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Research article

Bioactive role of plant stress hormone methyl jasmonate against lipopolysaccharide induced arthritis

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ABSTRACT

The current investigation was carried out to screen antiarthritic potential of Methyl Jasmonate (MJ) against lipopolysaccharide (LPS) induced arthritis. Cartilage damage was induced in experimental animals by intraplantar administration of LPS (1 mg/kg) and antiarthritic effect of MJ was screened in two doses of MJ-1 (20 mg/kg), MJ-2 (40 mg/kg) by intraperitoneally administration. Indomethacin (30 mg/kg p.o.) was used as standard drug. The severity of arthritis was evaluated by assessing arthritis score, secondary lesions, motility test, stair climbing ability, and dorsal flexion pain score method. The estimation of blood cytokine tumor necrosis factor- aplha (TNF-α),interleukine (IL-2 and IL-6) and thymus/spleen index was carried out to access the severity of inflammation. Estimation of hepaticenzymatic antioxidant activity superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), glutathione peroxidase (GPx)and radiological examination was carried out on 28th day. Results indicated that MJ showed significant reduction in severity of arthritis by decreasing arthritis score, secondary lesions where as significant increase in motility, climbing ability and flexion pain score was observed. Significant decreased in blood cytokine viz. TNF-α, IL-2, IL-6 andthymus/spleen index was observed in MJ treated animals in dose dependent manner. MJ treated animals showed significant increased and restoration of hepatic antioxidant enzymatic activityof SOD, CAT, GSH, GPx where asradiological examination indicates protective effect on joint structure as compared to LPS treated rats. These current studies conclude that MJ has protective role in arthritis.

1. Introduction

Researchers have recognized numerous classes of phytohormones (PTH) amongst them salicylic acid and its congener derivatives were considerably studied and implicated them as a potential theraputic candidate (Verma et al., 2016). The jasmonate family is an essential class of PTH and prominent members are cis-jasmone, jasmonic acid, and MJ (fatty acid-derived cyclopentanones). MJ is abundant within the vegetation and regulates plant developmental processes and edition to the environmental conditions (Cheong and Do Choi, 2003; Farmer and Ryan, 1990). MJ is important for normal growth, flowering, bearing fruit, in defense mechanism, plant survival in biotic/abiotic stressful conditions like wear and tear injuries, osmotic shock, extreme UV radiations, drought, insect attach, including cell death, etc (Blée 2002; Ahmad et al. 2016, 2019; He et al., 2018). Considering the important role of MJ in the survival of plants the researchers are keenly interested to screen the potential industrial as well as clinical benefits of MJ. In food industries MJ is used as flavoring and preservative (Del Nobile et al., 2012), in cosmetics, it is used as an important perfuming agent (Pichersky and Gershenzon, 2002) whereas in for agricultural application it is an effective antimicrobial agent (Fesenko et al., 2019), preservative and antioxidant (Karaman et al., 2013). MJ has screened for its clinical benefits such as anticancer (Flescher 2005; Cohen and Flescher, 2009), anti-inflammatory (Dang et al., 2008; Lee et al., 2011), anti-nociceptive (Umukoro and Olugbemide, 2011), anti-anxiety, anticonvulsant (Annafi et al., 2014) anti-stress (Aluko et al., 2015), anti-malarial (Oyinloye et al., 2015) and adaptogenic activity (Umukoro et al., 2016). Animal models for screening of anti-arthritic activity have been extensively studied and reported by several researchers (Bendele 2001), among them bacterial endotoxin-induced cartilage damage has gained

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more central attention as these can mimic the same pathological change as that of human being and activates components of the adaptive immune system (Jasin 1983). LPS is an important component of the bacterial cell was and systemic administration of it can lead to severe inflammatory responses through stimulation of synthesis of inflammatory mediators (TNF-α, IL's, PG's) (Lorenz et al., 2013). It was also reported that LPS extensively increases oxidative burden after systemic administration (Noworyta-Sokołowska et al., 2013). LPS induced inflammatory responses are initiated through intracellular activation of toll-like receptors and induce gene expression by activation of NF-κB and AP-1 signal transduction pathways leads to elaborated synthesis of inflammatory mediators (Lai et al., 2017; Rahim et al., 2019). LPS is universally accepted for induction of various inflammatory responces considering the same, arthritis and associated inflammation was induced in rat by LPS 1 mg/kg and MJ was tested for 2 doses 20 and 40 mg/kg I.P respectively. Physical, biochemical and radiological examination was carried out to investigate the potential effect of MJ. This study provides insights of protective mechanism of MJ in LPS induced arthritis in experimental animals. Study also highlighted pathological contribution of various mediators like free radicals, nociceptive response and inflammatory cytokines induced by LPS administration. The current research will provide basis to investigate MJ role at molecular level.

2. Material and methods

2.1. Experimental animals

Before the commencement of animal experimentation, the protocol approval was taken from the institutional animal ethical committee (Ref. No. IAEC/2017-18/001). Healthy, adult male Wistar rats weighing between 150-200 g were obtained from the departmental animal house. A total of 30 rats were divided into 5 groups. The animals were maintained at standard laboratory conditions. The animals had free access to food and water with ad libitum. The experiment was performed according to CPCSEA guidelines.

2.2. Chemicals

The reagent/chemicals used for experimental purposes were of the analytical grade and with high purity. MJ (95%) and LPS were obtained from Sigma Aldrich, St. Louis, USA. The cytokine levels were (TNF-α, IL-2, IL-6) assayed by ELISA kit Bio vision and Millipore.

2.3. Experimental protocol

Experimental animals were randomly divided into five groups containing six in each group. Control group animals received vehicle orally, LPS treated animals were injected intraplantar with LPS (1 mg/kg), Standard treated animals group was intraplantar injected with LPS (1 mg/kg) and indomethacin (30 mg/kg) p.o. MJ-1 and MJ-2- groups animals were injected intraplantar with LPS (1 mg/kg) and MJ 20 mg/kg and 40 mg/kg I.P. respectively. LPS was injected in all groups 0 days except control, the treatment was given for the 28 days.

2.4. Preparation of drug solutions

The dose selection and preparation of the MJ solution was carried out using previously reported studies. The molecular weight of MJ is 224.3 g. The 1M solution of MJ contains 224.3g in 1000 ml and is 100%. Since MJ obtained from sigma was supplied in the strength of 95% which is corresponding to 213.0 g in 1000 ml. The 5 ml of MJ (95%) contains 1.155 g of MJ. In each 1 ml of MJ contains 0.231 g or 231.0 mg of MJ. The dose was prepared from a stock solution of 231.0 mg/ml 95% ethanol was used for the dissolution of MJ and further diluted with distilled water. MJ was given to experimental animals in a dose of (20–40 mg/kg) intraperitoneal injection (null[). The gift sample of indomethacin standard drug was obtained from Alkem Pharmaceuticals, Mumbai. Indomethacin was prepared in 1% acacia and administered in a dose of 30 mg/kg orally (Sinnathambi et al., 2011).

2.5. Assessment of arthritic score

The assessment of arthritis severity become carried out using the previously reported arthritic scale method. The animals were scaled on 0–4, thinking about the dimensions as, Scale 0 = Complete absence of edema or swelling, Scale 1 = Presence of mild edema or swelling and partial erythema, Scale 2 = Presence of moderate edema or swelling and erythema from the ankle to the tarsal bone, Scale 3 = Presence of mild edema or swelling and erythema from the ankle to the tarsal bone. Scale 4 = The presence of extreme edema or swelling and erythema from the ankle to the entire leg. The assessment was carried out on the 7^{th}, 14^{th}, 21^{st} and 28^{th} days of the treatment protocol. 16 is considered as a max allowed scale by the sum of scale for 4 limbs of each rat (Kothavade 2015a; Wijekoon et al., 2019).

2.6. Assessment of secondary lesions

Assessment of secondary lesion became confirmed via absence or presence of lesion on non injected fore paws, hind paw, nose and tail. The percentage inhibition was carried out thinking about overall numbers of animals in each group and presence of lesion on number of animals. The assessment was carried out on 7^{th}, 14^{th}, 21^{st} and 28^{th} day of treatment protocol (Newbould 1963).

2.7. Motility test, stair climbing ability, and dorsal flexion pain

Assessment of motility and climbing capacity was achieved at the 7th, 14th, 21^{st} and 28^{th} day of the study protocol. The motility was observed for five min and scored on scale 0 to 2 points. Score 0 = Rats heading off touching ft to the ground, Score 1 = strolling with issue and toes touching the ground, Score 2 = walks easily. The climbing potential of rats were tested for at 5, 10 and 15 cm staircase. The water kept on the second one staircase (10 cm) and food changed into kept on a third staircase (15 cm) respectively and allowed to assess. Stair climbing ability of rats was scored from 0 to 3. Score 0 = rats did not climbed any step, Score 1 = climbed onto Step 1 (5cm), Score 2 = climbed onto Step 2 (10 cm), Score 3 = climbed onto Step 3 (15 cm). Dorsal flexion pain (DFP) was achieved at the ankle joint of the injected paw with an interval of five seconds with 5 repetitions on 7th, 14th, 21^{st} and 28^{th} of a study protocol. The injected paw joint turned into flexed dorsally until the toe touches the anterior leg part. The assessment of squeaking and leg withdrawal response turned into recorded by thinking about the subsequent score, score 0 = absence of squeaking and leg withdrawal response, 1 = presence of both squeaking or leg withdrawal response, 2 = presence of each squeaking and leg withdrawal response (Kothavade 2015a; Amdekar et al., 2012; Kothavade et al., 2015b).

2.8. Thymus and spleen index

After completion of a treatment protocol that is on 28^{th}day animals were weighed and sacrificed using a high dose of anesthesia. The abdominal cavity was opened and identified thymus and spleen. These immune organs were gently removed and weighed on a balance. The index was expressed as the ratio of wet spleen/thymus against rat body weight and calculated based on mg/g (Li et al., 2018).

2.9. Estimation of enzymatic antioxidant activity

SOD activity from liver homogenate was determined as the ability to inhibit the auto-oxidation of pyrogallol by measuring increases in the absorbance at 420 nm (Nam et al., 2006). Catalase activity was assayed according to the method described earlier using H_2O_2 as a substrate. The

rate of decomposition of a substrate (H_2O_2) was measured at 240 nm (Umar et al., 2012). NADPH consumed by the reduction of the oxidized form of glutathione was determined by measuring absorbance at 340 nm and glutathione peroxidase (GPx) activity was calculated (Sinnathambi et al., 2011; Nam et al., 2006). The thiobarbituric acid-reactive substance was measured as a marker of lipid peroxidation. TBA was measured at 532 nm spectrophotometrically (Nam et al., 2006). The GSH activity was measured mediating the reaction of DTNB (5,5′-dithiobis(2-nitrobenzoic acid)) with thiol-containing compounds like GSH and produces 2-nitro-5-thiobenzoic acid and glutathione disulfide (GSSG). The 2-nitro-5-thiobenzoic acid is a yellow-colored compound and can be detected by spectrophotometry at the wavelength of 412nm (Umar et al., 2012).

2.10. Estimation of serum cytokine levels

On 28th day, animals were mildly anesthetized under diethyl ether and blood was collected from retro-orbital plexus for a serum sample. The serum was separated using a cold centrifuge at 6000 rpm for 25 min and stored at -70°C until use. The serum inflammatory mediators TNF-α, IL-1, IL-6 were estimated using commercial rat cytokine ELISA assay kits (Bio vision and Millipore). The assay procedure was followed as per the manufacturer instructions and values are expressed pg/ml.

2.11. Statistical analysis

Statistical analysis was carried out using graph pad prism software (Version 8.4). The data were analyzed by applying a one-way analysis of variance (ANOVA) followed by Dunnet's multiple comparison tests. Data are expressed as mean ± S.D where n = 6. The nociceptive score of motility test, stair climbing ability, and dorsal flexion pain was analyzed by the Kruskal-Wallis test was used to compare the groups and expressed as median scores. $P < 0.05$ is considered as significant and represented with *.

3. Result

3.1. Effect of MJ on the arthritic score

The severity of arthritis was measured in arthritis score by physical observation on 7th, 14th, 21st and 28th day. Treatment with MJ did not show any significant ($P > 0.05$) change in arthritis score as when compared with LPS on the 7th day. Significant ($P < 0.05$) decrease in arthritic score was observed on the 21st and 28th day in MJ treated rats when compared against the LPS treated group. Significant decreased in arthritis score in MJ treated rats is dose-dependent (Figure 1a).

3.2. Effect of MJ on secondary lesions

The assessment of inhibition of secondary lesion was calculated by considering the absence or presence of a lesion on non injected fore paws, hind paw, nose, and tail. The MJ did not show any significant ($P > 0.05$) difference in inhibition of secondary lesions on day 7th when compared against LPS treated animals. Significant inhibition ($P < 0.05$) of the secondary lesion in the MJ treated group was observed. MJ-1 treated group showed 35.5 ± 2.10, 55.13 ± 3.51, 67.15 ± 4.50 percent inhibition whereas MJ- treated group showed 44.45 ± 3.50, 74.14 ± 5.27, 96.02 ± 4.42 percent inhibition on 14th,21st and 28th day respectively (Figure 1b).

3.3. Effect of MJ on motility test, stair climbing ability, and dorsal flexion pain

LPS induces a significant decrease in motility score when compared against control group animals and a median score of 0 was obtained on the 28th day. On day 7thMJ did not show any significant improvement in motility score when compared with LPS treated animals but on 21st and 28th day of treatment protocol significantly ($P < 0.05$) ameliorated motility and median score 2 was observed on 28th day (Figure 2a). Intraplantar injection of LPS in rat paws significantly affected the animals' stair climbing ability and a median score of 0 was obtained on 28th day compared with control group animals. Treatment with MJ resulted in a significantly improved stair climbing ability and median score from 2 to 3 was observed on 21st and 28th day compared to LPS treated group (Figure 2b.) The LPS treated group rats exhibited a significant ($P < 0.05$) low threshold of pain and a median score of 2 was observed on 28th day compared to the control group. Treatment with MJ showed significant ($P < 0.05$) enhanced antinociceptive response to pain induced by LPS and median score 0 to 1 was obtained on 28th day (Figure 2c.)

3.4. Effect of MJ on thymus and spleen index

A significant increase ($P < 0.05$) in the thymus and spleen index was observed in LPS treated animals compared to the control group. Treatment with MJ significantly decreases ($P < 0.05$) thymus and spleen index as compared to animals treated with LPS. The reduction in indices was observed in a dose-dependent manner (Table 1).

3.5. Effect of MJ on enzymatic antioxidant activity

The control group showed normal levels of antioxidant enzymes where a significant decrease in antioxidant enzyme levels was observed in LPS treated animals. Animals treated with MJ showed a significant increase ($P < 0.05$) in levels of antioxidant enzymes viz SOD, CAT, GSH, and GPx when compared with animals treated with LPS (Table 2).

3.6. Effect of MJ on serum cytokine levels

Animals injected with LPS show a significant ($P < 0.05$) increase in levels of pro-inflammatory mediators (TNF-α, IL-2, IL-6) as compared to the control group. MJ shows significant inhibition on ($P < 0.05$) the production of pro-inflammatory mediators in a dose-dependent manner (Figure 3).

3.7. Radiological examination

The radiological examination indicated that the treatment with MJ-1 and MJ-2 showed the absence of joint space narrowing and destruction when compared with LPS treated rats. Whereas MJ-1 animals showed the presence of joint erosion which was absent in MJ-2 treated animals (Figure 4). The examination was carried out by the certified radiologist.

4. Discussion

In the present study, we assessed the anti-arthritic potential of MJ in LPS induced cartilage damage in experimental animals. Bacterial LPS are the important cell wall component of gram-negative bacterial and systyemic administration of these polysaccharides in animals can stimulate the synthesis of several cytokines and initiates inflammatory cascades (Sweet and Hume, 1996; Gasparrini et al., 2018). Inflammation and associated pain perception are the body's self-defense mechanisms and characterized by redness, swelling, heat sensation, etc. These inflammatory cascades are important for the survival of human being but if remains persistent can lead to severe chronic inflammatory diseases like ulcerative colitis, arthritis, asthma, cancer, and Crohn's disease, etc (Lorenz et al., 2013). LPS induced inflammation is a highly validated model for the induction of pathological changes and able to produces multifacial vital organ damage (Suntres and Shek, 1996; Seemann et al., 2017). Numerous *in-vitro* experimental models are designed to investigate potential anti-inflammatory, anti-oxidant effect of test molecule against LPS stimulated diverse cell lines. Recent study showed that LPS stimulates normal airway cells and bronchial tissues were found with

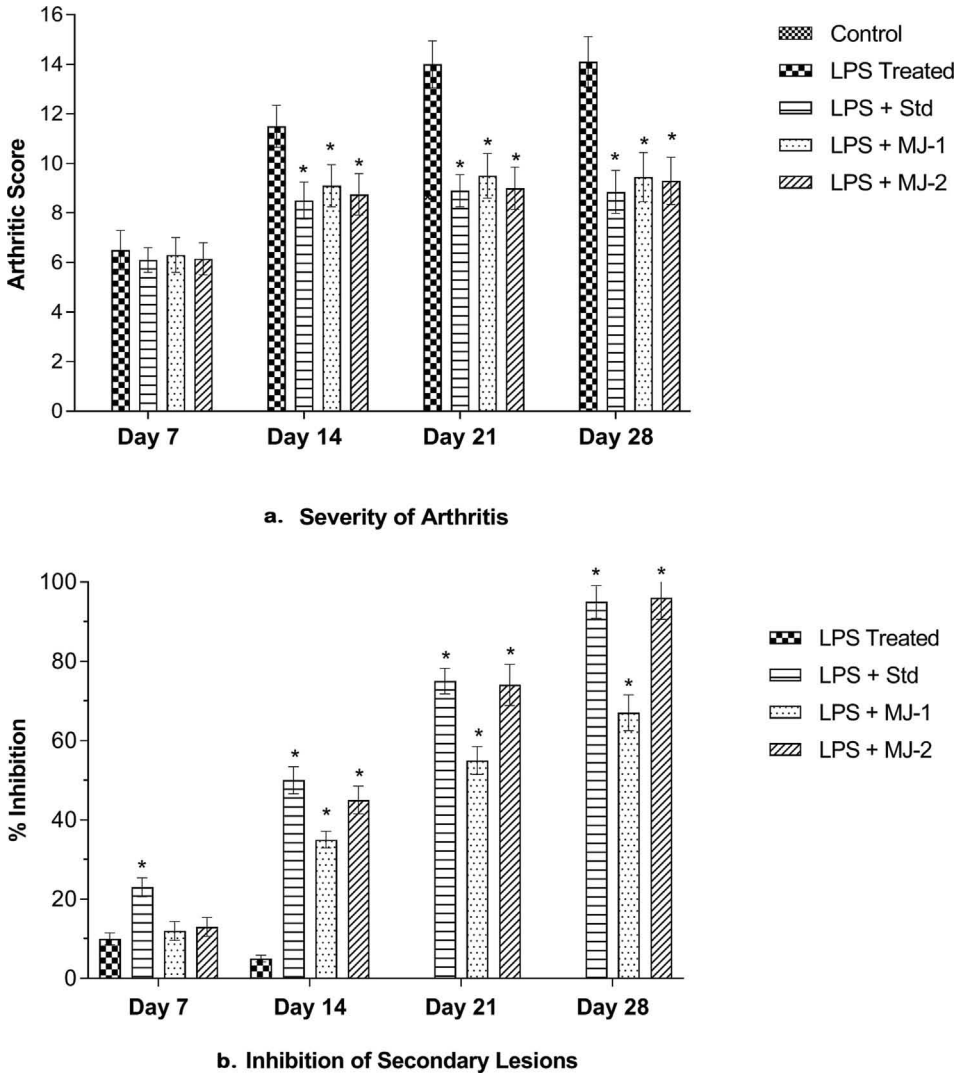

Figure 1. Effect of MJ on arthritis score and secondary lesions in LPS induced arthritis. a. Arthritic score, b Inhibition of scecondary lessions. Values are expressed as mean ± S.D., n = 6, *is $P < 0.05$, Control Vs. LPS Treated, LPS Treated Vs. LPS + Std., LPS Treated Vs. LPS + MJ-1, LPS Treated Vs. LPS + MJ-2. The experimental setup was repeated twice and all data were averaged.

significant increase in oxidative stress quantified by measuring H_2O_2, malondialdehyde (MDA) and NO activity, whereas a significant decrease in levels of GSH and SOD's. The study also showed that the LPS profoundly enhances levels of inflammatory mediators like IL-1β, IL-6, IL-8 and TNF-α and contributed for inflammatory responses. The underlying mechanism involved stimulation of neurokinin A (NKA) by LPS. The prominent inflammatory effect of NKA is mediated through enhanced levels of oxidant factors and inflammatory mediators by activation of NK-1 and NK-2 receptors (Calzetta et al., 2018; Cazzola et al., 2017). Another study illustrated effect of LPS in bovine mammary epithelial cells on proliferation, inflammatory factors and antioxidant enzyme activity. The result showed LPS treatment significantly decrease GPx, CAT and SOD activity and enhanced MDA content. IL-1, IL-6 and NO concentrations. The principle mechanism involved is activation of inflammatory cells by LPS (Shi et al., 2016). Another investigation advocated role of LPS induced expression of proinflammatory cytokine in H292 human lung mucoepidermoid carcinoma cells and THP-1 human monocytic cells. The cell viability assay showed significant cytotoxic effect, Significant enhanced levels of TNF-α, IL-6, 8, 10, TNF-α, MMP-9 and TIMP-1 in LPS treated H292 and THP-1 cells. The study reported that the LPS induces the activation of NF-κB signaling pathway causes profound expression of proinflammatory genes for TNF-α and proinflammatory interleukins in activated macrophages (Liu et al., 2018). The RAW264.7 cells are commonly used in screening of antioxidant and anti-inflammatory potential of test molecule. The study demonstrated role of LPS stimulated inflammatory response and oxidative stress in RAW264.7. The result of study indicates LPS dramatically increases the production of pro-inflammatory cytokines such as NO, iNOS, IL-6, and TNF-α in RAW264.7 cells by activation of NF-κB and increases the expression and synthesis of inflammatory cytokines (TNF-α, IL-6, IL-1β, NO, and iNOS2). In the conclusion it was summarized that inhibition or disorder of NF-κB pathway could be potential target in the treatment of inflammation and oxidative stress (Han et al., 2019). Activated NF-κB regulates gene transcription related to host immune, inflammatory responses and oxidative stress. LPS induces accumulation and infiltration of the immune cell through chemical signaling (Akira 2003; Asehnoune et al., 2004). On tissue injury, blood cells/fluid and protein get accumulated to the area of injury due to change in vascular permeability causes swelling of inflamed area (Li et al., 2007; Aukland and Reed, 1993). This effect is largely facilitated by inflammatory mediators like PG, IL's, TNF-α, histamine, and bradykinin, etc. The severity of arthritis can be directly assessed by measuring a change in paw thickness, paw volume, and arthritis index. Our previous study advocated that the administration of MJ showed a significant reduction in these parameters

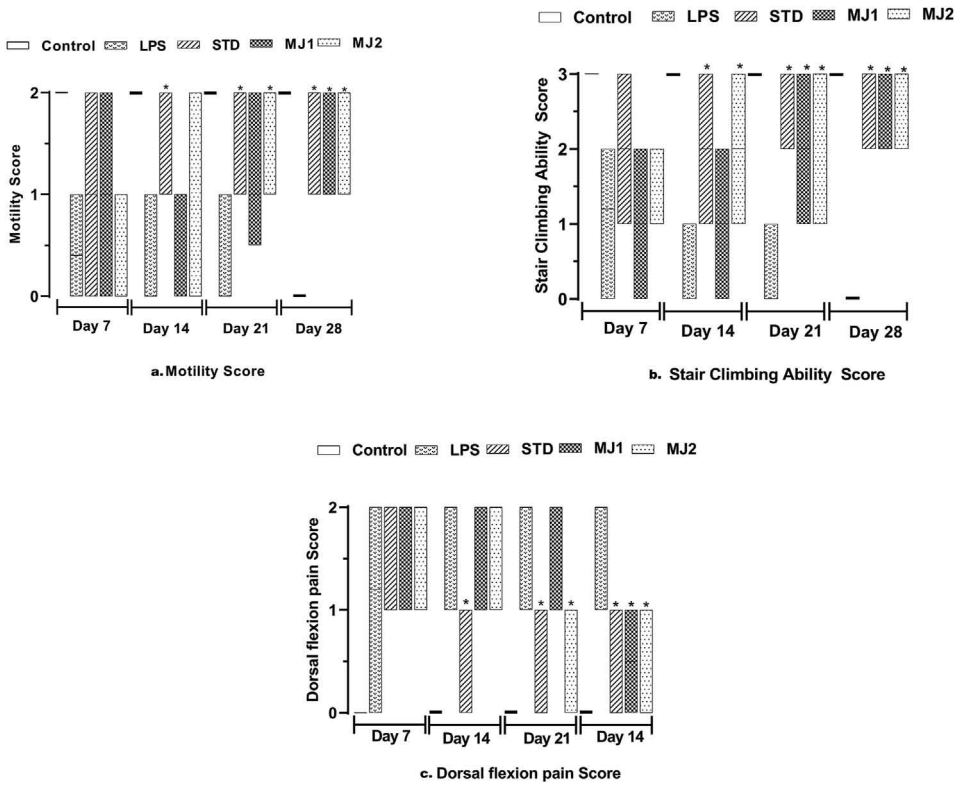

Figure 2. Effect of MJ on LPS induced inflammatory hyperalgesia. The scores of a. Motility b. Stair climbing abilityand c. Dorsal flexion pain shown in figure 3. Boxes represent interquartile ranges (25th and 75thpercentiles), bold line represents median values (n = 6); *is $P < 0.05$, Control Vs. LPS Treated, LPS Treated Vs. LPS + Std., LPS Treated Vs. LPS + MJ-1, LPS Treated Vs. LPS + MJ-2.

(Gunjegaonkar and Shenmugarajan, 2019). Assessment of arthritic score is also an important consideration in physical observation of inflammation and swelling. Treatment with MJ showed a reduction in arthritis score which indicates minimum accumulation of fluid, cell and protein due to inhibition of inflammation and associated mediators. LPS activates destructive intracellular signaling pathways through inflammatory mediators like TNF-α, IL, PG, LT's, histamine, etc (Lin et al., 2017). PG's especially PGE2 play a crucial role in the perception of pain to nociceptive responses (Kawabata 2011). Administration LPS results in the development of pain behaviors in experimental animals and is highly intense termed as hyperalgesia and assessed by physical methods viz. motility test, stair climbing ability, and dorsal flexion pain. The pain carrying sensory neurons (C fiber and Aδ-fiber) are innervated to brain higher center (DRG and TRG). The activation of these neurons is caused due to the activation of nociceptors present across the neuroaxis. The PGE2 and cytokines and main signaling molecules involved in the activation of nociceptors (Egan et al., 2004; Song et al., 2000). The treatment with MJ significantly controls these hyperalgesic responses produced by LPS injection. The enhanced motility, stair climbing ability, and reduced DFP indicate MJ anti-nociceptive effect may be contributed by a reduction in levels of PGE2 and IL's which was observed in our previous studies (Gunjegaonkar and Shenmugarajan, 2019). Administration of LPS leads to the activation of host immune systems and initiated defense mechanism through the synthesis of antibodies or cell-mediated immunity

Table 1. Effect of MJ on thymus and spleen index.

Group	Control	LPS Treated	Std	MJ-1	MJ-2
Thymus Index	0.39 ± 0.05	0.68 ± 0.08	0.45 ± 0.09*	0.52 ± 0.08*	0.048 ± 0.07*
Spleen Index	2.75 ± 0.50	3.95 ± 0.60	3.15 ± 0.55*	3.27 ± 0.42*	3.20 ± 0.35*

Values are expressed as mean ± S.D., n = 6, *is $P < 0.05$, Control Vs. LPS Treated, LPS Treated Vs. LPS + Std., LPS Treated Vs. LPS + MJ-1, LPS Treated Vs. LPS + MJ-2. The experimental setup was repeated twice and all data were averaged.

Table 2. Effect of MJ on enzymatic antioxidant activity.

Group	SOD (U/mg of protein)	CAT (H_2O_2/min/mg protein)	GSH (μmol/g of protein)	GPx (ηmol/mg of protein)
Control	7.55 ± 0.52	60.49 ± 0.92	8.45 ± 1.27^{b}	79.52 ± 1.72
LPS Treated	2.92 ± 0.89	29.55 ± 1.21	3.95 ± 0.85	48.53 ± 1.44
Std	6.41 ± 0.81*	49.13 ± 1.08*	7.55 ± 0.99*	66.13 ± 1.02*
MJ-1	4.82 ± 0.79*	36.20 ± 0.88*	5.61 ± 0.59*	55.15 ± 0.96*
MJ-2	5.90 ± 0.55*	43.16 ± 1.10*	7.81 ± 1.05*	63.50 ± 1.15*

Values are expressed as mean ± S.D., n = 6, *is $P < 0.05$, Control Vs. LPS Treated, LPS Treated Vs. LPS + Std., LPS Treated Vs. LPS + MJ-1, LPS Treated Vs. LPS + MJ-2. The experimental setup was repeated twice and all data were averaged.

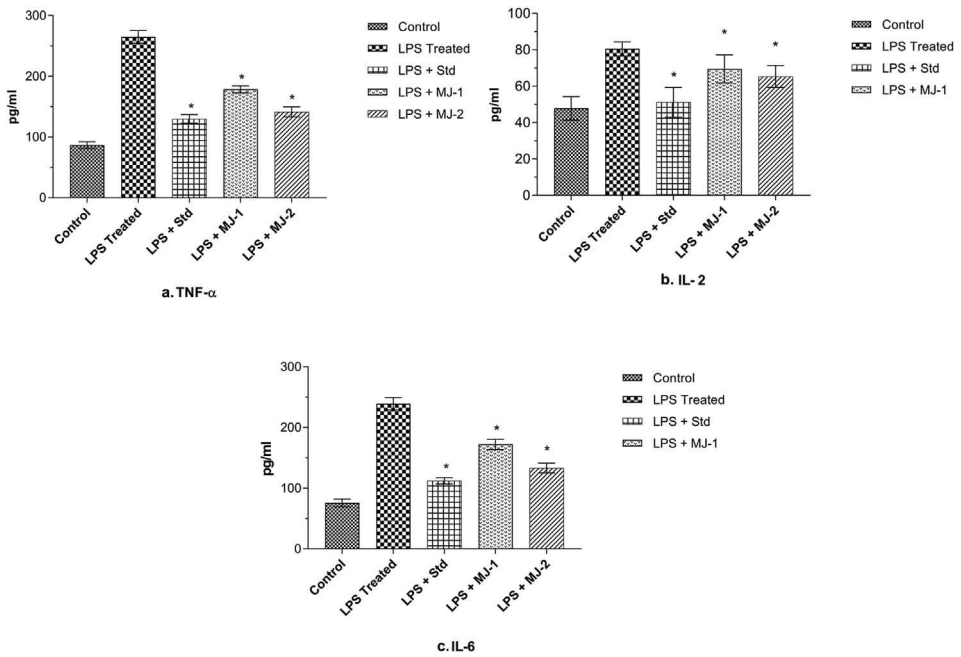

Figure 3. Effect of MJ on serum cytokine levels. a. TNF-α, b. IL-2, c. IL-6. Values are expressed as mean ± S.D., n = 6, *is $P < 0.05$, Control Vs. LPS Treated, LPS Treated Vs. LPS + Std., LPS Treated Vs. LPS + MJ-1, LPS Treated Vs. LPS + MJ-2. The experimental setup was repeated twice and all data were averaged.

responses (Anderson 2000; Rosadini and Kagan, 2017). The thymus gland is a specialized primary lymphoid organ of the immune system and important for maturation of T cells. T cells are essential to activate adaptive or acquired immune system to fight against foreign invaders (Thapa and Farber, 2019). LPS administration leads to an increase in thymus mass due to the activation of an adaptive immune response in the event of antigen invading and activation of T cells. In inflammatory cascades, the WBCs turnover has been found to increase to fight against the antigen. The spleen is primarily associated with the storage and recruitment of WBS's and was found enlarged in severe inflammatory conditions (Lewis et al., 2019). Administration of MJ showed minimum activation of the host immune system and WBC's. A significant reduction in the spleen thymus index was observed in MJ treated animals due to its anti-inflammatory effect. It was well addressed that LPS not only activates nuclear factor-κB (NF-κB) signaling pathway but induces severe oxidative stress primarily from macrophages and infiltrating neutrophils,

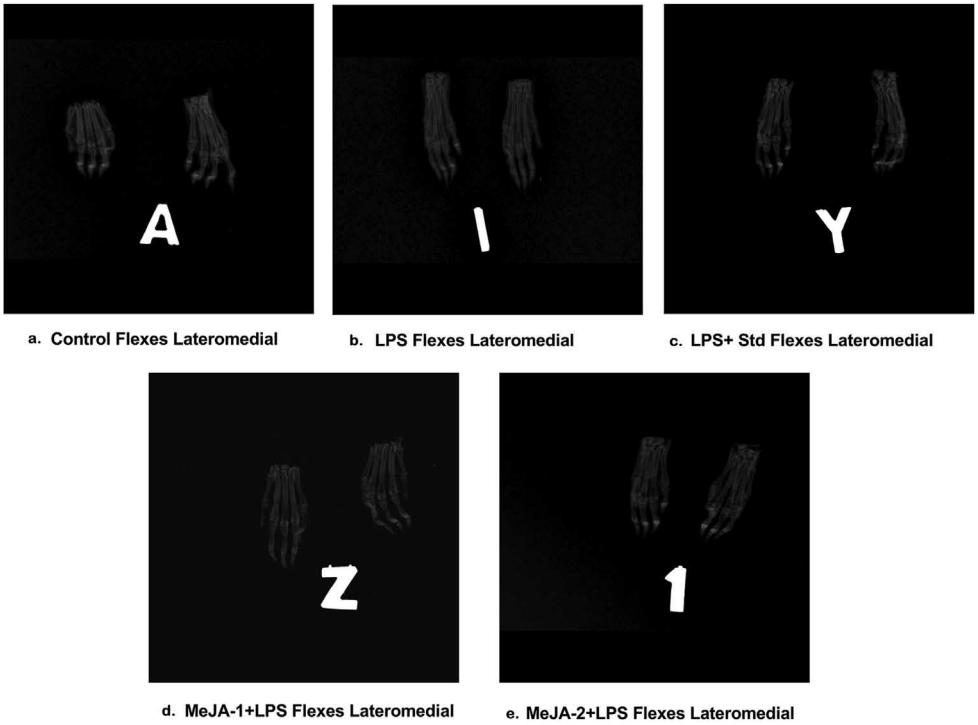

Figure 4. Effect of MJ on Rat joints (Radiological examination). a. Control, b. LPS Treated, c. LPS Treated Vs. Std., d. LPS Treated Vs. MJ-1, e. LPS Treated Vs. MJ-2.

increased pro-inflammatory cytokines such as TNF-α, IL and PG's (Ala'a-Ahmed and Willias, 2010). Oxidative stress-mediated by ROS/RNS is normally encountered by the endogenous enzymatic antioxidant system. In disturbed endogenous antioxidant system or uncontrolled generation of free radicals disturbs cellular redox status develops cellular oxidative stress (Laye et al., 2000). In the present study, it was observed that the LPS significantly depletes the levels of endogenous antioxidant enzymes viz. SOD, CAT, GPx, and GR. Treatment with MJ significantly stored the antioxidant enzyme levels as compared to LPS treated animals (Apel and Hirt, 2004). MJ has been well recognized for its potent antioxidant activity in plants and the same has been found in human being and suppress the free radical-mediated cellular damage (Gunjegaonkar and Shanmugarajan, 2018). The inflammatory cytokines play a central role in damaging the articular cartilage. Their causative contribution is studied to a greater extent from human and animal experimentations. Cytokines promote catabolic destruction process and disturb the homeostasis of articular joint tissues. The main cytokines involved in pathogenesis and signaling pathways are IL-1β, IL-6, IL-15, IL-17, IL-18, and TNF-α (Lotz, 2011; Goldring et al., 2011; Wojdasiewicz et al., 2014). IL-2 is a member of the γ-chain cytokine family and involved in generating pain hypersensitivity. IL-2R receptors are expressed by DRG neurons and activated by IL-2. Several studies reported that the administration of IL-2 enhances mechanical sensitivity is and pain sensation in OA patients (Song et al., 2000). IL-6 contains 184 amino acids and plays a significant role in the activation of host immune cells. Under the influence of IL-1β and TNFα, the synthesis of IL-6 is carried out by osteoblasts, chondrocytes, macrophages, etc. The IL-6 binds to membrane receptor mIL-6R and the soluble SIL-6R and follows the activation of cells. The intracellular signaling involves phosphorylation of tyrosine residues mediated by JAK kinase and phosphorylation of MAPK, and activation of the PI3 K/AKT pathway (Kamimura et al., 2003). The effect of IL-6 is similar to other cytokines and synergies them. The IL-6 is responsible to decrease in the production of type II collagen, increases the production MMPs, abnormal changes in the subchondral bone by bone resorption (Steeve et al., 2004). A significant decrease in serum level of IL-2 and IL-6 was observed in MJ treated rats as compared to LPS treated animals. TNF-α is a homotrimerictransmembrane protein secreted by the cells in the joint. TNF-α directs chondrocytes to produce MMP-1, MMP-3, MMP-13, and ADAMTS-4. TNF-α significantly induces apoptosis and limits the migration of chondrocytes (Zwerina et al., 2007). TNF reduces the efficacy of the respiration chain process to synthesis ATP within mitochondria and causes TNF mediated chondrocyte cell death. TNF-α induces the production of iNOS, COX-2, and PGE2 synthase and shows a synergize effect with IL-1β (Bodmer et al., 2002). Recent studies have demonstrated that anti-cytokine drugs are effective in controlling the pain and inflammation in experimental animals and would be effective in the treatment of joint-related diseases. The radiological examination showed significant protection against LPS induced damage in experimental animals treated with MJ. In conclusion, the study reveals that MJ a plant stress hormone reduces arthritis score, hyperalgesia and pain perception. The host immune system is less activated in response to endotoxin administration and indicated reduced spleen and thymus index, a significant increase in antioxidant enzyme levels and significant inhibition of cytokines, (TNF-α, IL-2, and IL-6) in MJ treated animals as compared to LPS injected animals. However, further investigations are required to address the effect of MJ on an expression of mediators and the underlying mechanism. Despite the extensive animal studies some translational research limitations are there like randomization and blinding was not performed in animal experimentations which is important consideration in study to address the unbiased findings. One of challenge for translational research is natural divergences between animal models and humans physiological and pathological system. Furthermore, investigation via single *in-vivo, in-vitro* disease model will not exactly mimic the similar pathological cascades in comparison to human beings (Pound and Ritskes-Hoitinga, 2018).

Declarations

Author contribution statement

S. Gunjegaonkar: Conceived and designed the experiments; Performed the experiments; Wrote the paper.

S.B. Wankhede: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

T.S. Shanmugarajan: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

S.D. Shinde: Analyzed and interpreted the data; Wrote the paper.

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The authors declare no conflict of interest.

Additional information

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