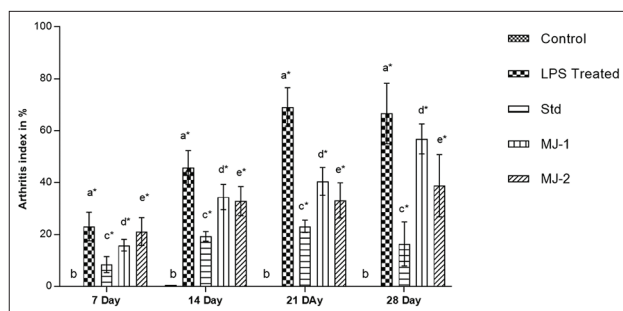
Arthritic index

The arthritis index was calculated on 7th, 14th, 21st, and 28th days of a treatment protocol. The LPS-treated group showed a significant increase in arthritis index as compared to the control group ($P < 0.001$). Whereas standard treatment, MJ-1 and MJ-2 group significantly reduces the in arthritis index as compare to LPS-treated group ($P < 0.001$). The reductions in the arthritis index in MJ groups are dose-dependent [Figure 2].

Body weight

The significant reduction ($P < 0.001$) in body weight was observed in LPS-treated group as compared to control group animals. Significant gain ($P < 0.001$) in body weight was observed in standard, MJ-1 and MJ-2 group on 14th, 21st, and 28th days as compared to LPS-treated group. No significant change is observed on the 7th day in standard, MJ-1 and MJ-2 group as compared to LPS-treated group [Table 2].

Effect of Methyl Jasmonate on Marker Enzymes in Serum

A consequence of arthritis induced by LPS, the levels of liver marker enzyme, i.e. AST, ALP, and ALT were significant increases ($P < 0.001$) in LPS-treated group in comparison control group on 14th and 28th days. A significant decrease ($P < 0.001$) in these marker enzyme levels was observed

Figure 2: Effect of methyl jasmonate on arthritis index in LPS induced arthritis. Values are expressed as mean±SD., $n = 6$, #is $P < 0.05$, $is $P < 0.01$, *$P < 0.001$, b(control group) versusa(LPS-treated group), a(LPS-treated group) versus c(LPS+Std.), a(LPS-treated group) versus d(LPS+MJ-1), a(LPS-treated group) versus e(LPS+MJ-2)

in standard, MJ-1 and MJ-2 treated a group as compared to LPS-treated group [Table 3].

Effect of Methyl Jasmonate on DPPH Radical Scavenging Activity

Effect of methyl jasmonate on DPPH radical scavenging activity has shown in Table 4. Significant percentage inhibition was observed for free radical scavenging [Table 4].

Effect of Methyl Jasmonate on Different Antioxidant Enzymes

Table 5 shows that the activity of antioxidant enzymes SOD, CAT, GST, Gpx, and GR levels is significantly ($P < 0.001$) reduced in LPS-treated group as compared to the control group while MJ-1 and MJ-2 treated animal shown significantly increase ($P < 0.001$) in antioxidant enzyme levels in comparison to LPS-treated group [Table 5].

Effect of Methyl Jasmonate on Blood Cells and ESR

LPS-treated group has significantly increased ($P < 0.001$) in white blood cell (WBC) and ESR count as compared to the control group. Treatment with MJ significantly shows a decrease in WBC and ESR rate. Inflammation is associated with a decrease in red blood cell (RBC) count and significantly decreases ($P < 0.001$) in LPS-treated group. RBC count is significantly restored in MJ and standard-treated rats [Table 6].

Histopathology

Control animal tarsal-metatarsal joint section showed normal articular structure, cartilage, and synovial lining cells. LPS-treated group animals section showed proliferation of synovial lining cells, damage to articular cartilage, pannus formation in plasma cells infiltration of lymphocytes leads to cartilage erosion, and inflammatory necrosis. Section of animals treated with standard and MJ showed a reduction of cellular flux and cartilage erosion, minimum necrotic lesion [Figure 3].

Table 2: Effect of MJ on mean change in body weight (g) on 7th, 14th, 21st, and 28th days					
Group	0th day	7th day	14th day	21st day	28th day
Control	162.8±1.65b	174.6±1.38b	180.7±1.08b	183.9±1.86b	188.7±0.96 b
LPS-treated	163.15±1.95a	168.7±0.85a*	165.7±1.14a*	163.1±1.14a*	160.2±2.06 a*
Std	163.6±1.12ac	168.2±2.37acNS	173.7±1.39ac*	174.6±0.98ac*	176.3±1.46 ac*
MJ-1	163.6±1.06ad	169.2±1.39adNS	174.2±1.34ad*	175.6±1.34ad*	179.1±1.38 ad*
MJ-2	163.3±0.80ae	168.1±2.33aeNS	176.7±1.46ae*	178.8±1.93ae*	181.8±1.86ae*

Values are expressed as mean±SD., $n=6$, #is $P<0.05$, $is $P<0.01$, *$P<0.001$, b(control group) versus a(LPS-treated group), a(LPS-treated group) versus c(LPS+Std.), a(LPS-treated group) versus d(LPS+MJ-1), a(LPS-treated group) versus e(LPS+MJ-2)

Table 3: Effect of MJ on different liver marker enzymes in serum of rats on 14th and 28st days

Day	Control	LPS-treated	Standard	MJ-1	MJ-2
14 days					
AST	65.83±2.31[b]	103±2.28[a]*	76.66±1.75[ac]*	84.83±3.31[ad]*	79.16±1.94[ae]*
ALP	105.83±3.31[b]	159.16±4.95[a]*	134.5±4.135[ac]*	146.67±4.13[ad]*	138.84±2.40[ae]*
ALT	65±1.09[b]	103.33±1.50[a]*	94.33±5.42[ac$]	97.67±2.50[ad]*	90.66±6.08[ae]*
28 days					
AST	66.00±2.60[b]	122.33±3.82[a]*	88.16±6.61[ac]*	101.0±5.25[adNS]	92±5.65[ae]*
ALP	124±5.05[b]	163.66±2.87[a]*	140.16±6.17[ac]*	146±4[ad]*	138.5±1.97[ae]*
ALT	65.50±1.87[b]	122.33±3.82[a]*	91.66±7.94[ac]*	101.16±6.17[ad]*	98.00±5.65[ae]*

Unit - IU/L, values are expressed as mean±SD, n=6, #is P<0.05, $is P<0.01, *P<0.001, [b](control group) versus [a](LPS-treated group), [a](LPS-treated group) versus [c](LPS+Std.), [a](LPS-treated group) versus [d](LPS+MJ-1), [a](LPS-treated group) versus [e](LPS+MJ-2)

Table 4: Effect of methyl jasmonate on different antioxidant enzymes (% inhibition)

Sample	50 µg/mL	100 µg/mL	200 µg/mL	400 µg/mL	500 µg/mL
MJ	14.13±0.347	18.35±0.589	23.97±0.485	30.54±0.891	39.33±0.987
αtocopherol	68.35±0.883	74.38±0.752	82.41±0.910	91.21±0.894	94.72±0.888

Values are expressed as mean±SD, n=3

Table 5: Effect of methyl jasmonate on different antioxidant enzymes

Group	SOD (U/mg of protein)	CAT (H$_2$O$_2$/min/mg protein)	GS H (umol/g of protein)	GPx (nmol/mg of protein)	GR (nmol/mg of protein)
Control	8.55±0.42[b]	54.49±1.32[b]	9.91±0.47[b]	97.03±2.57[b]	1.982±0.084[b]
LPS-treated	3.12±0.44[a]*	32.3±0.91[a]*	4.80±0.23[a]*	58.43±2.12[a]*	0.800±0.033[a]*
Std.	7.46±0.87[ac]*	45.83±0.98[ac]*	8.55±0.39[ac]*	86.50±2.2[ac]*	1.308±0.042[ac]*
MJ-1	4.62±0.49[ad]*	50.26±0.98[ad]*	6.61±0.29[ad]*	73.78±1.66[ad]*	1.002±0.073[ad]*
MJ-2	5.94±0.40[ae]*	53.86±1.36[ae]*	7.81±0.35[ae]*	84.95±2.48[ae]*	1.140±0.053[ae]*

Values are expressed as mean±SD, n=6, #is P<0.05, $is P<0.01, *P<0.001, [b](control group) versus [a](LPS-treated group), [a](LPS-treated group) versus [c](LPS+Std.), [a](LPS-treated group) versus [d](LPS+MJ-1), [a](LPS-treated group) versus [e](LPS+MJ-2)

**Table 6:** Effect of methyl jasmonate on blood cells and ESR

Group	RBC (millions/mm³)	WBC (thousands/mm³)	ESR (mm/Hrs)
Control	5.985±0.040[b]	7.802±0.136[b]	1.89±0.033[b]
LPS-Treated	4.373±0.132[a]*	11.478±0.272[a]*	6.27±0.037[a]*
Std.	5.182±0.044[ac]*	8.252±0.077[ac]*	4.29±0.034[ac]*
MJ-1	5.528±0.066[ad]*	8.903±0.049[ad]*	3.895±0.033[ad]*
MJ-2	5.815±0.048[ae]*	9.587±0.025[ae]*	5.757±0.035[ae]*

Values are expressed as mean±SD, n=6, #is P<0.05, $is P<0.01, *P<0.001, [b](control group) versus [a](LPS-treated group), [a](LPS-treated group) versus [c](LPS+Std.), [a](LPS-treated group) versus [d](LPS+MJ-1), [a](LPS-treated group) versus [e](LPS+MJ-2), RBC: Red blood cell, WBC: White blood cell

DISCUSSION

LPS-induced inflammation and cartilage damage are comprehensively studied and validated model for screening of antiarthritic activity. LPS is a biologically unique substance produced as a component of the Gram-negative bacterial cell wall.[34] LPS induces inflammation, hyperalgesia, and disturb immune system function.[35] These responses are mediated through the release and activation of pro-inflammatory cytokines such as TNF-α, interleukin-1b, and prostaglandins.[25] ROS are normally balanced by the endogenous antioxidant system. Disturbed balances in redox status may develop cellular oxidative stress. Failure of endogenous antioxidants to overcome ROS production, then external administration of antioxidants substances would be necessary to balance redox status.[36] LPS generates an abundance of ROS primarily from macrophages and infiltrating neutrophils.[37] In the present study, it was observed that the LPS generates oxidative stress
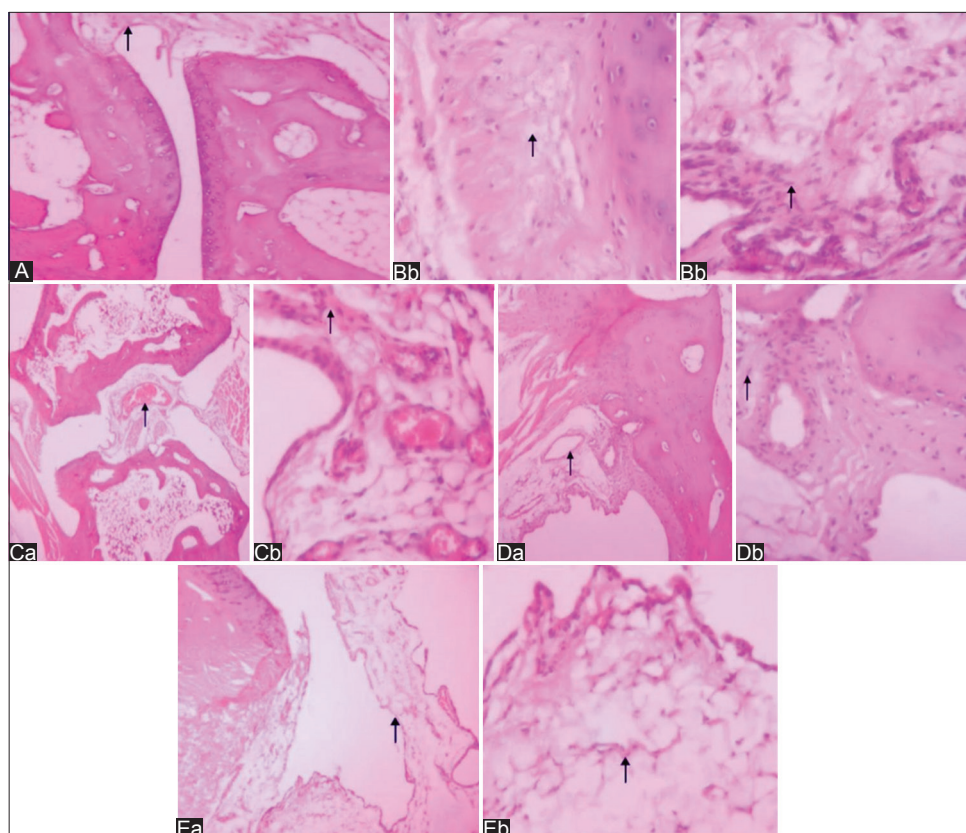
Figure 3: Effect of methyl jasmonate on histology of tarsal-metatarsal joint. (a) Section of tarsal-metatarsal joint treated with vehicle control in LPS-induced arthritis in rats (H and E, ×100), (b) section of tarsal-metatarsal joint treated with LPS 1 mg/Kg in LPS induced arthritis in rats. ([Ba] H and E ×100, [Bb] H and E ×400), (c) section of tarsal-metatarsal joint treated with LPS 1 mg/kg induced arthritis in rats followed by indomethacin (3 mg/kg b.w.) administration. ([Ca] H and E ×100, [Cb] H and E ×400), (d) section of tarsal-metatarsal joint treated with LPS 1 mg/Kg induced arthritis in rats followed by MJ (20 mg/kg b.w.) administration ([Da] H and E ×100, [Db] H and E ×400), and (e) section of tarsal-metatarsal joint treated with LPS 1 mg/kg induced arthritis in rats followed by MJ (40 mg/kg b.w.) administration ([Ea] H and E ×100, [Eb] H and E ×400)

and causes depletion of endogenous antioxidant enzymes, i.e., CAT, GSH, and SOD the depletion of endogenous antioxidant enzymes creates imbalance and causes ROS mediated cellular damage. SOD is major antioxidants in the cartilage, and its deficiency would lead to oxidant damage in the matrix and clinical disease. Several studies revealed that there is an association between decrease in extra cellar SOD and arthritis pain.[38] GSH is most studied antioxidants and plays roles as detoxification of electrophilic xenobiotics, regulates redox status.[39] In plant defense mechanism plant stress phytohormones such as methyl Jasmonate plays prominent role as an antioxidant and suppresses oxidative stress-mediated cellular damage.[8] The same evidences were observed in animals treated with methyl Jasmonate and minimize the depletion of endogenous antioxidant enzymes. Activated immune cells, such as macrophages and neutrophils in the host and, in turn, the stimulated cells synthesize pro-inflammatory factors, matrix proteases, and free radicals and lead to intense secondary inflammation in tissues begin transient synovitis-arthritis.[38] The inflammation and pain are associated with peri-articular tissue edema which causes an increase in paw swelling and ankle diameter. Assessment of arthritis effectively carried out by measuring the change in

paw volume using plethysmograph and swelling by measuring paw thickness.[40] In the present study treatment with methyl jasmonate reduces the paw volume and paw thickness which may be due to anti-inflammatory activity. Severity of arthritis is again an effective and simple way to calculate the arthritis index. The inflammation, paw swelling, and thickness are directly proportional to arthritis index. In the current study, the arthritis index is significantly reduced with the treatment of methyl jasmonate. This is again an indicating parameter which shows that MJ is potential anti-arthritis candidate.

Due to alteration in the metabolic activities of diseased group is underlying cause for mean reduction in body weight. Previous studies reveal that reduction in body weight during inflammation is due to deficient absorption of nutrients through the intestine.[41] Decrease in mean body weight was observed in LPS-treated rats due to inflammation. Methyl jasmonate shows gain in mean body weight as compared to disease group. The MJ effect on gain in body weight may be due to reduced inflammation or by improving the essential nutrient absorption through intestine.

In the clinical practice markers of bone are an excellent indicator of disease activity in bone disease, menopause, or

autoimmune processes, etc. Methyl jasmonate reduces the serum levels of ALP, AST, and ALT which is an indication for antiarthritic activity.

Change in hematological parameters is directly related to inflammation and is previous studies and reveals that there are changes in a count of RBC, WBC, and ESR. Chronic inflammation is associated with iron deficiency and disturbed body erythropoietin synthesis. Iron and erythropoietin are important for erythropoiesis in red bone marrow.[42] Methyl jasmonate compensates for control RBC count due to a reduction in inflammatory responses. Host defense cell-like WBC count is increased in inflammatory responses to fight against invading pathogen, bacteria, viruses, and initiate chemotactic cascades.[43] LPS-treated group showed an increase in WBC count as compared to methyl Jasmonate treated group. The ESR is common; a frequent simple test performed to assess the inflammation in arthritis. A fast sedimentation rate was observed in LPS treated a group as an incidence of severe infection. LPS causes severe damage to articular cartilage tissues which is observed in the disease control group. The observed changes were damaged synovial lining, loss of cellular integrity, pannus formation, and plump endothelial lining. The effective protection was observed in MJ-treated group and restores the normal articular cartilage structure which is directly related to its anti-inflammatory activity.

From the current study, we can conclude that the treatment with plant stress hormone Methyl Jasmonate ameliorates the biochemical and physical changes induced by LPS. The protective effect is presumably through its antioxidant, antiperoxidative, and anti-inflammatory activity. However, further studies are required to find out the exact mechanism at the molecular level, signaling proteins and a possible role of methyl jasmonate as a cartilage protective and antiarthritic candidate.

ACKNOWLEDGMENT

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